

# Topical application of a cleanser containing extracts of *Diospyros kaki* folium, *Polygonum cuspidatum* and *Castanea crenata* var. *dulcis* reduces skin oil content and pore size in human skin

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**Abstract.** The effects of skin pores on skin topographic features can be reduced by decreasing excessive production and accumulation of sebum and elimination of comedones. Therefore, a cosmetic cleanser that regulates sebum homeostasis is required. In the present study, the effects of a cosmetic cleanser that contained *Diospyros kaki* folium, *Polygonum cuspidatum* and *Castanea crenata* var. *dulcis* (DPC) was examined on the removal of sebum and on skin pore size. Healthy volunteers (n=23) aged 20-50 years were asked to apply the test materials to the face. Skin oil content, pore size, pore number and extracted sebum surface area were measured using various measurement methods. All the measurements were performed at pre- and post-application of the test materials. When the cosmetic cleanser containing DPC was applied to the skin, the oil content decreased by 77.3%, from 6.19 to 1.40. The number of skin pores decreased by 24.83%, from 125.39 to 94.23. Skin pore size decreased from 0.07 to 0.02  $\mu\text{m}^3$  (71.43% decrease). The amount of extracted sebum increased by 335% when the DPC cleanser was used. Compared to the control cleanser, skin oil content was significantly decreased when the cleanser that contained DPC was used. The cleanser containing DPC also decreased pore size and number. Finally, the DPC cleanser easily removed solidified sebum from the skin.

## Introduction

In skin, the sebaceous glands produce and secrete sebum that consists of a complex mixture of lipids that include functions, such as photoprotection, antimicrobial activity, delivery of fat-soluble anti-oxidants to the skin surface and pro- and anti-inflammatory activity exerted by specific lipids for homeostasis (1,2). Excreted sebum also maintains the skin surface moisture via the formation of a lipid film on the skin (3). Sebum is excreted to the skin surface through the skin pore, which is an opening of the pilosebaceous unit (2). The skin pores affect the topographical features at the skin surface. Under specific conditions, enlarged funnel-shaped skin pores may be present. Skin pore widening is induced by various exogenous and endogenous factors, such as gender, genetic predisposition, aging, chronic ultraviolet light exposure, comedogenic xenobiotics, acne and seborrhea (4). Various factors induce excessive sebum synthesis and accumulation in the pilosebaceous unit, which also contributes to skin pore size (5). The enlarged skin pores can be visible as 'orange peel skin', which is a significant cosmetic problem (6). In addition, lipid oxidation solidifies accumulated sebum, which generates comedone formation (7).

A cosmetic cleanser is required that reduces skin pore size by decreasing excessive sebum production and accumulation, and that eliminates comedones. The objective of the present study was to evaluate the effect of a cosmetic cleanser on the removal of sebum and on skin pore size. The cleanser contained a mixture of *Diospyros kaki* folium, *Polygonum cuspidatum* and *Castanea crenata* var. *dulcis* (DPC).

*Diospyros kaki* folium (persimmon) is widely cultured in eastern Asia (8,9). *Diospyros kaki* folium leaves are used for herbal medicines as they contain abundant flavonoid glycosides that are used for their microbial inhibition, radical scavenging, neuroprotection, blood-pressure reduction and thrombosis inhibitory effects (10,11). *Polygonum cuspidatum* and *Castanea crenata* var. *dulcis* are also used for herbal medicines in eastern Asia (12). *Polygonum cuspidatum* has been used in traditional medicine for the remedy of inflammatory diseases,

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hepatitis, tumors, diarrhea, dermatitis and osteomyelitis (13). Additionally, in recent studies, the resveratrol present in high concentrations in *Polygonum cuspidatum* has been found to be an anti-inflammatory via reduction of cytokine levels in plasma and an anti-oxidant (14,15). *Castanea crenata* var. *dulcis* is representatively used as an antiwrinkle agent in East Asia (16).

The overall purpose of the present study was to determine the effects of DPC added to a cosmetic cleanser on enlarged pores and sebum elimination. The associations between pore size and sebum emission were assessed in the absence and presence of DPC.

## Materials and methods

**Preparation of test materials.** DPC was purchased from Ami Cosmetics (Seoul, Korea). Each dried sample of *Diospyros kaki* folium and *Castanea crenata* var. *dulcis* (1 kg) was incubated for 3 h in 5 l water at 85°C. *Polygonum cuspidatum* was incubated for 3 h in 5 l water at 75°C. The extracts were filtered using a 0.45  $\mu\text{m}$  membrane and vacuum evaporated using an Evelyn-1100 evaporator (EYELA, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The concentrated extracts were freeze-dried using a Bondiro FD8508 freeze dryer (ilShinBioBase Co., Ltd., Dongducheon, Korea). All the extracts were sterilized using 60-Co  $\gamma$ -radiation (10 kGy/h, 25 kGy) prior to use.

The base cosmetic cleanser prepared for the study contained 3.5% dipropylene glycol, 2.5% hydrolyzed algin, 0.1% sodium hyaluronate, 2.5% panthenol, 1% disodium-EDTA, 0.01% triethanolamine and 0.03% sodium lauroyl sarcosinate. The cosmetic cleanser with the DPC extracts was prepared by adding 4% DPC extracts to the base cosmetic cleanser.

**Subjects and treatments.** The study protocols were approved by the Institutional Review Board of the Korea Institute for Skin and Clinical Sciences (Seoul, Korea). Informed consent was obtained from all the participants. In total, 23 patients (7 male and 16 female) were studied, aged 20–50 years, who had no skin disease or hypersensitivity. Each side of the subject's nose was washed with 70% ethanol prior to application of the test materials. The control cosmetic cleanser was applied to the left side and the DPC cosmetic cleanser was applied to the right side of the nose. After a 20-min application of the test materials, external solidified sebum was harvested using a curette. Oil content and skin pore count and area were measured. All the experiments were performed in a temperature- and humidity-controlled room. The temperature was  $22 \pm 1^\circ\text{C}$  and the humidity was  $45 \pm 5\%$ .

**Evaluation of oil content in skin.** The oil content in the skin was measured by first applying sebum tape to each side of the nose, prior and subsequent to application of the cleansers. The oil content of the sebum tape was measured using a DermaLab USB sebum probe (Cortex Technology ApS, Hadsund, Denmark).

**Evaluation of skin pore count and area.** A PRIMOS Lite skin measurement device (field of view 45x30-simple, flexible 3D measuring; GFMesstechnik GmbH, Berlin, Germany) was utilized for evaluation of skin pore count and area. Each side of the nose for each subject was measured three consecutive times.

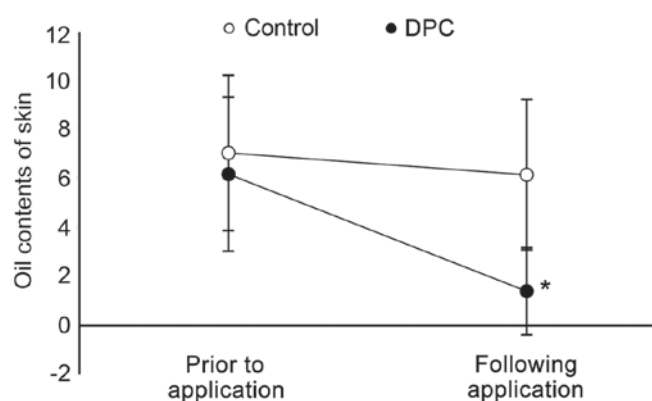


Figure 1. Change in oil content following application of a cosmetic cleanser containing *Diospyros kaki* folium, *Polygonum cuspidatum* and *Castanea crenata* var. *dulcis* (DPC) (filled circles), or a control cleanser (open circles). \* $P < 0.05$ , compared to the control. Results are expressed as the mean  $\pm$  standard error for the experiment, which was performed using 23 participants.

The measurements were taken prior and subsequent to application of the test materials. The images were fitted into the same position using 3-dimensional matching and were analyzed using the PRIMOS Lite software (PRIMOS Lite version 5.6E).

**Evaluation of external sebum.** For evaluation of external sebum, emitted sebum was harvested from a 1-cm<sup>2</sup> skin surface area using a comedone extractor. The sebum was diffused onto a glass slide and photographed. The sebum was calculated and quantified using Image J software (National Institutes of Health, Washington, DC, USA).

**Statistical analysis.** The statistical significance of the differences was determined using the Student's paired t-test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Effect of DPC on oil content of skin.** The effect of DPC on skin oil content was first assessed. A DermaLab USB sebum probe was used on each side of the nose where the control and DPC cosmetic cleanser was applied. As indicated in Fig. 1, skin oil content decreased by 12.70%, from 7.06 to 6.16, for the control cosmetic cleanser. The skin oil content decreased by 77.3% (from 6.19 to 1.40) when the cleanser that contained DPC was used.

**Effect of DPC on number and size of skin pores.** The numbers and sizes of skin pores were also evaluated to reveal the effects of the DPC cosmetic cleanser on the skin. Using the PRIMOS Lite, the numbers of skin pores were counted prior and subsequent to application of the control cosmetic cleanser. The number of skin pores increased by 3.05%, from 124.00 to 127.78 (Fig. 2). The number of skin pores decreased by 24.83%, from 125.39 to 94.23, when the cleanser containing DPC was used. PRIMOS Lite was also used to reveal the skin pore size. The control cleanser increased skin pore size, from 0.05 to 0.06  $\mu\text{m}^3$  (20% increase). The cleanser containing DPC decreased skin pore size, from 0.07 to 0.02  $\mu\text{m}^3$  (71.43% decrease) (Fig. 3).

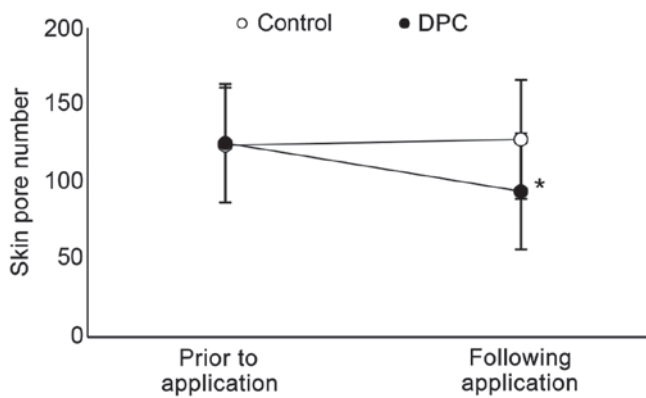


Figure 2. Change in skin pore number following application of a cosmetic cleanser containing *Diospyros kaki* folium, *Polygonum cuspidatum* and *Castanea crenata* var. *dulcis* (DPC) (filled circles), or a control cleanser (open circles). \* $P < 0.05$ , compared to the control. Results are expressed as the mean  $\pm$  standard error for the experiment, which was performed using 23 participants.

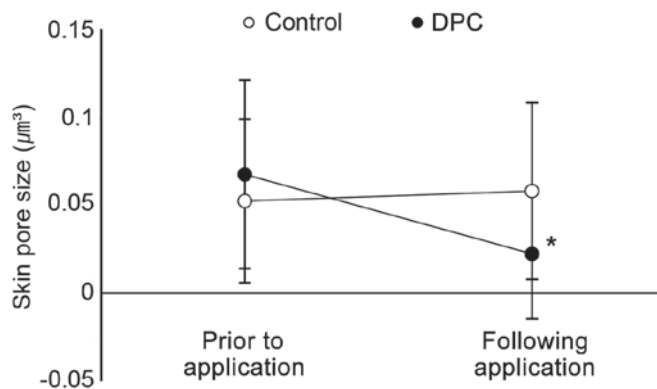


Figure 3. Change in skin pore size following application of a cosmetic cleanser containing *Diospyros kaki* folium, *Polygonum cuspidatum* and *Castanea crenata* var. *dulcis* (DPC) (filled circles), or a control cleanser (open circles). \* $P < 0.05$ , compared to the control. Results are expressed as the mean  $\pm$  standard error for the experiment, which was performed using 23 participants.

**Effect of DPC on amount of extracted sebum.** To quantify the amount of extracted sebum, the straightened area was measured using Image J software. The straightened area of sebum increased 335% when DPC cleanser was used (Fig. 4A). In addition, *Demodex mites* were detected in extracted sebum (Fig. 4B). Overall, this result suggested that the cleanser containing DPC extracted a greater amount of sebum compared to the control cleanser from human skin.

## Discussion

To the best of our knowledge, the present study is the first to reveal that a cosmetic cleanser containing DPC decreases skin sebum and decreases the numbers and sizes of skin pores. Oil content decreased by 12.60% when the control cleanser was applied to the skin. However, pore size and number were slightly increased, by 20 and 3.05%, respectively, when the control cleanser was used. These results indicated that the cleanser reduced skin oil content, but with the accompanying side effects of increases in pore size and number. This result was

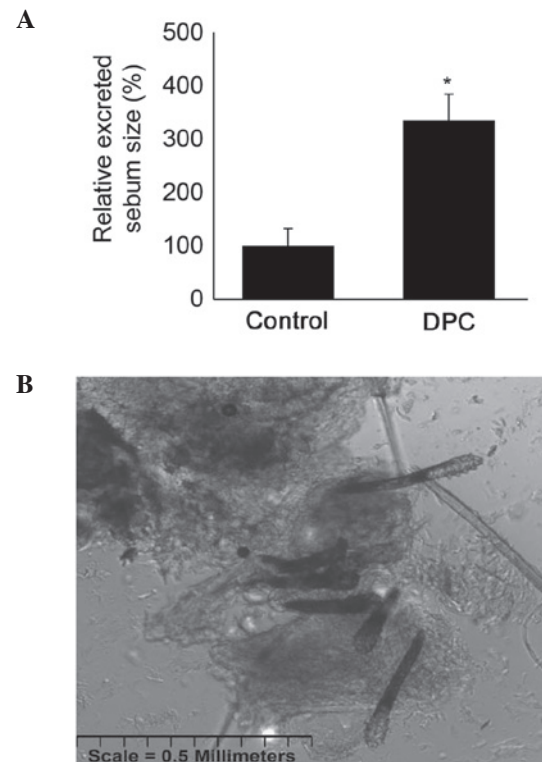


Figure 4. Evaluation of extracted sebum following application of cosmetic cleanser containing *Diospyros kaki* folium, *Polygonum cuspidatum* and *Castanea crenata* var. *dulcis* (DPC) and a control cleanser. (A) \* $P < 0.05$ , compared to the control. Results are expressed as the mean  $\pm$  standard error for the experiment, which was performed using 23 participants. (B) *Demodex mites* was detected by light microscopy (magnification,  $\times 100$ ).

consistent with the results of an earlier study, which indicated that the detergent contained in cleansers induces irritation, inflammation and increases androgens (17-19). In general, it is well-known that irritation, inflammation and androgen activation provokes intercellular reactive oxygen species, which induce cell death, inflammation, increase cytokine expression and can increase skin pore size and number (18-20).

Additionally, in the DPC-containing cleanser applied participants, extracted solidified sebum increased by 335%, compared to the control cleanser. In the extracted solidified sebum, *Demodex mites*, which is associated with the development of rosacea and seborrheic dermatitis feed on sebum (21,22), was detected by light microscopy. The results suggested that the DPC cleanser facilitated extraction of *Demodex mites*.

In conclusion, the skin oil content was significantly decreased by the DPC cleanser, compared to the control cleanser. The cleanser that contained DPC decreased pore size and number, and the DPC cleanser easily removed solidified sebum in skin.

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