Olive Branch Extract as a Potential Cosmetic Ingredient with Skincare Properties

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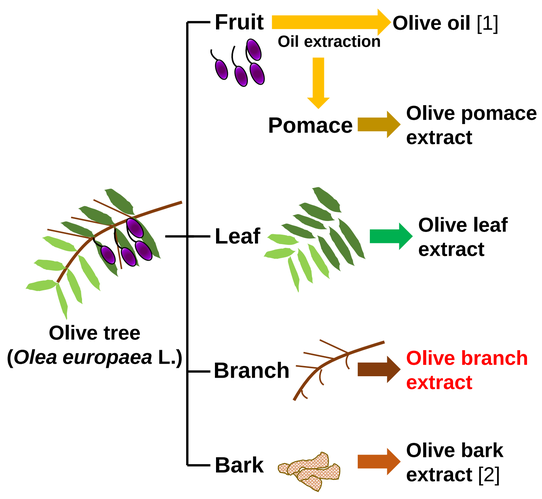
Introduction:

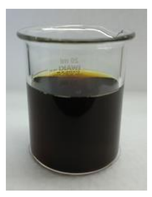
Olive tree (*Olea europaea* L.) is a traditional plant and its fruits are used for olive oil production. Olive oil extraction produces by-products such as pomace, leaves, and twigs, which have become a major environmental issue. In addition, a massive amount of the branches obtained during the pruning of olive trees and harvesting and cleaning of olive fruits before oil extraction is considered a waste product of the olive supply chain.

Previous studies have shown that by-products such as pomace, leaves, and bark from the olive industry are rich sources of bioactive compounds and that these by-products have added value as cosmetic agents. However, previous studies have not focused on the cosmetic value of olive branches. Thus, the objective of the present study was to evaluate the properties of the olive branch extracts as a potential skincare cosmetic component.



Materials & Methods:

Preparation of olive branch extract
Olive branches: 30% (w/w) ⇒ 5–30%
Solvent: 1,3-butylene glycol (BG)
Temperature: 50 ℃ ⇒ 40–80 ℃
Extraction time: 3 h ⇒ 0.5–3 h



***in vitro* assay**

- **Total phenolic content (TPC) assay:** Folin-Denis method.
- **Antioxidant activity assay:** Oxygen rapid absorbance capacity (ORAC) method.
- **Glycation inhibition assay:** Collagen and elastin glycation assay kit (Cosmo Bio).
- **Dermal enzyme activity assay**: *in vitro* methods described previously [1,2].

Cellular assay

- **Fibroblast activation assay:** Normal dermal fibroblasts (NHDFs) were used.
- **Melanin production assay:** Mouse B16 melanoma cells (4A5) were used.

Results & Discussion:

1. Determination of optimum conditions to obtain phenolic-rich extracts from olive branch

Possible interactions among the process operating parameters such as extraction temperature, extraction time, and input of the extracted material should be considered to optimize the extraction of components from plant materials. Based on these three parameters, extraction for each operation condition was evaluated using TPC as an index. Extractions at higher temperatures showed higher TPC values than those at lower temperatures (Fig.-1A). The TPC increased moderately by 2 h (Fig.-1B). The maximum input of the material selected as the optimum input of the extracted material was 30% (w/w) of the olive branch (Fig.-1C). The TPC value obtained under the optimum extraction conditions was eight times higher than the TPC value obtained under basic extraction conditions (Fig.-2A). The extraction obtained under optimum conditions could be an antioxidant and anti-glycation ingredient in functional cosmetical products (Fig.-2B and 2C).

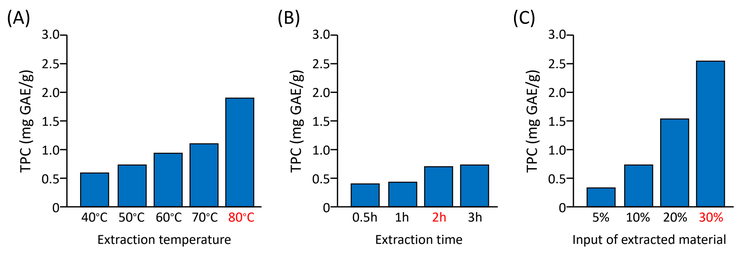


Fig.-1 Effect of the three independent variables on the behavior of TPC of OBrE.

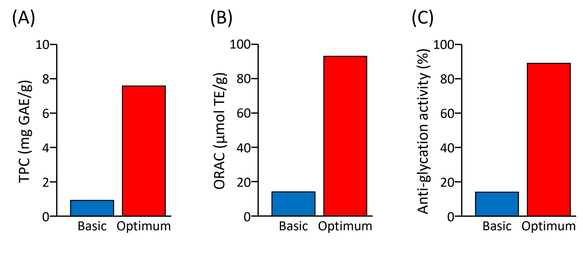


Fig.-2 Comparisons of TPC, antioxidant, and anti-glycation activities of OBrE obtained from the basic and optimum extraction conditions.

2. Assessment of the physicochemical stability of the OBrE

We conducted the following preliminary stability studies to assess the initial phases of product development. The OBrEs were subjected to thermal stress at 100 ℃ for 10 h, freeze-thaw cycles (-30 ℃/24 h and then 40 ℃/h per cycle); pH stress (exposure to a wide pH range); and light stress (exposure to natural sunlight for 10 h). These stability tests revealed that the phenolic compounds of OBrE were stable under the tested physicochemical conditions, except under alkaline conditions (Fig.-3).

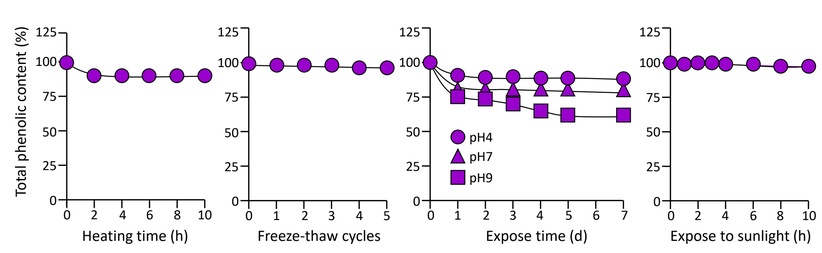


Fig.-3 Physicochemical stability of OBrE.

3. Evaluation of anti-dermal enzymatic activities of OBrE

We examined the anti-skin-aging activity of OBrE for use in skincare by measuring the *in vitro* inhibitory activity of dermal enzymes and found that OBrE inhibited the enzymatic activities of collagenase and hyaluronidase (Fig.-4A and 4B). However, OBrE did not inhibit the activity of elastase and tyrosinase (Fig.-4C and 4D). These results indicate that OBrE has an anti-aging activity, and thus, could be a useful ingredient in functional cosmeceutical products.

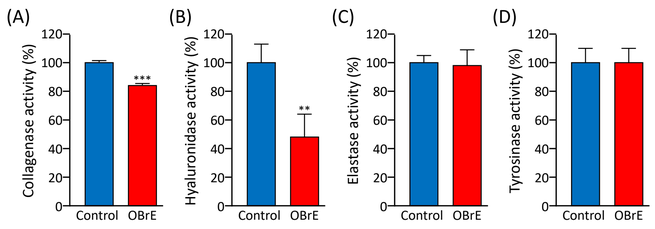


Fig.-4 Anti-dermal enzyme activities of OBrE. (**p<0.01, ***p<0.001; Student's t-test).

4. Effect of OBrE on the activation of human dermal fibroblasts

NHDFs were treated with OBrE at different concentrations for 24 h to examine the effect of OBrE on dermal proliferation. Treatment with OBrE stimulated the growth of NHDFs by 9–16 % at concentrations of 0.1 % and 0.3 % (v/v) when compared to that of the solvent-treated control cells (Fig.-5A). Treatment with OBrE enhanced collagen production by NHDFs by 4 % at concentrations of 0.03 %, 0.1 %, and 0.3 % (v/v) when compared with that of the solvent-treated control cells (Fig.-5B). These results showed that OBrE induced the mild activation of NHDFs.

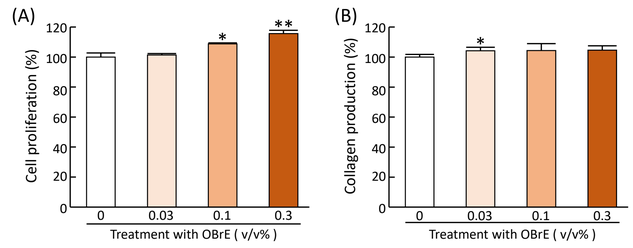


Fig.-5 Cell proliferation and collagen production by the OBrE-stimulated NHDFs. (*p<0.05, **p<0.01; Student's t-test).

5. Inhibitory effect of OBrE on melanin production in B16 melanoma cells

We determined the rate of melanin production using mouse B16 melanoma cells stimulated with or without α-melanocyte-stimulating hormone (MSH) to evaluate the effects of OBrE on melanogenesis. Mouse B16 melanoma cells were treated with various concentrations of the extract for 3 days. Treatment with OBrE at different concentrations (1.25, 2.5, and 5 μL/mL) did not reduce the rate of melanin production in the cells without α-MSH stimulation compared to that in untreated control cells (Fig.-6A and 6C). However, in cells stimulated with α-MSH, the rate of melanin production was significantly reduced by OBrE in a concentration-dependent manner (Fig.-6B and 6D). α-MSH stimulates melanogenesis via the G protein-coupled receptor-cyclic adenosine monophosphate-microphthalmia-associated transcription factor pathway. On the contrary, OBrE did not inhibit activity of tyrosinase, which is the rate-limiting enzyme in melanogenesis (Fig.-4D). These results suggest that OBrE may serve as an effective inhibitor of α-MSH-dependent melanogenesis in melanocytes.

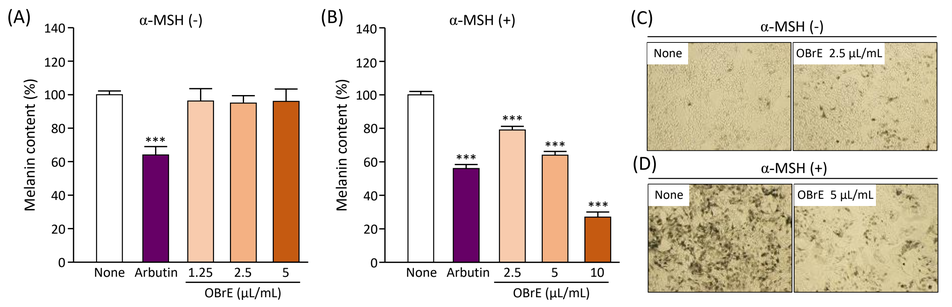


Fig.-6 Inhibitory effect of OBrE on melanin content. (***p<0.001; Student's t-test).

Conclusions:

The results of the present study suggest that a temperature of 80 ℃ with 30% input of dried olive branch, and an extraction time of 2 h should be employed as the optimum operating conditions to extract the highest total polyphenlic content from olive branch. The obtained OBrE showed high antioxidant, anti-glycation, and anti-dermal enzyme activities, which are stable against various physicochemical stresses. OBrE enhanced the skin fibroblast proliferation and collagen synthesis, and inhibited α-MSH-induced melanogenesis. These results show that OBrE could be a potential cosmetic ingredient with skincare properties.

References:

1. Kishimoto N., (2021) An innovative olive oil for attenuating age-related skin changes. IFSCC 2020 Yokohama: P-63.

2. Kishimoto N., (2020) *Olea europaea* (olive) bark extract as a potential cosmetic sunscreen agent with combined photoprotective and skincare properties. IFSCC 2018 Munich: P-2S-279.