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Evaluation of the safety of Sacha inchi seed oil (SIO) compared to olive oil for use in cosmetics

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Introduction

The cosmetic products are substances or mixture substances which to be applied externally to improve the appearance of the outside body[1, 14]. There may also be adverse side effects and lead to skin damage. The assessment of substances safety has typically involved the skin damage that concern for the skin structure arrangement with this procedure has been evaluated by skin organ tissue cultures that allows for the determination of skin damage by measuring epidermal thickness, this alternative *in vitro* methods[15]. The skin organ tissue cultures closely mimic the physiological and biochemical activities of the human skin when applied with substances in real-life situations[15]. The development of alternatives to skin organ tissue model is the test methods that avoid the use of laboratory animals. *In vitro* tests can be used to predict the potential of cosmetic products for further clinical trial.

The oils from the Sacha Inchi seed (SIO), is rich in Poly Unsaturated Fatty Acids (PUFA), particularly alpha linolenic acid (ALA) and linoleic acid (LA), which make up about 82% of the total oil content[5, 8]. The PUFA have been reported to have a strong inflammatory properties[3, 6] and an effective moisturizer capability including stratum corneum (SC) protectants and emollients[7]. ALA can decrease skin dryness and scaling as well as a variety of other inflammatory skin abnormalities[6]. LA plays a significant role in the skin, as a component of lipids in the sebaceous glands, assisting in the regeneration of the structure of the lipid barrier in the epidermal stucture[2, 12]. Both ALA and LA are able to be incorporated into the lipid components of the cell membranes to regenerate the skin damaged[6]. SIO also contains bioactive compounds including tocopherols and polyphenol compounds[4, 5] which have significant antioxidant capabilities for maintaining the human skin. Oxidation is a reaction of the chemical interaction with the cell constituents including DNA structure, protein and lipids that play a role in photosensitivity disorders of human skin[9, 11, 13]. With the above mentioned beneficial effects of SIO, they can be safely used as ingredients in cosmetic preparations for skin care.

The aim of this study was to investigate the safety of SIO when applied for skin care. Firstly, the anti-oxidation activity of SIO was determined, and was to determine whether epidermal arrangement is associated with increased epidermal by *In vitro* skin organ culture model.

Methods

Samples

The extraction of SIO by cold pressing provided a yellow to golden liquid. The oil samples used in our study were supplied by TAI.C.M.S. Standard Industrial Company Limited. The samples were stored at 4ºC until used.

Evaluation of radical scavenging activity

DPPH assay were used to evaluate the properties of SIO extracts for measure free radical scavenging activity. The SIO extracts, and the positive control (Trolox), were dissolved into various concentrations which were pipetted into a 96-well plate with 150 µl. of DPPH solution added. The solution mixtures were incubated at room temperature for 30 min. and kept from light. The absorbance was then read using a microplate

spectrophotometer (Spectra Count, Packin Elmer, USA) at 515 nm. The percentage of inhibition was calculated using the following equation.

$$\% \text{ inhibition} = \text{control} - \text{test/control} \times 100$$

Where 'control' was the absorbance of the control reaction and 'test' was the absorbance in presence of samples. Testing samples were replicated in triplicate.

In vitro skin organ culture model

In this study, the study protocol was approved by an institutional review board of Naresuan University, Phitsanulok, Thailand. (COA No.087/2016; Approval Date: Mach 22, 2016).

Skin culture

Skin culture was prepared as described previously by Varani (1993). Briefly, a skin tissue from a female in the age range of 50-65 years was prepared by punching 6 mm. diameter biopsy cultures, which were placed into a 24-well plate and immersed in a medium consisting of Keratinocyte Basal Medium (KBM) and 1.4 mM Ca^{2+} of $CaCl_2$ supplement. Previous studies had demonstrated that this condition was required skin cultures[10].The skin tissue cultures were incubated at 37ºC in a humid atmosphere containing 5% CO_2 for 12 hr., after which time the test samples were applied on the air-exposed epidermis for a further 48 hr.

Histological evaluation

Hematoxylin and Esosin (H&E) staining were used to study the skin tissue morphology and pathology. Hematoxylin is a dye for staining the basophilic tissue and the nuclei. Eosin is an acid dye used to stain the cytoplasm and extracellular matrix. The skin tissue samples were fixed in tissue freezing medium and stored at –80°C. The frozen tissues were sectioned using a cryostat with 8 µm. in thickness, which were placed on slides. The sections were examined histologically after staining with hematoxylin and eosin. The skin tissue morphology and pathology were measured using the AXIO software (Carl Zeiss Microscopy Ltd., Cambridge, UK).

Results

Evaluation of radical scavenging activity

The potential antioxidant activities of the cold-pressed SIO were evaluated using DPPH. The concentration of the test samples providing 50% inhibition of the radical scavenging activity (RSA) was 73.05± 0.02 mg/ml for DPPH. The antioxidant activities of SIO protected oxidation reaction and provide more maintaining the human skin. This suggests that further investigation of the skin benefits associated with this radical scavenging activity is warranted.

In vitro skin organ culture model

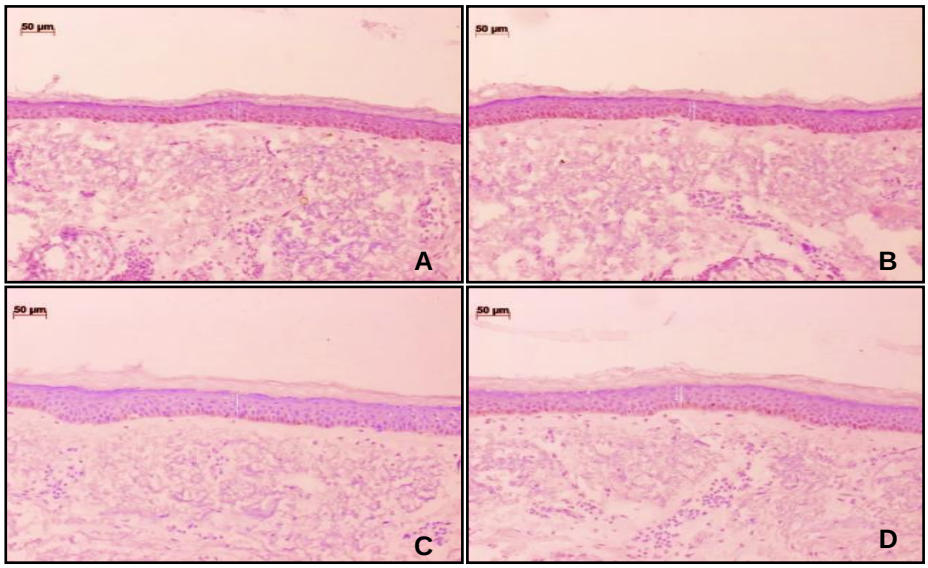

Figure 1 Histology of normal age skin at Time0 (the baseline) (A) and after 48 hr. of non-treatment (B), after 48 hr. of treatment with SIO (C) and after 48 hr. of treatment with olive oil (D), stained with hematoxylin-eosin, 20X.

The obtained this result, after applying both SIO and Olive Oil (benchmark) on the human skin tissue cultures, for 48 hr. no change in the thickness of the epidermis was compared to control conditions (non-treatment). Figure 1 shows the skin histology at Time0 (the baseline) and after 48 hours of treatment. The epidermis thickness was measured from the skin stained by H&E using AXIO program.

Discussion

The radical scavenging activity (RSA) of the SIO samples was analyzed using DPPH methods. The positive control (Trolox) showed an IC50 value of 23.06 ± 0.03 µg/mL which indicated that SIO has even lower radical scavenging activities than the positive control. In a similar study of the antioxidant activities of olive oil, Minioti et al. (2010) the RSA of SIO was shown to be higher than in most olive oils investigated using the DPPH method. However, a study by Follegatti-Romero et al. (2009) indicated that SIO comprises of tocopherols (2.39 g/kg), which could increase resistance against oxidation because they are the most active antioxidants in the oils.

The improvement of epidermal thickness is a sign of skin damage. In the present study, epidermal thickness was measured and compared between test samples including SIO and Olive oil (benchmark). After for 48 hours of treatment, no increase in epidermal thickness was observed in SIO and olive oil $p<0.05$ as compared to the non-treatment: this is a significant result, indicating that the oils did not induce skin damage. While most of the current interest in the extracts relates to their skin safety, the focus of our study was on identifying the safety for the compounds in SIO. Skin damage is a cosmetic problem, but it is also a significant cause of morbidity. If the damage of skin barrier can be proved, it might be possible to delay or prevent the serious consequences that can occur when the tissue is damaged.

Conclusion

Our observations and tests, in this study, suggest that SIO cannot induce a thickening of the epidermis, and is safe to use as an ingredient of cosmetic products. These results can be used to inform and guide further investigations into the skin benefits of SIO, and particularly the development of better skin care products.

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