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Evaluation of moisturizing and irritation potential of sacha inchi oil

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Abstract

Objective: The moisturizing and irritation effects of sacha inchi oil were evaluated.

Study Design : The moisturizing effect on the skin was clinically assessed using a regression study design. Sacha inchi oil or olive oil (benchmark) was applied on the left or right lower leg of the subjects for 14 days followed by application discontinuation for 2 days. The TEWL, skin moisture content and dryness appearance were observed.

Methods: The fatty acid composition and characteristics of cold-pressed sacha inchi seed oil were determined. Skin tissues cultured ex vivo were used to assess primary irritation induced by the oil by examining keratin 1 expression and TNF-α and IL-1α release from the oil-applied tissues.

Results: The sacha inchi oil contained 42.3% linolenic acid and 39.5% linoleic acid. This oil's saponification, iodine, acid and peroxide values were 168.58 ± 1.55 mg KOH/g, 203.00 ± 0.04 g I_2/100 g, 1.68 ± 0.03 mg KOH/g, and 1.95 ± 0.26 mEq peroxide/kg, respectively. Compared with nontreated skin tissues, induced secretion of TNF-α and IL-1α and disruption of keratin 1 integrity in the stratum corneum layer were not found in the sacha inchi oil-treated tissues. In a clinical study with 13 volunteers, the improvement in moisture content and skin dryness appearance at the sacha inchi oil-applied site was comparable with that observed at the olive oil-applied site.

Conclusions: The sacha inchi oil was mild to the skin and benefited dry skin.

KEYWORDS
irritation, moisturizing effect, olive oil, sacha inchi oil

1 | INTRODUCTION

Dry skin, which is associated with the loss of skin humidity and elasticity, is usually characterized by a rough, scaly, and flaky surface. Generally, it is caused by changes in stratum corneum (SC) composition and structure, leading to impairment of skin barrier function.[1] This impairment is associated with a decrease in the SC hydration level and an increase in transepidermal water loss (TEWL), which provokes the inflammatory process. Important components of the SC are dead corneocyte cells that contain keratin filaments surrounded by a lipid-enriched matrix. Keratin 1/keratin 10 dimers are reported to be the dominant keratin molecules in corneocytes and play a role in maintaining SC hydration and barrier and mechanical integrity.[2] The lipid matrix is composed of a mixture of ceramides, fatty acids, and cholesterols and organized into a lamellar bilayer. These nonaqueous components are important for barrier permeability. Moreover, a number of hygroscopic molecules considered "natural moisturizing factors or NMFs," such as lactic acid, pyrrolidone

carboxylic acid, and amino acids, are found in the SC and maintain efficient hydration.[3]

Dry skin can be treated with moisturizers to restore SC hydration and improve the visual signs of dryness. Ingredients with emollient, humectant, and/or skin protection functions are commonly included in moisturizers. Several natural oils have been reported as efficient moisturizing agents through their emollient and occlusive properties.[4,5] For example, olive oil, one of the most commonly used moisturizing products, has been reported to provide moisturizing, anti-bacterial, anti-inflammatory, and UV protection activities.[6-8] These activities are associated with the presence of fatty acids, including omega-3 (α-linolenic acid, 0.5%-0.9%), omega-6 (linoleic acid, 2%-10%), and nonessential omega-9 (oleic acid, up to 78%-80%) fatty acids, squalene and phenolics.[6-9] However, olive oil has been reported to disrupt the skin barrier, thereby acting as an irritant and causing erythema.[10,11] Because a high amount of oleic acid is present in olive oil, such disruption is possibly a result of the interaction between SC lipids and oleic acid.[12]

Sacha inchi oil is a cold-pressed oil from the seed of *Plukenetia volubilis* L. The oil has a higher omega-3 (≅ 40%-50%) and omega-6 (≅ 30%-40%) content and a lower oleic acid (≅ 10%) content [13,14] than olive oil. The omega-6 fatty acid linoleic acid plays a role in regeneration of the lipid barrier structure in the SC. Moreover, linoleic acid and the omega-3 linolenic acids are precursors of other polyunsaturated fatty acids and arachidonic acid, which are involved in differentiation of keratinocytes.[15,16] Natural antioxidants, including tocopherols and polyphenolics, have also been found in sacha inchi oil. Due to its fatty acid ratio and phytochemical composition, sacha inchi oil has been used in cosmetics as an emollient and skin protector. However, its moisturizing efficiency and skin irritation potential have never been reported. In the present study, a clinical study to assess the moisturizing efficiency and skin irritation potential of sacha inchi oil was conducted using a regression model. Olive oil was used as the control moisturizer. Additionally, the secretion of tumor necrosis factor-α (TNF-α) and interleukin-1α (IL-1α), which are pro-inflammatory cytokines released in response to inflammation induced by irritants, was observed in ex vivo skin tissue cultures treated with sacha inchi oil or olive oil.

2 | MATERIALS AND METHODS

2.1 | Materials

The sacha inchi tree was grown in Chiang Rai province, Thailand. Olive oil (*Olea europaea* fruit oil) was purchased from Filippo Berio. Keratinocyte basal medium (MCDB 153) was purchased from United States Biological Inc. Anti-cytokeratin 1 antibody (AB185629) and secondary antibody conjugated with HRP (AB97051) were purchased from Abcam plc. The Human IL-1α Picokine ELISA Assay Kit (cat no. EK0389) was purchased from Boster and human TNFα Ready-SET-Go Elisa Assay Kit (cat no. EK0389) was purchased from eBioscience, Fisher Scientific.

2.2 | Preparation of the sacha inchi oil

Plukenetia volubilis L., or sacha inchi, oil was prepared and supplied by TAI.CMS Standard Industrial Company Limited. The oil was obtained from seeds through cold pressing and kept in a tight container at room temperature until use.

2.3 | Determination of the fatty acid composition of the sacha inchi oil

First, fatty acids in the sacha inchi seed oil were converted into their methyl esters (FAMEs) and stored at 4°C until analysis. The FAME preparations were analyzed via gas chromatography with a flame ionization detector (GC–FID) (Auto system XL) with a capillary column (SP™-2560 Capillary GC Column fused silica, L * ID 100 m * 0.25 mm, *df* 0.20 μm) using the method of AOCS Ce 1e-91 (2001). Helium was used as the carrier gas. The injector and detector (FID) temperatures were set at 220°C and 275°C, respectively. The oven temperature was set at 150°C for 1 minute, increased to 220°C at a rate of 15°C/min, and then to 250°C at a rate of 2°C/min and held for 5 minutes. The sample size was 1 mL with a split ratio of 60:1. For data analysis, compounds were recognized based on their retention times (RTs) using standard FAMEs.

2.4 | Physicochemical characterization

2.4.1 | Determination of acid value (mg KOH/g)

Five grams of the oil was dissolved in 50 mL of isopropyl alcohol and gently mixed with a stirring bar on a magnetic stirrer for 5 minute. After that, the solution was titrated with 0.1 M KOH standardized solution in toluene with isopropyl alcohol. The acid value was calculated using the below equation, where A is the titrant solution volume (mL) used in the titration of the sample, B is the titrant solution volume (mL) used in titration of the blank, and C is the concentration of the titrant solution (M).

Acid value (mg KOH/g) = ([A-B] × C × 56.1)/g of sacha inchi oil.

*1 mL (0.1 N KOH) = 56.1 mg KOH.

2.4.2 | Determination of saponification value (mg KOH/g)

The oil in an amount of 2.5 g was slowly dissolved in 25 mL of 0.5 M alcoholic potassium hydroxide, and the obtained mixture was boiled in a steam bath for 30 minutes. After that, the solution was titrated with 0.5 M HCl using phenolphthalein as an indicator. The saponification value was calculated using the following equation, where A is the titrant solution volume (mL) used in the titration of the sample, B is the titrant solution volume (mL) used in titration of the blank, and C is the concentration of the titrant solution (M).

Saponificationvalue(mgKOH/g)=Acidvalue=([A-B]×C×28.05)/gof sacha inchi oil.

*1 mL (0.5 N HCl) = 28.05 mg KOH

2.4.3 | Determination of peroxide value (mEq/kg)

One gram of the oil and 1.0 g of powdered potassium iodide were dissolved in 20 mL of chloroform. The mixture was then boiled in boiling water for 30 seconds and quickly transferred to a flask containing 20 mL of 5% KI solution. After that, the solution was titrated with 0.01 M $Na_2S_2O_3$ solution using 0.5 mL of starch as an indicator. The peroxide value was calculated using the following equation, where A is the titrant solution volume (mL) used in the titration of the sample, B is the titrant solution volume (mL) used in titration of the blank, and C is the concentration of the titrant solution.

Peroxide value (mEq peroxide/kg sample) = ([A-B] × C × 1000)/g of sacha inchi oil.

2.4.4 | Determination of iodine value (g I_2/100 g)

One g of the oil and 15.0 g of CCl_4 were dissolved in 25 mL of Wijs solution. The solution mixture was incubated at room temperature for 2 hour and protected from light. Then, 20 mL of 10% KI solution and 150 mL of distilled water were added. After that, the solution was titrated with 0.50 M $Na_2S_2O_3$ solution using 0.5 mL of starch as an indicator. The iodine value was calculated using the below equation, where A is the titrant solution volume (mL) used in the titration of the sample, B is the titrant solution volume (mL) used in titration of the blank, and C is the concentration of the titrant solution.

Iodine value (g I_2/100 g) = ([A-B] × C × 12.69)/g of sacha inchi oil.

2.5 | Effects of the sacha inchi oil on the release of cytokines and expression of keratin 1

In this study, the protocol for skin collection was approved by an institutional review board (COA no. 087/2016; approval date: March 22, 2016). Briefly, skin tissues from surgery to remove excess skin from females in the age range of 50-65 years old were collected using 6-mm, full-thickness punch biopsies. The obtained biopsies were divided into 6 groups (n = 3 for each group): nontreated tissue group (control group), sacha inchi oil-treated tissue group, and olive oil-treated tissue group for nonirradiated and irradiated tissues.

2.5.1 | Human skin tissue culture and treatment

The skin biopsies were transferred into a 24-well dish, and the dermal layers were immersed in keratinocyte basal medium (KBM) containing 1.4 mmol/L Ca^{2+} from $CaCl_2$.[17,18] The samples were incubated at 37°C in a humid atmosphere containing 5% CO_2 for 12 hours. Next, 20 μL of sacha inchi or olive oil was applied to the air-exposed epidermis, and the oil-treated tissues were further incubated for 24 hours. After that, the tissues and medium were collected for further studies.

Moreover, the levels of cytokines released from UVB-irradiated tissues pretreated with the tested oils were assessed. The epidermal layers of the cultured tissues were treated with 20 μL of sacha inchi oil or olive oil and then irradiated using a UVB source (200 mJ/cm^2, TL-D lamp, wavelength in a range of 280-350 nm; Philips) in irradiation chambers (Dr Gröbel Elektronik GmbH). The irradiated skin tissues were further incubated for 24 hours, and the tissues and medium were collected for further studies.

2.5.2 | Histological evaluation

At 24 hours after treatment with the tested oil with or without UVB irradiation, the skin tissue samples were immediately fixed in tissue freezing medium and stored at −80°C. The frozen tissues were sectioned at 8 μm thickness using a cryostat. The section samples were placed on slides, rehydrated in acetone at −20°C for 10 minutes, and stained with hematoxylin and eosin (H&E) to observe skin tissue morphology under an inverted microscope (Carl Zeiss Microscopy Ltd).

2.5.3 | Determination of keratin 1 (KRT1) in the skin tissues

To detect KRT1 protein, the sectioned tissues were placed on poly-L-lysine coated slides, fixed with acetone at −20°C for 10 minutes, and washed with PBS (pH 7.4). The tissue samples were immersed in antigen retrieved with 1% sodium dodecyl sulfate in PBS for 5 minutes and washed with PBS. The samples were then permeabilized using 0. 25% Triton-X in 1X Tris-buffered saline (1X TBS) for 5 minutes and washed twice. Nonspecific antigens were blocked using 10% bovine serum albumin (BSA) in PBS in a humidified chamber at room temperature. After blocking, the tissue samples were incubated with anti-cytokeratin 1 (AB185629) polyclonal (1:200 dilution) primary antibody diluted in TBS containing 1% BSA overnight in a humidified chamber at 4°C. The samples were washed with 0.025% Triton-X in TBS for 5 minutes (2 times) and incubated with secondary antibody conjugated with HRP (AB97051) for 1 hours at room temperature. Then, the samples were incubated with the substrate 3,3′-diaminobenzidine to develop the brown-colored substrate product and enzyme-conjugated antibody. Finally, the samples were dehydrated with absolute ethanol for 1 minutes (2 times), immersed in xylene for 1 minutes (2 times), mounted and covered with a coverslip.

2.5.4 | Measurement of tumor necrosis factor-α (TNF-α) and interleukin-1α (IL-1α) released from the skin tissues

The tissue medium was collected from each sample, and then, the concentrations of TNF-α and IL-1α in the medium were measured via enzyme-linked immunosorbent assay (ELISA) using the appropriate ELISA kits according to the manufacturers' instructions. The level of cytokines released from irradiated tissues that were not treated with oil was adjusted to 100%.

2.6 | Evaluation of the moisturizing and irritation potential of sacha inchi oil

In this study, the protocol for determination of skin moisture content and dryness appearance after application of sacha inchi oil on the

lower leg of subjects was approved by an institutional review board (COA no. 087/2016; approval date: March 22, 2016).

2.6.1 | Study design

The study was designed as a randomized, double-blind, benchmark (olive oil) controlled trial, and home-use study. The study was conducted from May to June 2016. The assigned application area was both lower legs of the subjects. A simple randomization was used to designate whether the recruited subjects applied the sacha inchi oil or the olive oil (benchmark) on their left or right lower leg. All subjects were followed up for 16 days to measure skin hydration and transepidermal water loss (TEWL) using biophysical instruments and to observe skin dryness via visual grading based on the overall dry skin (ODS) scale described in the EEMCO guidelines.[19] The scale ranged from 0 to 4 as follows: grade 0 = absent; grade 1 = faint scaling, faint roughness, and dull appearance; grade 2 = small scales in combination with a few larger scales, slight roughness, whitish appearance; grade 3 = small and larger scales uniformly distributed, definite roughness, possibly slight redness, and possibly a few superficial cracks; and grade 4 = dominated by large scales, advanced roughness, redness present, and cracks.

2.6.2 | Subjects

Subjects were recruited through advertising on the institutional information board. Healthy females in an age range of 20-60 years were initially screened for recruitment. They were then informed of the study protocol and instructions. After consent was obtained, the subjects with dry skin (ODS scale ≥1) at the test site (both sides of the lower leg) were first included. Subjects were excluded if they were pregnant; had skin diseases that might interfere with measurement of skin properties; had a history of allergy or sensitivity to any topical product; or used a topical medication or product containing steroids, hormones, and/or other agents specifically indicated for improvement of dry skin on the lower leg within 4 weeks of the beginning of the study.

2.6.3 | Study protocol and direction for use of the assigned products

The study model utilized a mini-regression approach modified from previous studies.[20] After signing the informed consent form, the subjects who met the inclusion criteria were enrolled in the study. Both sides of their lower legs were shaved two or 3 days before starting the study. On the first visit (day 0), subjects arrived in the study room and waited for 30 minutes before proceeding to the next step of scoring the skin dryness level and measuring the baseline skin properties, including moisture content using a Corneometer®825, which was mounted on a Multi Probe Adapter® MPA5 (Courage and Khazaka, Electronic GmbH), and the TEWL value using a TEWAMETER TM210 (Courage and Khazaka, Electronic GmbH) according to the guidelines provided by the Standardization Group of the European Society of Contact Dermatitis. Subjects then received either the sacha inchi oil or the olive oil. Olive oil was selected as the benchmark because it is a well-known moisturizing oil available in the market. The appearance, including color, viscosity, and tackiness, of the olive oil was quite similar to that of the sacha inchi oil, and both were presented in the same packaging design. Subjects were instructed to apply each product (in an amount of 0.5 mL) on their left or right lower leg twice a day: in the morning and before bed. The duration of application was 14 consecutive days. During the application period, subjects were instructed not to apply any product at the test site before or after application of the assigned product. Any cleansing product that did not contain steroids, hormones, or agents with specific indication for improvement of dry skin was approved for use. Subjects were arranged for a second (day 7) and third (day 14) visit to assess adverse events, including erythema, scaling, and edema, using the visual grading scale of Frisch and Kligman and COLIPA.[21] The scale was set from 0 to 4 as follows. For erythema, grade 0 = absence of erythema; grade 0.5 = minimal or doubtful erythema; grade 1 = slight redness, spotty, and diffuse; grade 2 = moderate and uniform redness; grade 3 = intense redness; and grade 4 = fiery redness. For scaling, grade 0 = no evidence of scaling; grade 0.5 = dry without scaling or appears smooth and taut; grade 1 = fine or mild scaling; grade 2 = moderate scaling; and grade 3 = severe scaling with large flakes. For edema, grade 0 = absence of edema, and grade 1 = presence of edema. Additionally, on the third and fourth visits (2 days after application stoppage, day 16), the skin dryness level and skin properties at the application sites were measured. For each visit day, the subjects were instructed to continue application of the tested oil in the morning (except the fourth visit) and arrive at the study room before noon for skin evaluation. To determine subject compliance, subjects were asked to return the used product and receive a new supply on the second and third visit. The returned product was weighed to assess the amount used.

2.7 | Statistical analysis

Descriptive statistics were used to report all results in terms of the mean (SD) and percentage (%). The mean difference in each of the skin parameters between applications was analyzed using a Wilcoxon's signed-rank test. Differences were considered to be statistically significant at $P < .05$.

3 | RESULTS AND DISCUSSION

3.1 | Fatty acid composition and physicochemical characteristics of sacha inchi oil

Extraction of the oil from seeds via cold pressing (mechanical expression) provided a yellow to golden liquid. The physicochemical properties of the oil were as follows: saponification value = 168.58 ± 1.55 mg

TABLE 1 The fatty acid profile of the sacha inchi oil

Fatty acid	Palmitic acid (C 16:0)	Stearic acid (C 18:0)	Oleic acid (C 18:1)	Linoleic acid (C 18:2)	α-Linolenic acid (18:3)
(%)	4.2	3.4	10.2	39.5	42.3

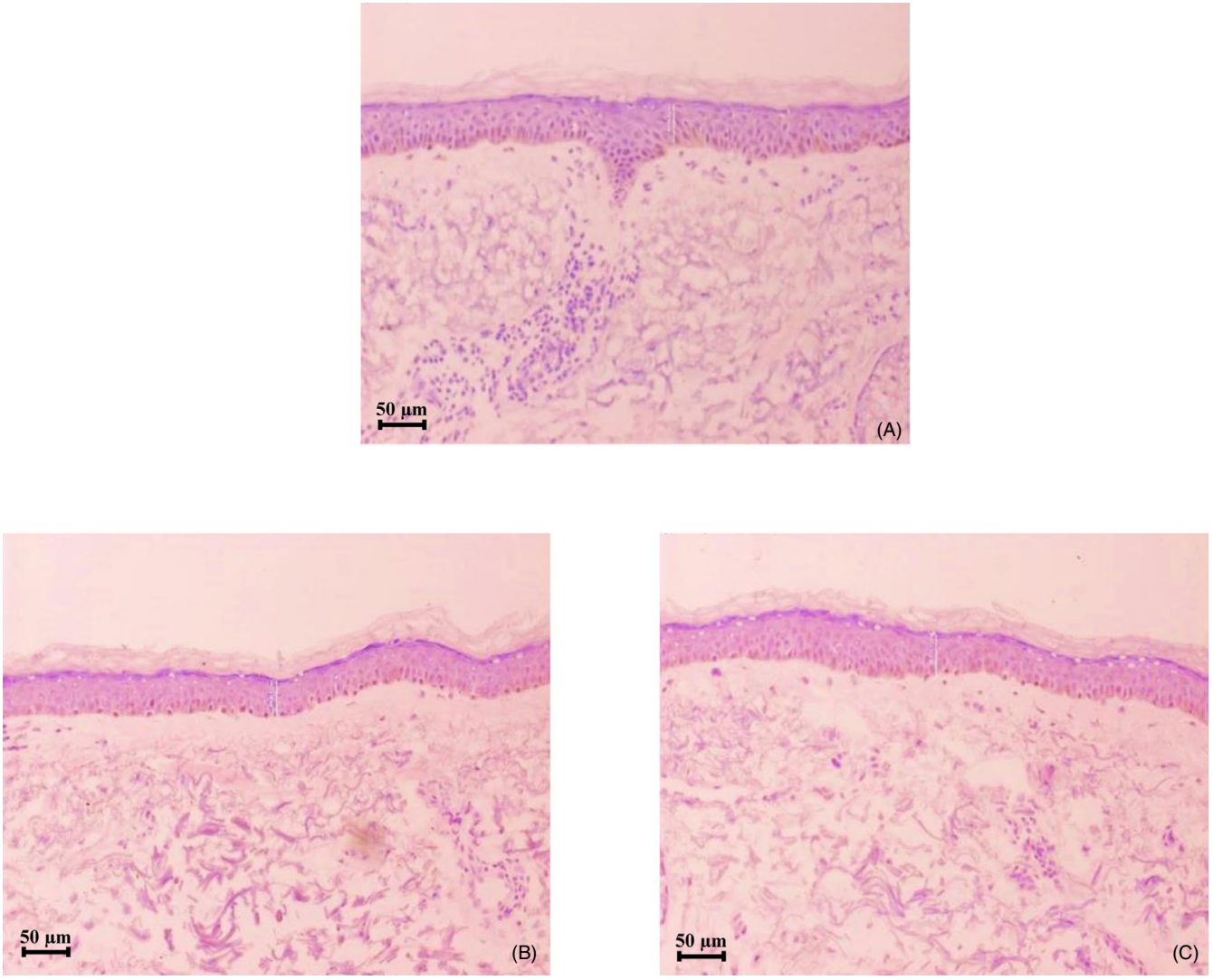
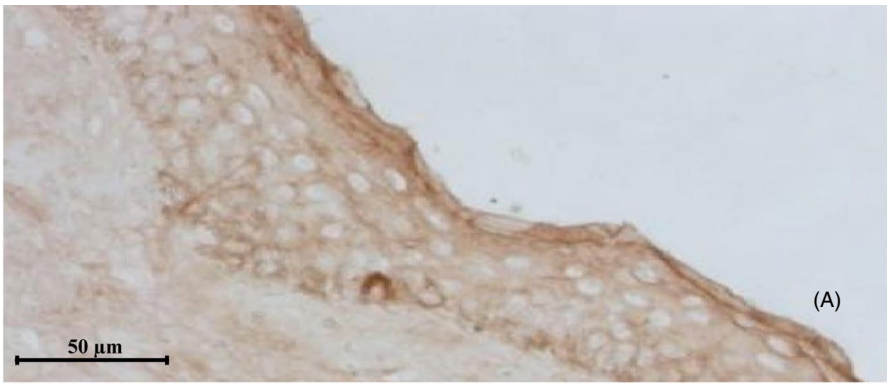


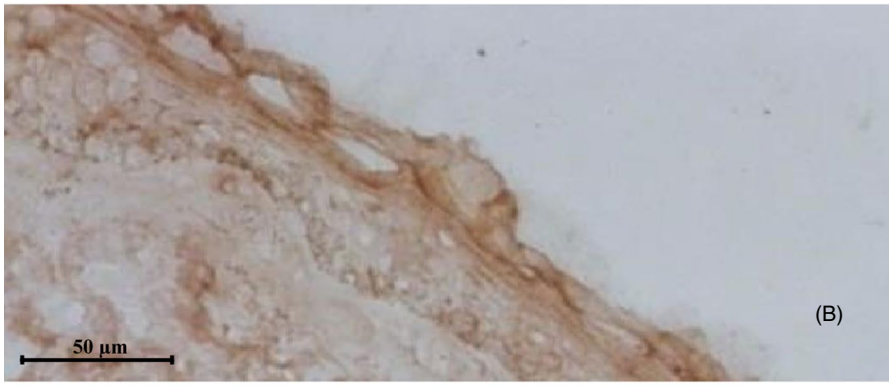
FIGURE 1 Photomicrographs of H & E-stained skin sections under a light microscope (magnification 20×) showing the morphology of human skin tissues after ex vivo cultivation for 36 h A, followed by treatment with the sacha inchi oil B, or the (benchmark) olive oil C, for 24 h

KOH/g, iodine value = 203.00 ± 0.04 g I_2/100 g, acid value = 1.68 ± 0.03 mg KOH/g, peroxide value = 1.95 ± 0.26 mEq peroxide/kg sample, viscosity = 81.98 ± 0.23 cPs, density = 0.9204 g/cm^{3,} and refractive index = 1.5, which coincide with the previously reported properties of sacha inchi oil from Colombia.[14] According to GC-FID analysis, the fatty acid profile of the oil is shown in Table 1. The oil was found to be rich in linolenic acid (omega-3, 42.3%) and linoleic acid (omega-6, 39.5%). Similar to recent reports,[13,14] the fatty acid composition of the sacha inchi oil was dominated by linolenic and linoleic acids, which were present at higher levels than found in other vegetable oils, such as olive oil and linseed oil.[10,14,22] Previous studies have found that topical application of linoleic acid and/or linoleic acid can restore the permeability barrier and improve skin appearance.[23-25] Therefore, sacha inchi oil might be an alternative source of essential fatty acids for improvement of skin appearance and function through topical administration.

3.2 | Effects of sacha inchi oil on skin morphology and keratin 1 expression

For the nontreated tissue group (control), after 36 hours of ex vivo cultivation, no change in skin morphology was observed compared





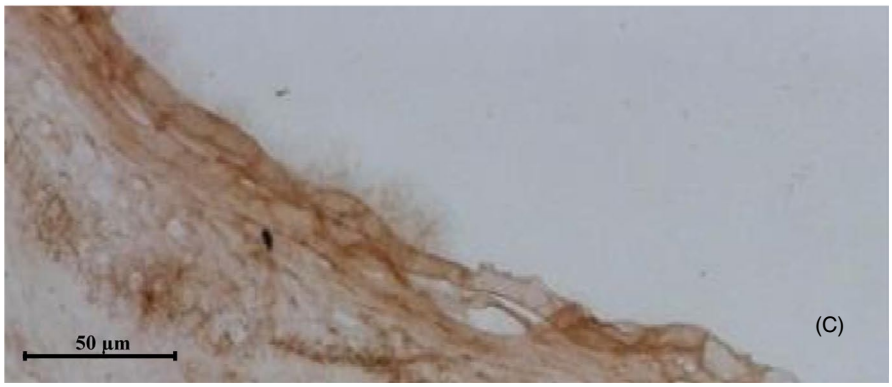


FIGURE 2 Photomicrographs of immunohistochemistry of skin sections under a light microscope showing keratin 1 expression on the outer layer of the human skin tissues after ex vivo cultivation for 36 h A, followed by treatment with the sacha inchi oil B or the (benchmark) olive oil C for 24 h

with the fresh skin tissue according to the H&E staining results (Figure 1A). After treatment of the human skin tissues with sacha inchi (Figure 1B) or olive oil (Figure 1C) for 24 hours, the skin morphology was not changed compared against the group of control tissues. The SC layer and rete ridge structure located at the epidermal-dermal junction were clearly seen. The expression of keratin 1 in the SC (Figure 2) was also observed to determine the integrity of the skin barrier layer of the cultured tissues. Generally, keratin 1 is a specific marker for terminal differentiation of epidermal cells. Damage and/or abnormal expression of keratin 1 results in skin disorders related to skin barrier defects.[2,26] We found that neither of the tested oils reduced k1 expression or the integrity of the SC layer compared with the appearance of the control tissues. A previous study reported a mild irritant effect of olive oil associated with a reduction in SC integrity. However, such effect was seen after topical application of olive oil for 4 weeks.[11]

3.3 | Effects of sacha inchi oil on the release of TNF-α and IL-1α

In the skin, TNF-α and IL-1α are pro-inflammatory cytokines primarily produced by keratinocytes. These cytokines are expressed at a minimal level to exert biological activities that contribute to maintaining healthy skin. Keratinocytes activated in response to skin injury and/or barrier disruption induces the release of TNF-α and IL-1α, leading to stimulation of signals for barrier repair. On the other hand, the initial release of these cytokines is a sign of SC disruption.

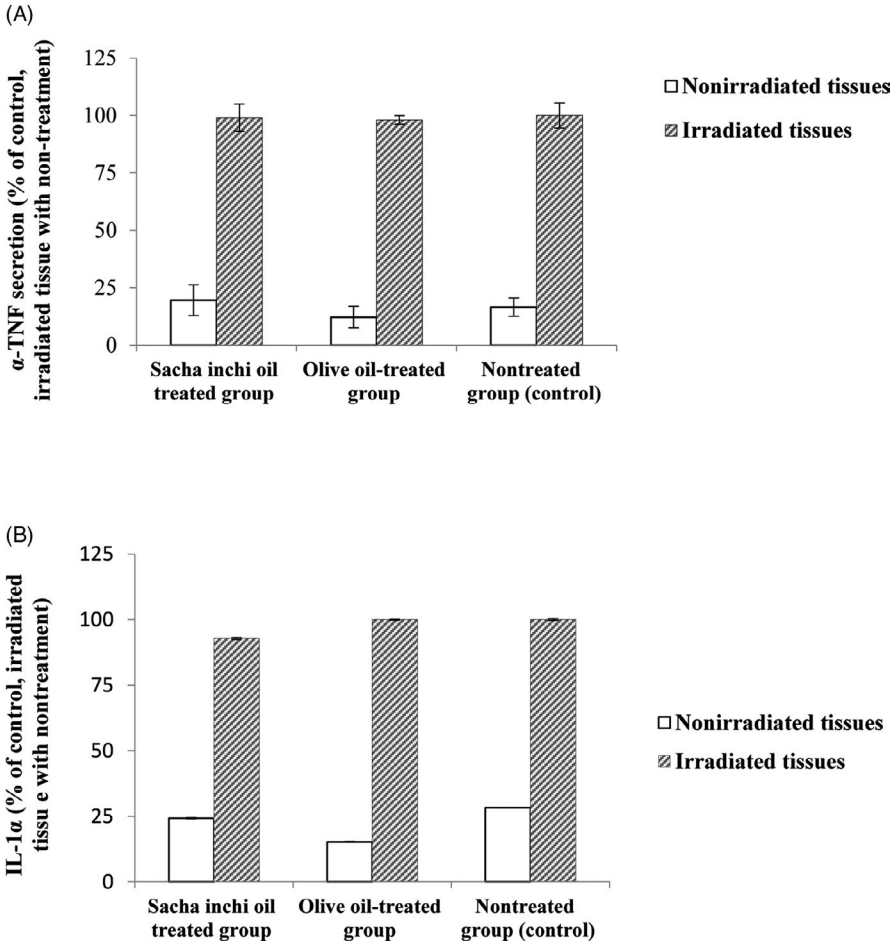


FIGURE 3 Effects of the tested oils on the release of pro-inflammatory cytokines, specifically TNF-α A, and IL-1α B, from nonirradiated and irradiated tissues pretreated with the sacha inchi or (benchmark) olive oil. Each bar represents the mean ± SD of triplicate studies

The effect of sacha inchi oil on inflammatory cytokine release in an ex vivo skin culture model has never been reported. Compared with the group of nontreated tissues (control), alterations in TNF-α (Figure 3A) and IL-1α secretion (Figure 3B) were not found in the sacha inchi- or olive oil-treated tissues. The results obtained correspond with the results of the skin morphology study, revealing that the tested oils did not disturb the skin barrier.

As expected, an increase in TNF-α and IL-1α secretion from UV-irradiated skin tissues, compared with the nonirradiated tissues was found. UV, particularly UVB, has been reported to be one of the external factors that can deteriorate barrier function, consequently leading to the release of pro-inflammatory cytokines.[27,28] Barrier deterioration at least partially results from disorganization of lipids in the stratum corneum.[27] Pretreatment of the tissues with sacha inchi or olive oil before UV irradiation did not provoke over-secretion of the cytokines compared with the levels of cytokines secreted from UVB-irradiated tissues. Again, all the obtained results indicate that the tested oils do not disturb barrier function.

3.4 | Effects of sacha inchi oil on SC hydration and the TEWL value

For the clinical study, 15 volunteers were enrolled and randomized according to the inclusion and exclusion criteria. The skin properties and ODS scale values obtained from 13 volunteers who completed all 16 days of the study were averaged. The enrolled volunteers had an average age of 29.1 ± 6.5 years and were instructed to apply the sacha inchi oil or olive oil (benchmark) on the left or right lower leg twice daily for 14 days, and the skin properties and ODS scale values were determined at baseline (day 0), day 14, and the two days after application stoppage (day 16). The occurrence of adverse events was assessed on day 7 and day 14 of the study, and the observation revealed no signs of erythema, scaling, or edema at the application site.

Figure 4 shows the moisture content and TEWL values before (baseline, day 0) and after repeated application over 14 days and after discontinuation of application for 2 days (day 16, after a 2-day regression period). This design was proposed to evaluate the moisturizing efficacy of the tested oils in maintaining skin hydration even after application stoppage. At day 0, the average moisture content values, which were measured with a Corneometer® on the lower leg, for participants that applied the sacha inchi oil and olive oil were 36.7 ± 3.0 AU and 36.7 ± 2.7 AU, respectively. These measured values, which were in the range of 30-40 AU, indicate dry skin at the application site [29] and are related to the observed ODS scale values, which ranged from grade 1 to 3 (Table 2). Application of the tested oils for 14 days improved visual dryness; no subject was given a score of grade 3 or higher on the ODS scale. These results were associated

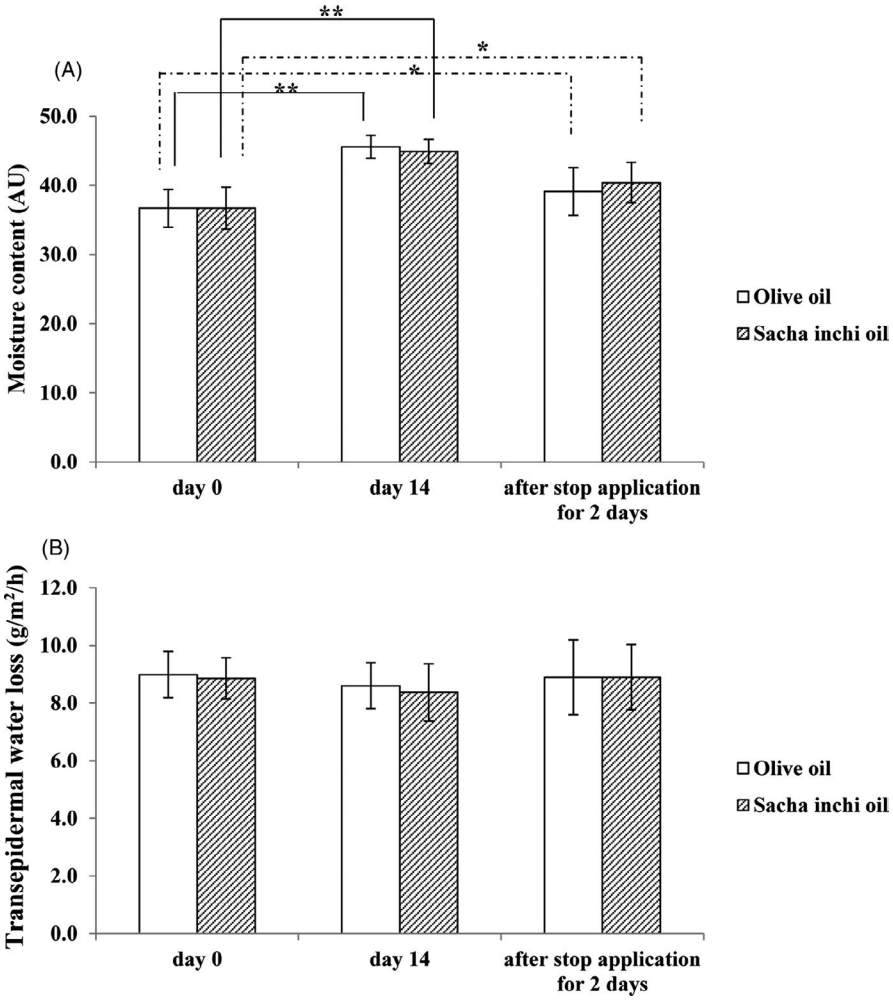


FIGURE 4 Average moisture content values (one arbitrary unit, AU, represents a stratum corneum water content of 0.02 mg/cm^2) A and transepidermal water loss (g/m^2/h) B. The value at day 0 is the baseline value. Each bar is the average value obtained from 13 subjects, and the error bars represent the SD of the mean; * $P < .05$ and ** $P < .01$ when compared between the two groups

TABLE 2 The overall dryness grading scales of 13 subjects at before (baseline, day 0) and at after continuing application over 14 d followed by stopping application for 2 d (day 16, 2-day regression period)

	Day 0 (baseline): Application site for		Day 14 of application: Application site for		Day 16 (after stop application for 2 d): Application site for	
Grade	Sacha inchi oil	Olive oil	Sacha inchi oil	Olive oil	Sacha inchi oil	Olive oil
Grade 1	6 (46.2%)	6 (46.2%)	10 (76.9%)	11(37%)	9 (69.2%)	9 (69.2%)
Grade 2	4 (30.8%)	4 (30.8%)	3 (23.1%)	2 (16%)	3 (23.1%)	4 (30.8%)
Grade 3	3 (23.0%)	3 (23.0%)	0 (0%)	0 (0%)	1 (7.7%)	0 (0%)

with the statistically significant increase ($P < .01$) in skin hydration (44.9 ± 1.7 AU for the sacha inchi oil-applied site; 45.6 ± 1.7 AU for the olive oil-applied site). The TEWL value at day 14 of application (8.4 ± 1.0 for g/m^2/h for the sacha inchi oil-applied site and 8.6 ± 0.8 g/m^2/h for the olive oil-applied site) also decreased compared with the baseline value (8.9 ± 0.7 g/m^2/h for the sacha inchi oil-applied site and 9.0 ± 0.8 g/m^2/h for the olive oil-applied site), but the decreases were not significant. After application discontinuation for 2 days, the skin hydration values (40.4 ± 2.9 AU for the sacha inchi oil-applied site; 39.1 ± 3.5 AU for the olive oil-applied site) were still significantly better than the baseline value ($P < .05$). The obtained results indicate that the moisturizing capacity of the sacha inchi oil is equivalent to that of the olive oil. The moisture content value obtained from the Corneometer® is related to the electrical capacity of the skin surface, which reflects the level of SC hydration. This hydration level is associated with water-binding ability, which depends on the components and structural organization of the SC and is of importance both for barrier properties and the clinical appearance of the skin. The improvement in moisture content and skin dryness appearance after application of the tested oils might be due to their occlusive effect. Additionally, the increase in skin moisture content might be achieved by the presence of natural moisturizing factors (NMFs), such as fatty acids, in both oils. Both olive and sacha inchi oils consist of mixed fatty acids, such as oleic acid, linoleic acid, and

α-linoleic acid. Several studies have suggested that the percentage of linoleic acid and α-linoleic acid in sacha inchi oil (up to 80%) [13,14] is generally higher than that in olive oil (up to 11%),[10] while oleic acid percentage in olive oil is relatively high (up to 70%).[10] Both linoleic acid and α-linoleic acid are essential for skin barrier function and homeostasis [15] and are precursors of prostaglandin that can promote the keratinization process.[16] Additionally, as mentioned above, the irritant effects associated with the high oleic acid content in olive oil has never been reported. However, differences in SC hydration, skin appearance, and tolerance between the skin treated with sacha inchi oil and that treated with olive oil were not observed. Further studies with a larger number of subjects and a longer duration should be conducted to confirm the beneficial effects of sacha inchi oil.

4 | CONCLUSIONS

The cold-pressed sacha inchi seed oil contained a high concentration of linolenic acid. The results of ex vivo skin tissue culture and a clinical study revealed that the oil was mild to the skin and had a beneficial effect on dry skin. Its moisturizing effect was comparable to that of olive oil. Our findings preliminarily indicate that sacha inchi oil is safe to use and provide evidence supporting the performance of the oil as an active moisturizing ingredient.

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