

Sacha Inchi: The Promising Source of Functional Oil for Anti-Aging Product

Nattaya Lourith^{1,2*}, Mayuree Kanlayavattanukul^{1,2}, and Puxvadee Chaikul^{1,2}

¹ School of Cosmetic Science, Mae Fah Luang University, Chiang Rai 57100, THAILAND

² Phytocosmetics and Cosmeceuticals Research Group, Mae Fah Luang University, Chiang Rai 57100, THAILAND

Abstract: Sacha inchi (*Plukenetia volubilis*) oil is constituted with macronutrients and the health benefit fatty acids. In this context, the efficient of Sacha inchi oil for anti-aging product is presented. The light-clear yellowish seed oil of Sacha inchi was revealed on its physicochemical properties that are in the same range of the commercializing plant-oil supplied for topical products. The oil was GC/MS exhibited to be constituted with α -linolenic (51.72%) and linoleic (24.3%) acids, with unsaturated/saturated fatty acids ratio of 21.26. The oil was noted onto its potent *in vitro* antioxidant activity assessed by ABTS, DPPH and FRAP assays. In addition, the oil (1-3%) was proved to be safe in normal human fibroblast cells. Furthermore, the oil exhibited cellular antioxidant with inhibitory effect against MMP-2. Sacha inchi oil is therefore highlighted as a potential source of nutraceutical especially for anti-aging product. The oil is specified for the product development in terms of physicochemical, chemical and biological profiles. Innovative processing of Sacha inchi is therefore encouraged as the promising plant for anti-aging product.

Key words: *Plukenetia volubilis*, bio-oil, fatty acids, anti-aging, nutraceutical

1 Introduction

Sacha inchi or *Plukenetia volubilis* L. (Euphorbiaceae) is the important crop of South America^{1,2)}. The seed oil is documented to be used for medical treatments of coronary heart disease, arthritis, diabetes, attention deficit hyperactivity disorder and inflammation³⁾. Its seed oil is edible and was shown to reduce serum total cholesterol and LDL cholesterol, increase HDL cholesterol and increase total protein during a course of oral administration at 10 or 15 ml for 16 weeks as examined in adult volunteers⁴⁾. Its therapeutic effects are governed by the biologically active fatty acids¹⁾, i.e., α -linolenic and linoleic acids (35-60%), which are of significance for topical products, including those of cutaneous and hair importance⁵⁻⁹⁾ that are oleic, palmitic and stearic acids as well as amino acids such as phenylalanine, tyrosine, leucine and isoleucine. Furthermore, the health importance minerals for example potassium, magnesium and calcium as well as vitamin E, carotenoids and polyphenols are reported as the constituents of Sacha inchi oil^{4, 10, 11)}. The oil is majorly produced and supplied for food industry¹⁰⁻¹⁵⁾, and applicable for several health promotion products, particularly anti-aging products, at which the expectation on natural products are dramatically increased^{5, 6)}. Sacha inchi oil prepared by a cold-pressed, solvent free

method, is therefore becoming of interest as a superfood due to its nutritional and health benefits worldwide especially for the European market, which drives the price of oil very expensive¹⁰⁾. Nonetheless, the sufficient data applicable for topical product industry is sparsely to be reported especially for its safety and efficacy claims.

The present study was therefore aimed to challenge on the possibility of the commercially available, cold-pressed Sacha inchi oil with required parameters enabling its uses for cosmetic, pharmaceutical and nutraceutical products.

Abbreviations: ABTS; 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid), DPPH; 1,1-Diphenyl-2-picrylhydrazyl, DMEM; Dulbecco's Modified Eagle's Medium, EC; equivalent concentration, ECM; extracellular matrix, EDTA; ethylenediaminetetraacetic acid, EtOH; ethanol, FAMES; fatty acid methyl esters, FBS; fetal bovine serum, FRAP; ferric reducing ability of plasma, HDL; high-density lipoproteins, IC₅₀; effective concentration at 50%, LDL; low-density lipoproteins, LSD; the least significant difference, NHF; normal human fibroblast, MeOH; methanol, MMP-2; matrix metalloproteinase-2, rpm; round per minute, SD; standard deviation, SDS-PAGE; sodium dodecyl sulfate polyacrylamide gel electrophoresis, SEM; standard error of the mean; SFA; saturated fatty acids, SRB; sulforhodamine B, TPTZ; 2,4,6-tri(2-pyridyl)-S-triazine, UFA; unsaturated fatty acids

*Correspondence to: Nattaya Lourith, School of Cosmetic Science, Mae Fah Luang University, Chiang Rai 57100, THAILAND
E-mail: nattayal@mfu.ac.th

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In this presenting context, physicochemical, fatty acids and *in vitro* antioxidant activities of seed oil of Sacha inchi cultivated in Thailand were reported with the cellular safety and efficacy in normal human fibroblasts (NHF), the cells responsible for aging of skin. Innovative processing of Sacha inchi oil for nutraceutical product is encouraged accordingly, especially for anti-aging product.

2 Experimental

All of the chemicals used were of analytical grade unless otherwise specified. The standards (vitamin E; α -tocopherol and vitamin C), chemicals and reagents were from Sigma-Aldrich (USA) unless otherwise stated. The absorbance of each assay was recorded using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany).

2.1 Sacha inchi oil

The oil that is currently commercializing by the local-cooperative farmers in Chiang Rai, Thailand was used in this study. Sacha inchi cultivated in Chiang Rai, was seed harvested. The seed oil was cold-pressed and claimed as per the process for an extra-virgin oil.

2.2 Physicochemical properties

The acid, iodine, peroxide, saponification, and unsaponification values were determined in accordance with the official methods¹⁶⁾. Color parameter of the oil (glass chamber, HunterLab, USA; 30 mL) in terms of CIELAB was examined using chromameter (Ultrascan Vis, HunterLab, USA). Viscosity of the oil (200 mL in 250 mL beaker) was determined with a viscometer (Brookfield RVDV2T extra, USA) under room temperature using spindle no. 2 at a speed of 200 rpm and torque (%) of more than 80%.

2.3 Fatty acid profiles

Fatty acid profiles of the oil in the form of fatty acid methyl esters (FAMES) were analyzed. The oil was esterified by the mild and quantitative yield condition. Briefly, the oil was mixed with toluene (J.T. Baker, USA), MeOH (Merck, Germany), and 8% HCl (VWR, USA), and incubated at 45°C for 24 h. The resulting reacted solution was partitioned with *n*-hexane (Merck, Germany), the organic phase was dried over MgSO₄ anhydrous (Panreac, Spain) to collect the filtered FAMES solution. An aliquot (1 μ L) of the FAMES solution was injected (220°C) in the splitless mode into a gas chromatograph (Agilent, 6890N, USA). Chromatographic separation was by a HP-5MS capillary column (Agilent, 30 m $\times$ 250 μ m, 0.25 μ m film thickness) with the oven program starting at 50°C (5 min), rising to 65°C at a rate of 2°C/min, and then 200°C (5°C/min, 5 min), and 250°C (10°C/min) and held for 10 min, at which the carrier gas was helium (1.0 mL/min). Mass spectrophotometer

(Agilent, 5973N) and the reference mass spectrum (MS-Wiley7n.1 database) were analyzed¹⁷⁾.

2.4 *In vitro* antioxidant activities

2.4.1 ABTS^{•+} scavenging activity

In short, absorbance for ABTS containing potassium persulfate and EtOH (with an absorbance of 0.700 ± 0.200 at 750 nm) mixed with samples was recorded following 5 min of incubation at 750 nm. The antioxidant efficiency (%) was compared to that of standard ascorbic acid in different concentrations (1–8 μ g/mL; $r > 0.999$). Measurements performed in triplicate¹⁸⁾.

2.4.2 DPPH[•] scavenging activity

Antioxidant activity, on the basis of DPPH assay, was assessed in parallel. Briefly, DPPH in absolute EtOH was allowed to react with ascorbic acid to generate a calibration curve ($r > 0.999$). The scavenging activity of the sample against DPPH[•] was monitored at 517 nm with the microplate reader. The radical terminating capability (%) was calculated in comparison with the standard values. The experiments were performed in triplicate¹⁸⁾.

2.4.3 Ferric reducing ability of plasma (FRAP)

A FRAP reagent was prepared in a 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ) solution with HCl, FeCl₃ (20 mM), and acetate buffer. Shortly, the samples were reacted with FRAP reagent, and absorbance was recorded at 595 nm with the microplate reader. The reducing power of 1 mg of extract was expressed as an equivalent concentration (EC) of 1 μ g FeSO₄. The assay was determined in triplicate¹⁸⁾.

2.5 Safety and biological activities in NHF cells

NHF (ATCC[®] CRL 2097, USA) at 6–13th passage, were cultured in 75-cm² flask in DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C under 5% CO₂. Cells were grown and harvested by 0.25% w/v trypsin and 0.06 mM EDTA in phosphate buffer saline.

2.5.1 Cell cytotoxicity assay

The sulforhodamine B (SRB) assay was used for cell cytotoxicity determination. Cells were seeded at the density of 1×10^4 cells/well in 96-well plate and incubated for 24 h. Cells were then treated with different concentrations (1–5%) of the oil for 72 h. The adherent cells were fixed, washed and dyed prior to the absorbance measurement at 540 nm with the microplate reader. The cell viability was compared with the control treated with absolute ethanol⁷⁾.

2.5.2 Antioxidant activity assay

NHF were seeded in 96-well plate (1×10^4 cells/well) and incubated for 48 h. Cells were treated with various concentrations of the samples and the solvents (absolute ethanol or culture medium) for 24 h prior to treat with the fresh medium containing 150 μ M H₂O₂ and further incubated for 3 h. The cells were fixed, washed, dyed with SRB, and solubilized with 10 mM Tris base and recorded the absorbance at 540 nm for cell viability calculation¹⁹⁾.

2.5.3 Inhibitory activity against MMP-2

NHF (5×10^5 cells/well) in 6-well plate, cultivated without the supplement of fetal bovine serum, were incubated for 24 h. The cells were treated with the sample and incubated for 72 h. The supernatant was collected, MMP-2 quantification was carried out by SDS-PAGE zymography using gelatin as the substrate. The inhibitory effect was calculated in a comparison with that of the control⁷⁾.

2.6 Statistical analysis

The data are presented as the mean $\pm$ SD of three independent experiments. The results from the cellular studies are expressed as the mean $\pm$ SEM. One way analysis of variance (ANOVA) and LSD test were used for the analysis of the test results at the significant level of $p < 0.05$ using the program SPSS version 16.0.

3 Results and Discussion

3.1 Sacha inchi oil

Sacha inchi oil is an emerging plant-oil of interest for health promotion products, including for personal care industries. Nonetheless, sufficient information for personal care products is sparsely to be addressed. Appointment application of this plant-oil for personal care industries is therefore explored in this presenting context with a solidification data on physicochemical, chemical and biological profiles. Sacha inchi oil that was cultivated and produced by the cooperative farmers in Chiang Rai that is currently commercializing was examined in this study. The light-clear yellowish transparent liquid of a cold-pressed Sacha inchi oil with the indicated color parameters, was exhibited to be possibly applied for personal care/cosmetic industrial

applications. Which, physicochemical properties of the studying Sacha inchi oil are proved to be in the same ranges of the commercially available plant-oils supplied for topical products as exemplified in Table 1. It should be noted that saponification value of Sacha inchi oil was indicated to be lower than almond, macadamia, rice bran and wheat germ oils. Acid, iodine, peroxide and saponification values are oxidative indicators of the oil account for free fatty acids, degree of unsaturation, primary oxidation products and molecular weight of the fatty acids. Which, iodine and saponification values are common quality indices of fats and oils for industrial products²⁰⁾. Acid value of the studying Sacha inchi oil was higher than that of Peruvian oil prepared by Soxhlet extraction with ether¹¹⁾. On the means time, iodine value of the oil was lower than the extra-virgin Sacha inchi oil marketing in Peru¹⁰⁾ as well as that was Soxhlet with *n*-hexane¹³⁾. It should be noted that the cold-pressed oil prepared from Thai cultivated Sacha inchi seed was greater in peroxide value than the Peruvian oil¹⁰⁾. Which, may refer to its greater proportion of the unsaturated fatty acids. Although an acid value of Sacha inchi oil was greater than the acceptable value for oil in food application (0.6%)²¹⁾, on the basis of physicochemical properties, particularly iodine, saponification²⁰⁾ and peroxide values; the oil is industrial applicable for topical products without additional purification that economically feasible for industrial application. Viscosity of Thai Sacha inchi oil was higher than those of the Peruvian ones, which might be ruled by a difference of the measurement condition and the instrument as well. Because in this presenting study, the measured condition was undertaken in line with the guideline for cosmetic application in terms of volume, vessel size with the controlled torque. Which, these parameters crucially govern the determined viscosity; that torque

Table 1 Physicochemical properties of Sacha inchi oil compared with those of commercialized plant-oils in topical products.

Oil	Physicochemical property*									Ref.**
	Color			IV	AV	PV	SV	USV	Visc.	
	L*	a*	b*							
Sacha inchi	81.37 ± 0.05	-5.68 ± 0.01	47.20 ± 0.02	106.53 ± 1.95	0.84 ± 0.00	4.63 ± 0.03	180.43 ± 3.02	0.61 ± 0.02	130.6 ± 0.2	This study
				116.0-220.9		3.75-15.43		0.91-2.88	44.85-65.78	10)
				0.37 ± 0.01		2.90 ± 0.20				11)
				0.31 ± 0.03		1.83 ± 0.04				
				193.10 ± 1.00				185.20 ± 0.50	35.4 ± 0.4	13)
					3.40 -106				14)	
Almond				101.10			190.60			Parchem
Macademia				68.00-82.00		10.00				Sictia
Rice bran				70.00-130.00		10.00	190.00-200.00			RITA
Wheat germ				128.00			186.00			Parchem

* IV, iodine value (g I₂/100 g oil); AV, acid value (mg KOH/g oil); PV, peroxide value (meqO₂/ kg oil); SV, saponification value (mg KOH/g oil); USV, unsaponification value (%); Visc., viscosity (cps)

** data of commercialized bio-oils used in cosmetic from different suppliers were retrieved from www.cosmetics.specialchem.com

reflects onto the precision of the analysis. Chemical profile of the oil in term of fatty acid constituents was thereafter examined.

3.2 Fatty acid profiles

Pharmaceutics and cosmetic importance fatty acid constituents of the oil was GC/MS analyzed. The oil was noted to be high in α -linolenic acid (51.72%), followed by linoleic acid (24.30%) and palmitic acid (4.16%), giving the unsaturated/saturated fatty acids ratio (UFA/SFA) of 21.26 (Fig. 1). The oil rich with unsaturated fatty acids and high UFA/SFA is highlighted as the promising oil with health benefits²². In addition, linoleic acid/ α -linolenic acid ratio of the oil (0.47) offers important health and nutritional benefits, such as providing protection against cardiovascular disease²³, in harmony with its medical treatment for coronary heart disease³.

Linoleic and α -linolenic acids are the cosmetic active ingredients with skin, hair and nail nourishing efficacies. They are applicable to be used for inflammatory, dandruff and hair loss treatments with the potency for hair growth stimulation⁹. Their anti-inflammatory activities efficiently versatile their used in acne care products⁶. Palmitic acid is known to lowering cholesterol level and it is commonly compounded in cleansing products and widely incorporated in topical products in dosage forms of cream and lotion²⁴.

Sacha inchi cultivated in Thailand was noted onto the oil's profile that was higher in α -linolenic acid content than that of Peruvian ones that prepared by compression and supercritical fluid extractions (46.8 and 50.41%)^{12, 25} as well as those of Soxhlet extraction (45.62 ± 0.29 and $50.80 \pm 0.03\%$)^{13, 21}. Plant-oil with high constituents of UFAs are more of importance for health than SFAs²², which plant-oil

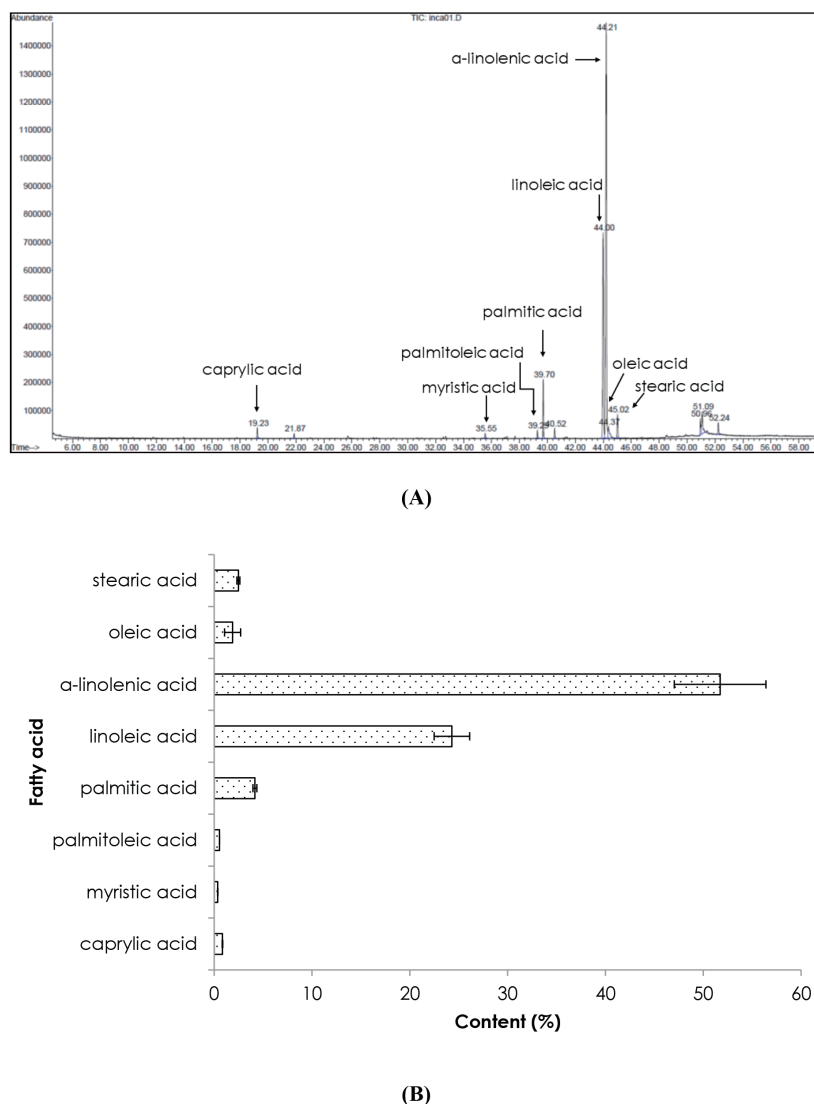


Fig. 1 GC chromatogram (A) and fatty acids (B) of Sacha inchi oil.

riches in fatty acids are emerging as the valuable substances for health promotion products²⁶⁾. Of particular, the C18 homologues, i.e., α -linolenic, linoleic and oleic acids that are crucial for epidermal aesthetical conditions^{5,6)}. Accordingly, the oil's antioxidant activity that is one of the key hallmarks of active ingredient against aging²⁷⁾, was examined.

3.3 *In vitro* antioxidant activities

The oil was *in vitro* antioxidant activity assessed with 3 different assays in a comparison with vitamin E, the benchmark active, in regards with their lipophilic natures. Sacha inchi oil was exhibited as a stronger antioxidant as examined at the same test concentration with the standard vitamin E as exhibited by ABTS and DPPH assays (examined at 30 and 15 mg/mL, respectively). Of which, that of FRAP was comparable (1 mg/mL) as illustrated in Fig. 2. These *in vitro* potency activities of the oil highlight its potential to be explored as the cosmetic active for anti-aging product. Thereafter, cellular biological activity assessment in the culture models relevant with skin wrinkle was undertaken in an order to verify the oil capability for anti-aging uses⁵⁾.

3.4 Cellular safety and biological activities

The seed oil of Sacha inchi is edible and has been utilized in foods¹⁰⁾. However, safety of the oil specified for topical product in NHF model has not yet reported. Safety assessment in the mammalian cells refer to the target applied cell is of crucial to be undertaken²⁸⁾. The cosmetic substances, including plant-oil may cellular cytotoxic at higher concentration, which governed by its components¹⁹⁾. In this presenting study, safety of the oil was therefore confirmed in NHF as well as cellular activities. Cell viability was challenged with the oil (1-5%) prior to an evaluation for biological activity. The oil at higher concentration (4 and 5%) caused cytotoxicity with the cell viability of less than 80% as depicted in Fig. 3A. Thereafter, its IC_{50} on NHF was calculated to be 3.86% ($y = -11.47\ln(x) + 94.293$; $R^2 = 0.9876$). Cellular antioxidant activity of the oil was thereaf-

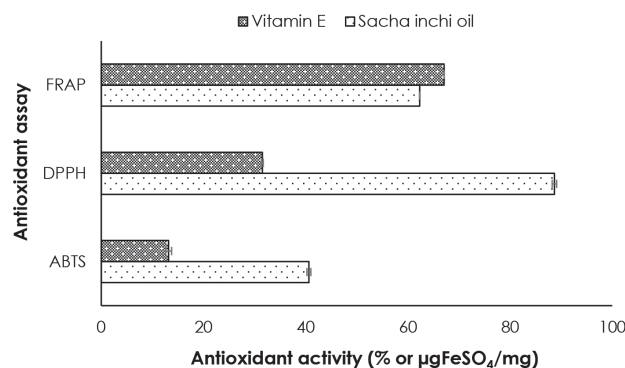


Fig. 2 *In vitro* antioxidant activity of Sacha inchi oil.

ter examined at its safety concentration range (1-3%) in a comparison with the standard vitamin C (0.1 mg/mL), the well-known and widely used anti-aging agent⁵⁾. H_2O_2 -cellular oxidative stress was induced, the cellular healing efficacy of Sacha inchi oil was monitored following the oxidative stress. Sacha inchi oil and vitamin C were comparable ($p > 0.05$) in cellular antioxidant activity (Fig. 3B). The oil and vitamin C were thereafter revealed on their cellular MMP-2 inhibition at the maximum safe antioxidant concentration (Fig. 4A). MMPs are zinc-dependent endopeptidases that degrade ECM enzymes. Those that are compulsory for skin are collagenase (MMP-1, MMP-8, MMP-13 and MMP-18), gelatinase (MMP-2) and elastase (MMP-9 and MMP-12). MMP-2 is a type I, II, III and IV, elastin and fibronectin degradation enzyme that results in skin aging. Fatty acids especially those with carbon numbers greater than 18 and at least 1 double bound, exhibit inhibitory effects against MMPs. In search of antiaging ingredients, especially those containing fatty acids as the active principles, the activity against MMP-2 has been widely examined²⁹⁾. It should be noted that Sacha inchi was comparable ($p = 0.118$) with

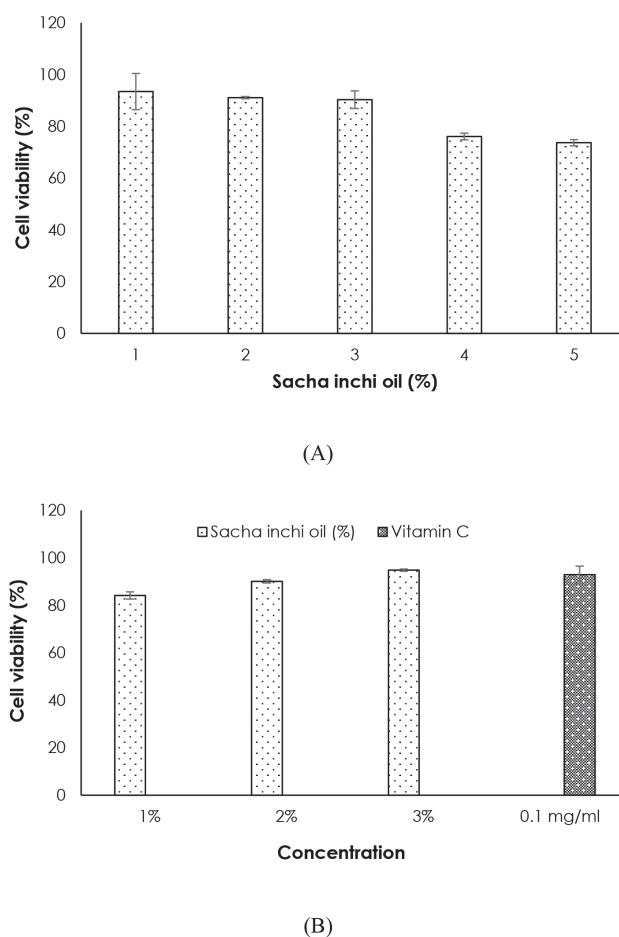


Fig. 3 Safety (A) and cellular antioxidant activity (B) of Sacha inchi oil in NHF.

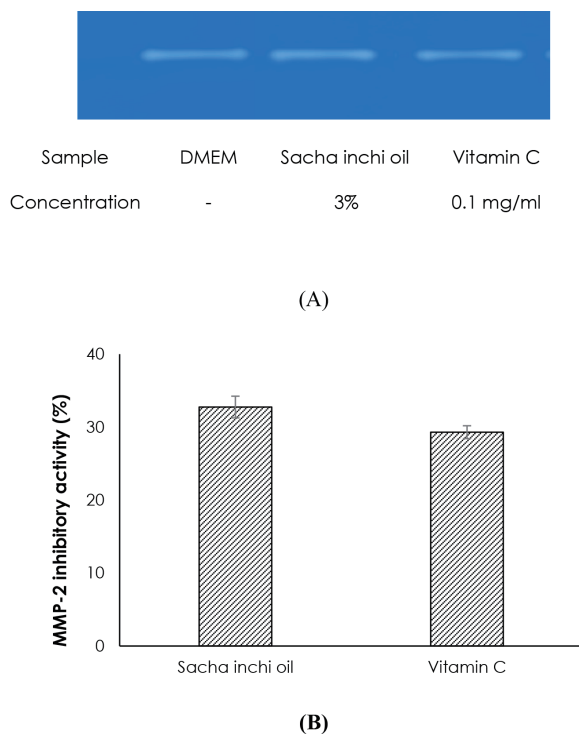


Fig. 4 Inhibitory activity of Sacha inchi oil against MMP-2 in NHF.

vitamin C against the enzyme and as depicted in Fig. 4B. Accordingly, Sacha inchi oil is indicated as the promising functional oil for anti-aging product on the basis of its cellular antioxidant and inhibitory effect against MMP-2.

4 Conclusions

Sacha inchi is proved to be the promising plant for nutraceutical product rich with α -linolenic and linoleic acids, the health benefits fatty acids. The plant-oil is evidently revealed as a safe and efficient active for anti-aging product in regard with the supportive physicochemical, chemical and biological profiles. This plant-oil is economically feasible in accordance with its availability with a cost-effective preparation. The presented cold-pressed extra-virgin Sacha inchi oil is industrial applicable for topical products without additional purification as indicated by its physicochemical, chemical and biological profiles. In addition, physicochemical and chemical specifications for Sacha inchi oil presented are useful indicators in industrial applications for cosmetics, pharmaceuticals and nutraceuticals. Its functional property for anti-aging product indicated with cellular antioxidant and anti-MMP-2 activities is reported for the first time abided with its *in vitro* antioxidant activity. Thus, innovative utilizations of Sacha inchi rich in bioactive constituents for anti-aging products are encouraged. Application of this plant for health promotion

products in addition to the conventional uses in food is appointed accordingly.

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