



Moisturizing and antioxidant evaluation of *Moringa oleifera* leaf extract in topical formulations by biophysical techniques



R. Álvarez-Román^a, P.G. Silva-Flores^a, S.A. Galindo-Rodríguez^b, A.A. Huerta-Heredia^{c,f},
W. Vilegas^d, D. Paniagua-Vega^{a,e,*}

^a Departamento de Química Analítica, Facultad de Medicina, Universidad Autónoma de Nuevo León, Av. Fco. I. Madero y Dr. E. Aguirre Pequeño s/n, 64460 Monterrey, Nuevo León, México

^b Departamento de Química Analítica, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Av. Pedro de Alba s/n, 66455 San Nicolás de los Garza, Nuevo León, México

^c Instituto de Biotecnología, Universidad del Papaloapan, Av. Circuito Central No. 200 Col. Parque Industrial, Tuxtpec, Oaxaca, CP 68301, México

^d Instituto de Biociências – Câmpus do litoral Paulista, UNESP – Universidade Estadual Paulista, Praça Dom Infante Henrique s/n, Parque Bitaru CEP 11330-900, São Vicente, São Paulo, Brasil

^e Cátedras CONACYT-UANL, México

^f Cátedras CONACYT-UNPA, México

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ABSTRACT

The phytochemical profile, formulations (nanoparticles and gel), moisturizing and antioxidant potential of a hydroalcoholic fraction of *Moringa oleifera* leaves (HFM) were determined. These were obtained through the application of eco-friendly strategies of cultivation and processing. The HFM phytochemical analysis by ESI-IT-MSⁿ showed the presence of seven compounds (flavonoids and phenolic acids). The viscosity, pH and particle size of HFM formulations ensure the suitability for topical application. Antioxidant activity was determined by inhibition of lipid peroxidation and ferric thiocyanate in vitro; it was confirmed that HFM and in formulations showed a significant inhibition of oxidation. In relation to the in vitro skin biophysical evaluation for topical formulations of HFM, water content, lipids and trans-epidermal water loss on the skin were favored with the presence of HFM. In addition, skin pH was not been modified. Our results emphasize the moisturizing and antioxidant potential of the moringa-formulation as a new skin drug delivery system.

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CE	crude extract
Phfm	partial hydroalcoholic fraction of moringa
HFM	hydroalcoholic fraction of moringa
ESI-IT-MS ⁿ	Multi-stage mass spectrometry with ion trap
TEWL	Trans-epidermal water loss
SCW	stratum corneum water content
HFM-NP	HFM-loaded nanoparticles

1. Introduction

Moringa oleifera (Lam.) is an easily cultivable and fast growing multipurpose tree that belongs to the Moringaceae family. It is commonly known as the ‘drumstick tree’ or the ‘horseradish tree’

*Corresponding author at: Departamento de Química Analítica, Facultad de Medicina, Universidad Autónoma de Nuevo León, Av. Fco. I. Madero y Dr. E. Aguirre Pequeño s/n, 64460 Monterrey, Nuevo León, México

E-mail address: david.paniaguav@uanl.edu.mx (D. Paniagua-Vega).

(Gopalakrishnan et al., 2016). *M. oleifera* is a plant with much research that supports its nutritional, medical, environmental, and agricultural applications (Alegebeye, 2018). All these applications are attributed to the wide range of phytochemicals produced by this plant (Mulugeta and Fekadu, 2014). Most of its biological activity is produced by its high content of flavonoids, glucosides, glucosinolates (Rani et al., 2018) and chlorogenic acids (Djande et al., 2018).

Leaves, seeds, roots, flowers and cortex from *M. oleifera* have a medicinal value, however leaves are more widely used (Popoola and Obembe, 2013). It has been reported that its leaves are the main source of antioxidant compounds due to their high content in carotenoids, ascorbic acids, glucosinolate and other bioactives (Saini et al., 2016). Its antioxidant activity, due to phytochemicals from *M. oleifera* leaves has been correlated with different biological activities because these antioxidant phytochemicals protect the cell from reactive oxygen species and reduce oxidative damage in lipids, proteins, and nucleic acids (Tshabalala et al., 2019). Indeed, in *M. oleifera* leaf extracts, a positive linear relationship was found between polyphenol

and flavonoid content and DPPH scavenging activity (Jahally and Puchooa, 2017).

The antioxidant activity of herbal- phenolic acids and flavonoids has gained considerable attention for topical application. The function of the skin is maintained by an important balance between the water content of the stratum corneum and skin lipids. Exposure to external factors; i.e., air humidity, ultraviolet radiation, and temperature, may disrupt this balance (DalBelo et al., 2006). Ultraviolet radiation acts as an initiator of several skin disorders such as wrinkling, scaling, dryness, photo-aging, and skin cancer. Thus, moisturizers containing antioxidants and phenolic compounds are the bastion of management for dry skin (Ali et al., 2013a). In this way, the application of moisturizing formulations, such as gel and nanoparticles would be very interesting. On the other hand, objective methodologies are considered appropriate to prove that substances improve skin hydration. Among these are non-invasive skin bioengineering techniques, which are often used to evaluate cosmetic products.

The objectives of this study were to investigate the moisturizing and antioxidant properties of a hydroalcoholic fraction of moringa (HFM) obtained through the application of eco-friendly strategies of cultivation and processing. The HFM formulations (nanoparticles and gel) were evaluated on excised porcine skin using biophysical techniques.

2. Materials and methods

2.1. Moringa crop conditions

Moringa oleifera seeds were germinated in multicell polystyrene trays (200 cells/tray). Fifty seeds were placed per tray in peat moss pellet (Vita®); trays were kept in the shade and each cell was irrigated with 50 mL of purified water every three days. When the seedlings reached between 10 and 15 cm tall, they were transplanted into black plastic bags containing a mixture of bokashi organic fertilizer- tezontle (volcanic rock)- black earth (2:2:6). The crop was established in a medium technology greenhouse (average temperature on the day of 45 °C in summer and 30 °C in winter, located at 18°26'09.8 "N 96° 20'33.8" W) with a daily drip irrigation system. Additionally, a nutrient solution of Bayfolan® Forte (Bayer®) at 200 mL/100 L was added every 15 days during the first three months. Leaves with petiole of *M. oleifera* were harvested from one-year-old plants, once the plants reached between 2 and 2.5 m tall. The plant was authenticated in the UANL School of Biology, prior to the usage of the leaves for the study.

2.2. Chemicals and materials

Linoleic acid ($\geq 99\%$), α -tocopherol ($\geq 95.5\%$), polyoxyethylene sorbitan monolaurate (Tween-20) and 2,2'-azobis (2-amidinopropane) dihydrochloride (ABAP), xanthan gum, methanol Chromasolv LC-MS-grade were obtained from Sigma (Sigma-Aldrich, Sternheim, Germany). HPLC-grade methanol, ACS-grade ethanol and chloroform were obtained from productos Quimicos Monterrey, S.A. de C.V. Ferromont, México. All other chemicals used were analytical grade. Purified water was from a Milli-Q water-purification system (Veolia, USA) and a Eudragit L100-55 was obtained from Evonik Industries, Germany.

2.3. Sample preparation

Leaves with petiole were washed with tap water, drained and dried in an oven with cross ventilation air flow design (Shellab, Model 1408, Sheldon Manufacturing, Inc., OR, USA) at 50 °C for 12 h. The leaves of the petioles were separated and the particle size of the leaves was reduced in an industrial food processor (Model FP 41, HOBART, Germany); the particle size was homogenized to ≥ 1.4 mm. The moringa leaf meal was stored in a container with an airtight top, and in the dark at room temperature until its use.

In a 250 mL Erlenmeyer flask, 8 g of moringa leaf meal was placed with 80 mL of 80% aqueous ethanol (v/v) at 190 rpm (Unimax 1010, Heidolph Instruments GmbH & CO. KG) for 20 min at room temperature. The extraction was repeated three times successively and the recovered volumes were collected and filtered. To determine the extraction yield of the crude extract (CE), the volume of the three extractions was adjusted to 250 mL with 80% (v/v) aqueous ethanol; 3 aliquots of 2 mL were taken to dryness in an oven at 80 °C for 8 h. The rest of the extraction volume was concentrated under reduced pressure. The extraction was done in duplicate.

The SPE cartridge (500 mg, Thermo Scientific™, HyperSep™ C18, IL, USA) was first preconditioned by the consecutive passing of 3 mL of methanol (HPLC grade) and then 3 mL of 85% (v/v) aqueous methanol by gravity. A total of 10 mg of the CE obtained was solubilized with 80% aqueous methanol (37 μ L) and it was completed to 1.5 mL of 85% methanol. The sample was loaded on to the cartridge and was eluted with 1.5 mL of 85% methanol (only this fraction was recovered and named partial hydroalcoholic fraction of moringa: pHFM). The cartridge was further eluted with 3 mL of 100% methanol and finally with 3 mL water (Milli-Q-grade). This process was repeated 40 times (using a new cartridge every 20 repetitions). To obtain the recovery percentage, 6 pHFM were randomly selected and dried in an oven at 80 °C; this SPE strategy was done in duplicate. Additionally, the performance of the cartridges was evaluated with respect to the amount of CE (with 20 mg).

As a quality control strategy, aliquots of all pHFM were analyzed using a thin layer chromatography plate (TLC, silica gel 60, 20 \times 20 cm, UV fluorescence 254 nm, Merk KGaA, Darmstadt, Germany; Eluent: chloroform-methanol 4:1 v/v) and revealed with an anisaldehyde-sulfuric acid mixture and heat (to detect secondary metabolites). Finally, the pHFM verified in the TLC were collected and dried in an oven at 40 °C. This dry sample of the hydroalcoholic fraction of moringa (HFM) was analyzed by mass spectrometry and used in the activity assays.

2.4. ESI-IT-MSⁿ analyses

Mass spectral data were obtained using the direct flow infusion of the samples on an LCQ Fleet ion-trap mass spectrometer (Thermo, San Jose, CA, USA) with an electrospray interface (Thermo, San Jose, CA, USA) (DFI-ESI-IT-MSⁿ) in negative mode. It used a fused silica capillary tube at 280 °C, a spray voltage of 5.00 kV, a capillary voltage of -35 V, a tube lens of -100 V, a 10 μ L min⁻¹ flow and a collision energy of 20–30%. The second scan event was an MS/MS experiment performed by using a data-dependent scan on the molecules from the compounds of interest at a collision energy of 20–30% and an activation time of 30 ms. The product ions were then subjected to further fragmentation in the same conditions, until no more fragments were observed. The compounds were identified by comparing mass spectra of the components with data from the literature and the mass spectral library from MoNA (MassBank of North America, USA) (<http://massbank.us/>) and the European MassBank (NORMAN MassBank) (<https://massbank.eu/MassBank/>). The HFM samples were dissolved in 80% methanol at 1 mg/mL and then were diluted to 10 μ g/mL in methanol grade masses.

2.5. Preparation and evaluation of topical formulations

2.5.1. Gel

The topical gel was formulated in xanthan gum (1.3% (w/v)). The HFM (30 mg) was dissolved in 20 mL of water under constant and magnetic stirring (50 rpm), the xanthan gum was added and stirred well to improve the consistency of the gel (HFM-gel). The control gel (C-gel) was prepared with the same method as the HFM-gel, omitting the moringa extract.

The formulated gels were evaluated by physicochemical parameters to assess their suitability for in vitro topical application. The pH was measured using a pH indicator strip. The viscosity of the formulated gels was evaluated using the Brookfield viscometer (DV-II, Brookfield, Germany) with a #63 spindle.

2.5.2. Nanoparticles

The hydroalcoholic fraction of moringa-loaded nanoparticles (HFM-NP) was prepared by a previously described (Fessi et al., 1989) solvent displacement procedure. An acetone-methanol fraction of HFM containing 175 mg of Eudragit L100-55® was injected into 25 mL of water. The organic phase was removed under reduced pressure at $35 \pm 2^\circ\text{C}$ for approximately 15 min. The HFM-NP suspension was concentrated to the desired final volume (20 mL) by removal of water under the same condition. The control NP (C-NP) was prepared by the same method as the HFM-NP, omitting the moringa fraction.

The mean size, and polydispersity (index from 0.0–1.0) and zeta potential of the NP was measured with a Zetasizer Nano-ZS90 (Malvern Instruments, USA). Measurements were made in triplicate for all prepared formulations.

The physical stability of each NP was determined by assessment of particle size and polydispersity (index from 0.0–1.0) with a Nano-ZS90 (Malvern Instruments, USA).

2.6. In vitro anti-oxidant assay

2.6.1. Inhibition of lipoperoxidation of linoleic acid emulsion

The HFM antioxidant efficiency was expressed in terms of its capacity to protect linoleic acid from peroxidation as described by Ruberto and Baratta (2000). A solution of dodecyl sulfate sodium (50 mM, SDS) was prepared in aqueous Na_2PO_4 (5 mM) and adjusted to pH 7.4. Linoleic acid was added, immediately before each experiment, to a concentration of 1.3 mM. A stock solution of ABAP in water (35 mM), stored at $4 \pm 1^\circ\text{C}$ was used within a week. Extract solutions (50 and 100 $\mu\text{g}/\text{mL}$) were prepared in methanol immediately before use. An aliquot (2 mL) of the micellar suspension of linoleic acid was stirred at 50°C . After an equilibration period (20 min), 10 μL of the radical initiator solution and 50 μL of the antioxidant solution (extract or control) were added and the progress of peroxidation was monitored at 232 nm for 15 min. The absorbance value of the linoleic acid subjected to full peroxidation (without antioxidant) measured at the 15th minute was used as a control of peroxidation. The thymol and carvacrol solutions were used as positive control samples. The percentage antioxidant index (%) was calculated using the formula:

$$\%I = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

where A_{control} is the absorbance value of the fully oxidized control and A_{sample} is the absorbance of the test sample.

2.6.2. Total antioxidant activity determination by the ferric thiocyanate method in linoleic acid emulsion

The antioxidant activity of HFM was determined according to the ferric thiocyanate method (FTC) as described by Gülçin et al. (2012). The stock solutions were prepared dissolving 10 mg of HFM in 10 mL ethanol. The linoleic acid emulsion was prepared by mixing and homogenizing 15.5 μL of linoleic acid, 17.5 mg of Tween-20® as an emulsifier, and 10 mL of PBS (40 mM, pH 7.0). The sample solution (HFM or α -tocopherol), which contains different concentrations of HFM in PBS (15–45 $\mu\text{g}/\text{mL}$), was added to 2.5 mL of linoleic acid emulsion in PBS. The control solution was composed of 2.5 mL of linoleic acid emulsion and 2.5 mL of PBS (40 mM, pH 7.0). The solutions were incubated at 37°C in a polyethylene tube, followed by the addition of 0.1 mL of ammonium thiocyanate solution (30% (w/v)) precisely 3 min after adding the 0.05 mL of ferrous chloride (20 mM)

in hydrochloric acid (3.5% (w/v)) (Lee et al., 2004). The peroxide levels were determined by reading the absorbance at 500 nm in a spectrophotometer (Thermo Scientific, Genesys 10 s, UV–vis Spectrophotometer, USA). This step was repeated every 5 h. The inhibition percentage of lipid peroxidation in linoleic acid emulsion was calculated at this point (30 h) (Elmastaş et al., 2006). The α -tocopherol solutions were used as positive controls of lipid peroxidation inhibition.

The inhibition percentage of lipid peroxidation in linoleic acid emulsion was calculated by the following equation:

$$\%LPI = 100 - \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the control reaction which contains only linoleic acid emulsion in PBS, and A_{sample} is the absorbance in the presence of HFM (Gülçin, 2006).

2.7. In vitro skin permeation and non-invasive biophysical measurements

2.7.1. Pig skin preparation

Full-thickness porcine ear skin was obtained from slaughterhouse (Nuevo León, México). The subcutaneous fat was removed from the skin and the tissue was stored frozen for a maximum of four weeks before use (Sekkat et al., 2002).

2.7.2. In vitro skin permeation study

The porcine skin was thawed and clamped into position between the donor and the receptor compartment of a vertical diffusion cell. The donor compartment (area = 3.14 cm^2) was then filled with 3 mL of the control or moringa formulations and covered to prevent evaporation. The formulations were placed in contact with the skin for 1 and 2 h. At the end of the permeation experiment, the excess formulation was removed from the skin surface. The skin was washed 3 times with PBS (pH 7.4) and dried gently with a cotton swab. All measurements were made in a draught-free room, with controlled temperature ($23 \pm 2^\circ\text{C}$) and relative humidity (30–40%). The effect of the formulations on the skin was evaluated in terms of:

2.7.3 Trans-epidermal water loss (TEWL)

The measurements were performed with a Tewameter® TM300 (Courage&Khazaka Electronics GmbH, Cologne, Germany), the results are expressed as an averaged TEWL ($\text{g}/\text{m}^2\text{h}$). The tewameter was calibrated according to guidelines of manufactures.

2.7.4 The stratum corneum water content (SCW)

The SCW was measured with a capacitance meter Corneometer® CM825 (Courage&Khazaka Electronics GmbH, Cologne, Germany), the results are expressed as an average SCW (arbitrary units) by measuring the water content of the superficial epidermal layers down to a depth of about 0.1 mm.

2.7.5 The skin sebum content (SSC)

The SSC was measured with a Sebumeter® SM815 (Courage&Khazaka Electronics GmbH, Cologne, Germany) with a noninvasive cassette. The measuring head of the cassette represents a 64 mm^2 measuring section of an opaque plastic tape which is slightly pushed into the skin for 30 s to collect sebum. The resulting change in transparency of the tape was measured and the displayed values correspond to the sebum amount on the face in μg of sebum/ cm^2 .

2.7.6 pH skin surface

The pH was measured using a skin-pH-meter PH905® (Courage&Khazaka Electronics GmbH, Cologne, Germany). The pH meter was calibrated daily with pH 4.0 and pH 7.0 buffer solutions (Courage&Khazaka Electronics GmbH, Cologne, Germany). The pH electrode was applied directly to the skin surface. In order to maintain contact between the

probe and the skin, the electrode was dipped in distilled water immediately prior to application.

2.8. Statistical analysis

The results are expressed as means \pm S.D. Statistical comparisons were performed using one-way analysis of variance with Tukey's comparisons, with Graph-Pad Prism 6 software (California, USA).

3. Results and discussions

3.1. Crop system

In order to obtain homogeneous plant material, a germination strategy was implemented in Peat Moss[®] with a germination percentage of $87.8 \pm 4.3\%$ and a protected *Moringa oleifera* crop was established in a greenhouse under drip irrigation conditions with the addition of nutrients. This allowed homogeneous crop growth, with a high density of 195,000 plants ha^{-1} . Foidl, Makkar, and Becker (2001) found that trials separation affects final density for moringa crops, reaching 1 million plants per ha. Moreover, Sánchez et al. (2006) studied the effect of planting densities and cutting frequencies. They found that 250,000 plants per ha increased biomass production under Nicaragua growth conditions. In order to explore nutritional quality, Mabapa et al. (2018) studied crops of *M. oleifera* in a range of cultivation density from 1250 to 5000 plants ha^{-1} and observed a positive relationship between crop density and dry leaves production (from 205 to 448 kg ha^{-1} respectively). Our proposed cultivation system can contribute to a high production of plant material with desirable characteristics, such as innocuousness and homogeneity (of growth and phytochemicals production), that help comply with the quality control of applications aimed at pharmaceutical products.

3.2. Crude extract and spe fraction

In order to perform an eco-friendly extraction method, the crude extract (CE) of *M. oleifera* leaf meal was obtained by successive hydro-ethanolic extractions under orbital agitation; this method showed an extraction efficiency of $29.2 \pm 2.0\%$.

To extract the secondary metabolites of the CE and remove components that can interfere with the mass spectrometry analysis, we use an extraction in reversed-phase cartridges C-18 (RP18 SPE). The sample was eluted with 85% methanol in the conditioned cartridges and the performance of the cartridges was evaluated with respect to the amount of CE (10 and 20 mg). The recovery percentage for 10 and 20 mg of CE was $81.3 \pm 0.6\%$ and $69.5 \pm 0.5\%$ respectively. According to the last result, 10 mg of the CE was used to load the cartridges because the decrease in recovery may be due to saturation problems. Thin layer chromatography (TLC) analysis on silica gel plates showed that all the partial hydroalcoholic fraction of moringa (pHFM) maintained a very similar profile of spots (Fig. 1). The small standard deviation obtained in the percentage of recovery of pHFM and the maintenance of the profile of spots by TLC, showed the stability of the cartridge performance during the 20 times it was successively reused.

3.3. DFI-ESI-IT-MSⁿ analyses

In order to obtain a fingerprint of the chemical composition of *M. oleifera*, HFM was directly injected into the ESI source of the ion trap. We have tested different mass spectrometry conditions and decided to use negative ionization. Table 1 shows the tentative identification of seven phytochemicals, mostly flavonoids and phenolic acids. These compounds have been previously identified in *M. oleifera* and

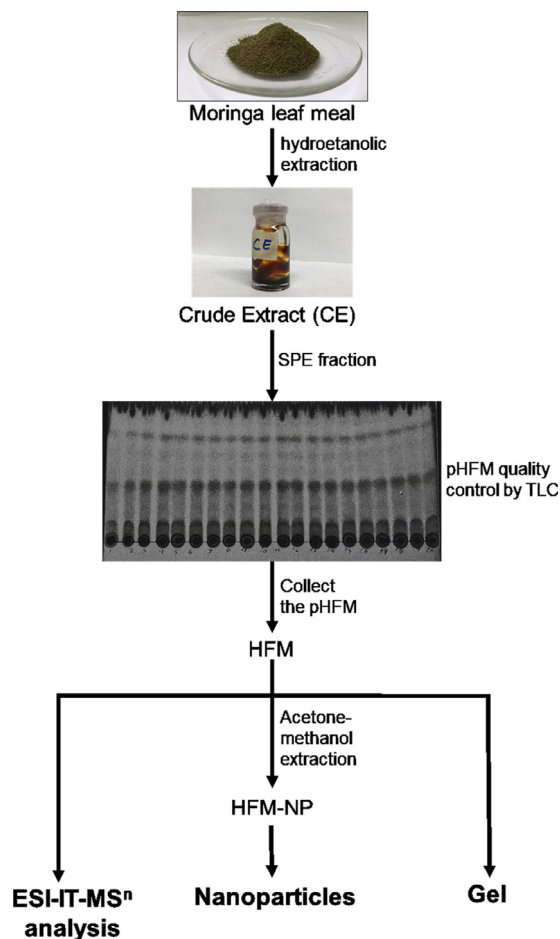


Fig. 1. General diagram of extraction, analysis, and formulations.

reported as phloretic acid, caffeic acid, quinic acid, caffeic acid O-glucoside, chlorogenic acid, apigenin glucoside and quercetin-O-glucoside.

3.4. Preparation and evaluation of topical formulations

The topical gel formulations were assessed for their pH and viscosity to ensure the suitability for in vitro application (Table 2). The pH observed for gel can be attributed to the properties of the excipients added. The close to neutral pH of HFM-gel, ensures non-irritancy and biocompatibility for its in vitro application (Kaci et al., 2018).

With the solvent displacement technique, it was possible to reproducibly prepare HFM-NP. The size of HFM-loaded NP was 169.83 ± 2.25 nm with a homogeneous distribution (Fig. 2) and a zeta potential of -10.14 mV. The results in Fig. 3, show that the NP prepared with HFM were stable after evaporation, and that no aggregation was detectable after 6 months. The absence of any aggregate or sediment attested to good association of the polymer with HFM (Martínez-Rivas et al., 2017).

3.5. In vitro anti-oxidant assay

Lipid peroxidation consists of a series of chain processes that involve oxidative degradation of lipids mediated by free radicals and associated with cell membrane damage (i.e., lipid denaturation). Free radicals can easily initiate peroxidation of membrane lipids, which leads to the accumulation of peroxides. Linoleic acid is the most abundant polyunsaturated fatty acid in the skin. Its presence ensures its health, as well as contributing to the formation of essential

Table 1

Precursor ions and their corresponding fragments obtained by ESI-IT-MSⁿ (negative ion mode) analyses of the hydroalcoholic fraction extracts of moringa (HFM).

[M-H] ⁻	Major fragments MS ²	MS ³	Tentative compounds identified	Background in moringa extracts
165	147*, 149, 129, 121, 105, 75	ND	phloretic acid ^a	Oka et al. (2016)
179	135*, 151, 107, 75	ND	caffeic acid ^a	Devisetti et al. (2016)
191	85*, 127, 111, 173, 93	ND	quinic acid ^{a, b}	Tan et al. (2015)
341	179*, 161	135	caffeic acid O- glucoside ^b	Makita et al. (2017)
353	191*, 179, 173	ND	clorogenic acid ^a	Djande et al. (2018)
431	311*, 341, 413, 283, 153	283	apigenin glucoside ^a	Karthivashan et al. (2013) and Djande et al. (2018)
463	301*	179, 151, 257, 193	quercetin-O-glucoside ^a	Karthivashan et al. (2013) and Makita et al. (2016)

Identification method: The identification is based on computer matching of the massspectra with those of the ^a Massbank and/or ^b MoNa libraries and comparison with literature data.

ND not determined.

* base peak.

Table 2

Physical parameters of gel formulations used in vitro skin permeation (mean ± S. D.; n = 3).

Formulation	Viscosity (cP)	pH
C-gel	1026±15	6–7
HFM-gel	1067±22	6–7

ceramides directly involved with the structure of the epidermal barrier (Dzialo et al., 2016).

Inhibition of lipoperoxidation of linoleic acid emulsion assay is based on the spectrophotometric determination of the conjugated dienes formed by the oxidation of linoleic acid in the absence and in the presence of a potential antioxidant. Based on the results (Table 3), it was observed that the HFM-NP, at 50 µg/mL, has the ability to inhibit peroxidation of linoleic acid in 6.16%. This percentage represents low inhibition percentage five times less than carvacrol and thymol (compounds with proven antioxidant activity). However, when the HFM-NP is 100 µg/mL, its ability to inhibit peroxidation of linoleic acid increased to 26.80%. There is a inhibition percentage similar to carvacrol and thymol at 50 µg/mL. DFI-ESI-IT-MS analysis indicated the presence of five phenolic acids and two flavonoids (Table 1). Polyphenols have been

known to exert a powerful antioxidant effect in vitro. They inhibit lipid peroxidation by acting as chain-breaking peroxy-radical scavengers, and can protect linoleic acid from oxidation (Chumark et al., 2008). It should be mentioned that the low%I obtained with HFM-NP, is probably due to the fact that all HFM encapsulated in the NPs have not been released in these 15 min. Complementary in vitro studies of HFM-NP release would be necessary.

On the other hand, the total antioxidant activity of HFM, HFM-NP and α-tocopherol (positive control) was determined by the ferric thiocyanate method in the linoleic acid system. This assay is based on the spectrophotometric determination of the colorful complex formed by Fe³⁺ and ammonium thiocyanate. During the lipoperoxidation of linoleic acid, hydroperoxides are formed and these oxidize Fe²⁺ to Fe³⁺, which subsequently participates in complex formation (Gülcin et al., 2012). In the absence of an antioxidant agent, the oxidation of linoleic acid is greater; thus, complex formation is also greater, and an increase in absorbance is observed and therefore the %LPI value is low. The effect of different concentrations of HFM or HFM-NP (15, 30 and 45 µg/mL) in preventing the peroxidation of linoleic acid emulsion is shown in Fig. 4. The absorbance of the formulations and control at 500 nm were plotted as a function of time. Fig. 4 shows that the absorbance of the negative control (linoleic acid

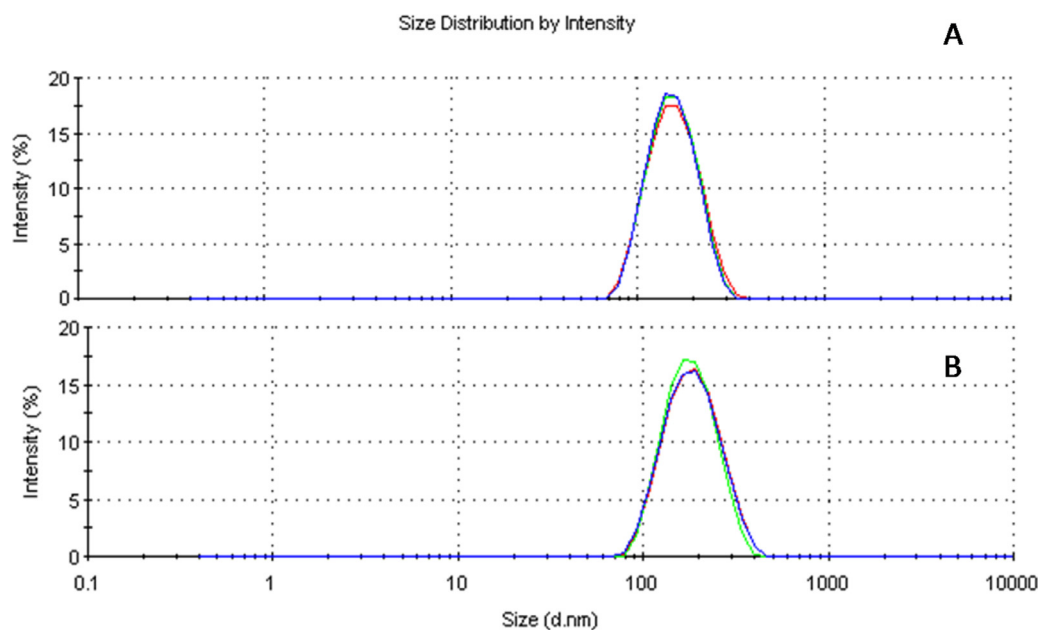


Fig. 2. Size distribution of nanoparticles: (A) control NP (C-NP) and (B) hydroalcoholic fraction of moringa-loaded nanoparticles (HFM-NP) measured by dynamic light scattering (DLS).

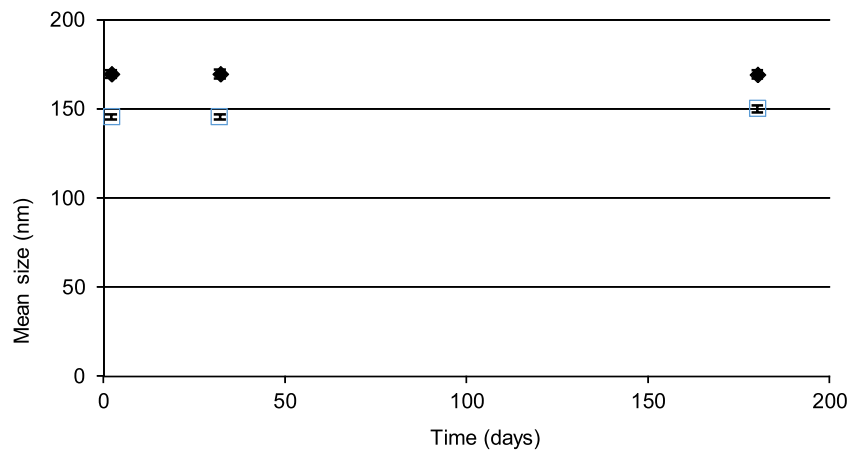


Fig. 3. Influence of time on nanoparticle size. (□) Control nanoparticles (C–NP) and (◆) hydroalcoholic fraction of moringa-loaded nanoparticles (HFM-NP).

Table 3

Determination of percentage inhibition of lipid peroxidation (linoleic acid).

Sample	Inhibition of lipid peroxidation (%) ^a
Carvacrol (50 µg/mL)	30.95 ± 1.06
Thymol (50 µg/mL)	30.11 ± 0.93
HFM-NP (50 µg/mL)	6.16 ± 0.87
HFM-NP (100 µg/mL)	26.80 ± 1.41

^a Percentage of inhibition of inhibition lipid peroxidation values determined after incubation period (15 min).

in the absence of a potential antioxidant), increased directly to the incubation time, and this higher absorbance indicates a higher colorful complex formation and therefore a low %LPI. On the other hand, α -tocopherol (15, 30 and 45 µg/mL) demonstrated a %LPI of 60.5, 62.0 and 69.10, respectively. These value were slightly lower than those obtained by Topal et al. (2016) for α -tocopherol at 30 µg/mL (%LPI = 73.88%). As shown in Fig. 4, the absorbance of the HFM and HFM-NP decreased depending on its concentration, therefore an increase in the %LPI dependent on the HFM concentration, was

observed. Interestingly, it was observed that, after 30 h of incubation, HFM and HFM-NP (at 45 µg/mL) presented a very close %LPI (62.2 ± 0.43 and 59.9 ± 0.24 , respectively), probably due to the release of HFM-compounds from the NC. It was demonstrated that HFM (free and nanoencapsulated) inhibits the oxidation of linoleic acid.

There is an important correlation between antioxidant activity and the amount of flavonoid compounds in the HFM. In this context, the antioxidant activity of *M. oleifera* could be attributed to the presence of relative concentrations of flavonoids, such as quercetin, in the free and nanoencapsulated HFM.

3.6. In vitro skin permeation and non-invasive biophysical measurements

In this study, electrical measurements in the short-term study are reported in Fig. 5(A) and (B). A significant increase in the stratum corneum water content (SCW) reading relative to untreated skin was observed 1 and 2 h after application of the HFM-gel formulations,

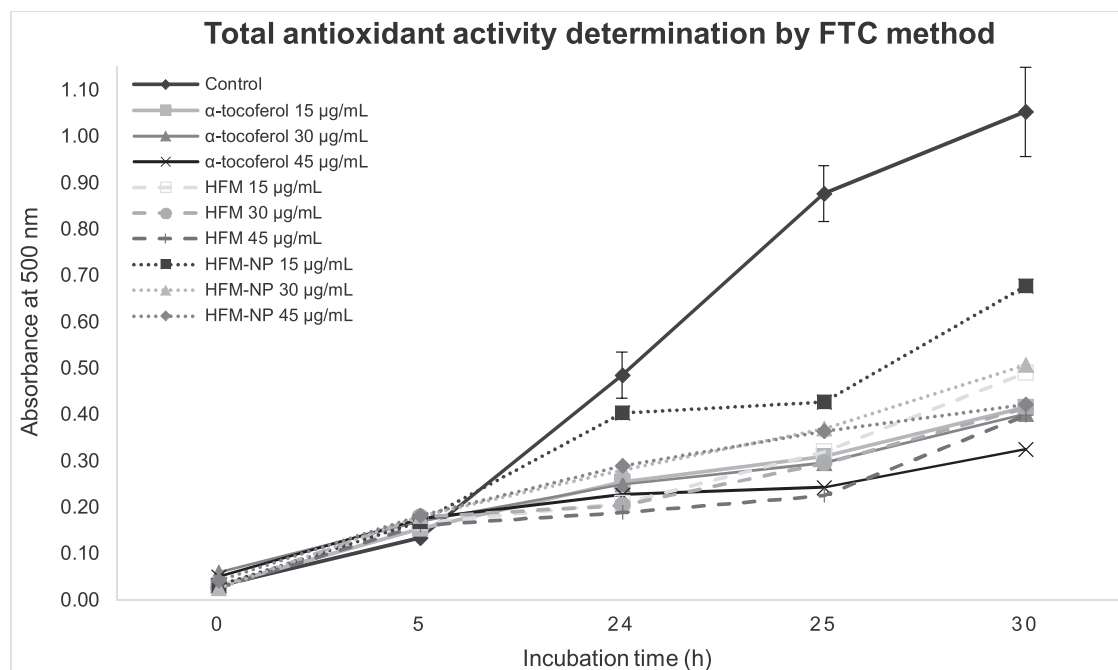


Fig. 4. Absorbance of hydroalcoholic fraction of moringa (HFM), hydroalcoholic fraction of moringa-loaded nanoparticles (HFM-NP), and standard of α -tocopherol (15, 30 and 45 µg/mL) by FTC method modified (mean ± SD, $n = 3$).

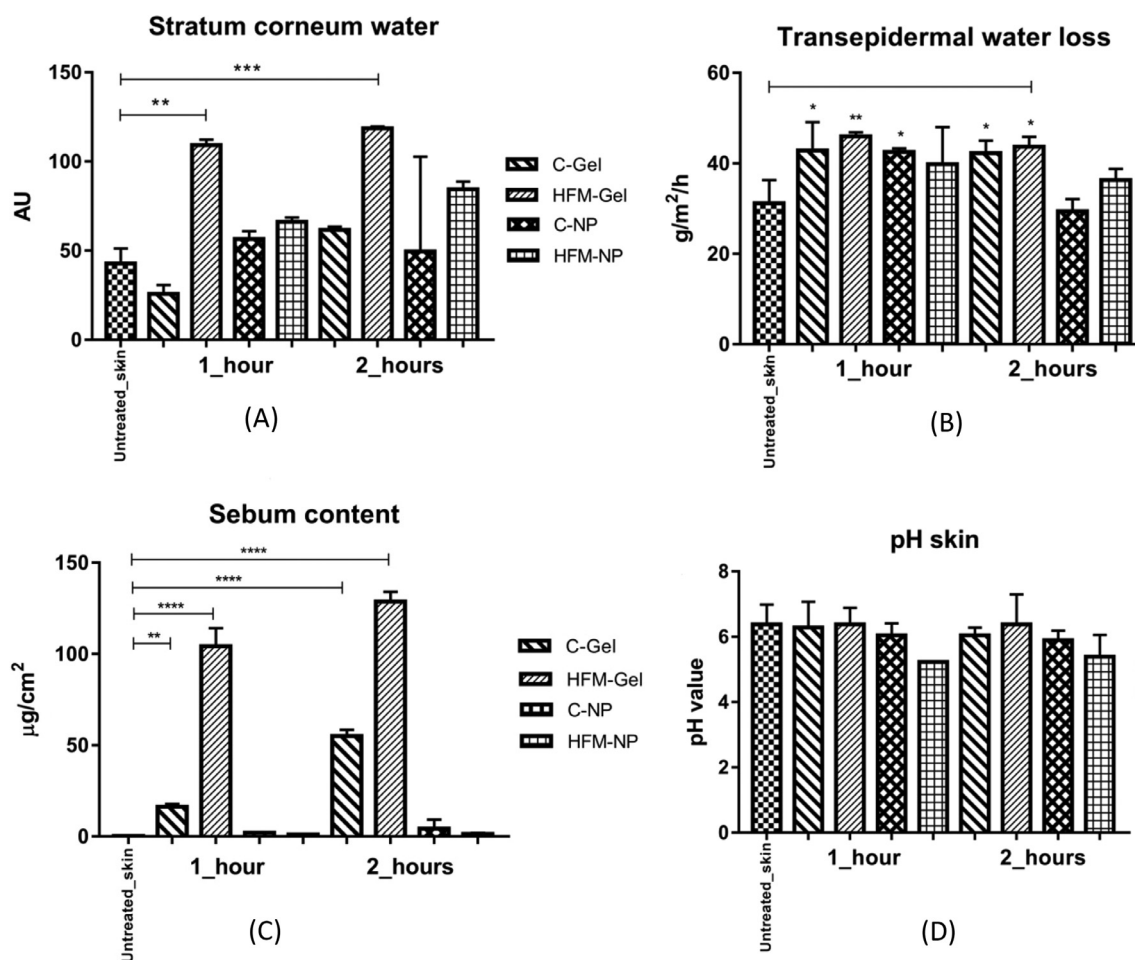


Fig. 5. Biophysical evaluation of hydroalcoholic fraction of moringa (HFM), hydroalcoholic fraction of moringa-loaded nanoparticles (HFM-NP) and hydroalcoholic fraction of moringa-loaded gel (HFM-Gel) and control formulations (C-Gel, C-NP): A) Stratum corneum water content, B) transepidermal water loss, C) sebum content and D) pH. All values are mean \pm SD ($n = 3$). * Indicates samples that are significantly different * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ compared with the untreated skin.

Fig. 5(A). However, when compared with the control-gel, SCW values were not modified 1 and 2 h after skin application, which means that the gel formulation does not increase the water content of the stratum corneum. This result was in agreement with previous studies, which reported that the in vivo skin application of cream containing *Moringa oleifera* leaf extract improved the moisturizing effect on the skin (Ali et al., 2013).

On the other hand, when NP formulations were applied on the skin, the SCW values were not modified after 1 and 2 h.

One and two hours after application, almost all formulation studied increased TEWL values significantly ($p < 0.05$) when compared with untreated skin values Fig. 5(B). In addition, when compared with the gel and NP vehicle, moringa-loaded formulations did not change TEWL values as well, which means that the presence of HFM in the formulations did not alter skin barrier function.

In relation to the lipid content in the skin, after application of the control-gel, the sebum content increased significantly (Fig. 5(C)). It should be mentioned that when the gel contained HFM, the sebum content increase was more than doubled, and a correlation was observed with the contact time of the formulations with the skin. However, Ali et al. (2013b) reported that *Moringa oleifera* cream significantly reduces undesirable skin sebum after cream application in human during 12 weeks. Quantitative studies are necessary to establish the dose-based biological activity relationship. Finally, in Fig. 5(D) it can be seen that free and encapsulated HFM do not alter skin pH.

4. Conclusions

Given the growing popularity of *Moringa oleifera* because of its multiple health benefits, it is necessary to establish production strategies and evaluate formulations that enhance its benefits in health care. In this work, we explored environmentally friendly *M. oleifera* growing and processing strategies. Also, the analysis of HFM by IT-ESI-MSⁿ allowed the identification of seven phytochemicals, mostly flavonoids and phenolic acids, to which activity is attributed to inhibit peroxidation of linoleic acid, the most abundant polyunsaturated fatty acid in the skin. In conclusion, the results of this study emphasize the moisturizing and antioxidant activity of moringa-formulation as a new skin drug delivery system. Complementary studies of HFM quantification would be necessary to establish the dose-based biological activity relationship.

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2019.10.011.

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