MARROCAN ORGANIC ARGAN OIL IMPROVING SKIN BARRIER FOR DRY SKIN

Argan oil improving skin barrier

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Abstract

Cold pressed Argan oil, prepared from the argan tree unroasted kernels, is traditionally used by Berber women for the care of skin, nails and hair. Although argan oil has a long history in traditional cosmetic use, to maintain the health of skin, its effect on the skin barrier has never been scientifically reported. Therefore, we have attempted to elucidate the effect of argan oil on epidermis using reconstructed human epidermis (RHE) model and clinically on volunteers.

First, RHE model from our laboratory were treated with argan oil for 5 days. Epidermal permeability assay was performed using Lucifer yellow, and 3 epidermal differentiation marker proteins were analyzed by immunofluorescence. Second, fifteen healthy female volunteers have applicated daily argan oil during seven days and their superficial aspect of the skin was measured at day 0 and 7 using a digital dermoscope (C-Cube).

After 5-days treatment, argan oil decreases the penetration of lucifer yellow and increase involucrin, filaggrin and transglutaminase 1 protein expression on RHE model.

Clinically, a 74% reduction in the quantity of scales and 45% of the sharpness of the microrelief were recorded after one week treatment.

By its composition rich in triglycerides (unsaturated and saturated fatty acid), argan oil is a moisturizer agent increasing proteins involved in the maintenance of skin barrier. These

results demonstrate that Argan oil helps to reduce the symptoms of dry skin, and visibly improve the skin.

Keywords: Argan oil, dry skin, skin barrier

Introduction

Argan tree (*Argania spinosa* (L.) Skeels) is only endemic in south-western Morocco, where it covers an area of 3200 square miles that constitutes a unique biotope and has an essential agro-economic function [1]. Because the argan tree is drought-resistant, it is also a powerful weapon for slowing down desertification [2] and is very well adapted to the extreme conditions of Atlas region. Cold pressed argan oil is prepared by pressing the slightly roasted kernels of the argan tree fruit and is composed of mono-unsaturated (UFA, 80%) and saturated (SFA, 20%) fatty acids (FA). It contains polyphenols, tocopherols, sterols, squalene, and triterpene alcohols. Traditionally, argan oil has been utilized in cooking, in the treatment of skin infections, and in skin/hair care products. Daily topical application of argan oil has also been shown to improve skin elasticity [3] and skin hydration by restoring the barrier function and maintaining the water-holding capacity [4]. Additionally, topical applications onto skin provide a softening and relaxing effect on the skin [5, 6].

The structure of *stratum corneum* (SC) is like a brick wall, in which the corneocytes are surrounded by the intercellular lipid lamellae. The skin's barrier function depends mainly on the integrity of the SC [7]. During differentiation, the plasma membrane of outer keratinocytes is replaced by the specialized cornified envelope (CE) of corneocytes. The CE gives corneocytes their rigidity. The development of the CE is attributed to the crosslinking of involucrin (IVL) and loricrin (LOR) by transglutaminases (TGM). Some of the lipids are synthesized in the keratinocytes at the stratum granulosum (SG) and then released from the lamellar bodies (LBs) into the SG-SC interface. The permeability barrier is provided by the intercellular lipid-enriched matrix, which is composed of ceramides, Free-FAs (FFAs), and cholesterol. SC lamellar membranes are mostly composed of saturated FFAs of significantly longer chain length, which varies between C16 and C26. The main FFAs in the lamellar membranes are palmitic acid (C16:0), stearic acid (C18:0) behenic acid (C22:0), lignoceric acid (C24:0), and hexacosanoic acid (C26:0) [8]. Other FFAs that present less in the SC include oleic acid (C18:1, n-9), eicosapentaenoic acid (C20:5, n-3), arachidonic acid (C20:4, n-6), docosahexaenoic acid (C22:6, n-3), linoleic acid (C18:2, n-6) as well as its derivatives. In fact, linoleic acid is the most abundant polyunsaturated fatty acid [9]. Hydration of the SC is also crucial for the SC integrity and the maintenance of the skin barrier homeostasis. Natural moisturizing factor (NMF) components within the corneocytes contribute to the

hydration of the SC. Filaggrin (FLG), one of the terminal differentiation markers of the epidermis, also aids in SC hydration and is degraded into free amino acids as a part of NMF. This makes filaggrin one of the major factors influencing the hydration status of the SC. The disruption of the barrier function of the skin results in an increase in transepidermal water loss (TEWL) [10], and consequently a decrease in water content of the epidermis (WCE), which constitute the two main characteristics of dry skin [11]. The normal functioning of the SC is disturbed under dry-skin conditions. When this occurs, the effectiveness of the SC-barrier function stops and a cycle of events initiates, such as superficial dehydration of the SC, subsequent release of inflammatory mediators, induction of epidermal keratinocyte hyperproliferation, and disruption of epidermal cellular differentiation [12]. Topical applications of SFAs and UFAs in healthy volunteers showed differences in TEWL and irritant skin response [13]. Linoleic acid maintained the integrity of the water permeability barrier of the skin whereas oleic acid is detrimental to skin barrier function and causes barrier disruption [14, 15]. Oils composed mostly of monounsaturated oleic acid increased skin permeability more than oils containing an almost even mixture of both monounsaturated and polyunsaturated fatty acids. Moreover, the concentration of FFAs such as oleic acid with respect to triglycerides correlates with clinical measures of skin barrier function (TEWL). This ratio determines molecular interactions with SC lipids and the extent of their penetration within the epidermis [16].

Argan oil used in this study is a fair and organic oil and its picking is governed by a sustainable picking charter and training in good environmental practises. Its composition is characterized by triglycerides (95% of constituent), with UFA (82.5%), small amounts of SFAs and minor components (squalene, sterols, triterpene alcohols and Vitamin E). We used a reconstructed epidermal model from our laboratory to study argan oil biological activity and performed a clinical study for assessing the effect of the Argan oil on the superficial aspect of the skin.

Materials and Methods.

Analytics

For sample preparation, 2g of argan oil was introduced into glass tube with isooctane, KOH methanolic solution and $NaHSO_4$ were added and, supernatant was analyzed after agitation. The analysis was performed with a Thermo Electron TRACE GC ULTRA coupled to a mass spectrometer ISQTrace with a column TR WAX (60 mm x 0.25 µm x 0.25 mm). Unsaponifiables, sterols and triterpenes alcohols or tocopherols were analyzed by HPLC using NF EN ISO 3596, NF EN ISO 12228 or NF EN ISO 9936 methods, respectively.

Reconstructed Human Epidermis (RHE) culture

NHEK extracted from a 60-year-old donor (female, breast) were used at passage 2. Cells were seeded on a polycarbonate cell culture inserts in multi-well plates (NuncTM) in proliferation medium (DMEM/HAM:F12, fetal calf serum 10%, hydrocortisone 0.4 µg/ml, epidermal growth factor 10 ng/ml, insulin 0.12 UI/ml, 3,3′,5-Triiodo-L-thyronine sodium salt 2 nM, adenine 24.3 µg/ml, apo-transferin 5 µg/ml, pituitary extract 50 µg/ml, isoprenaline hydrochloride 1 µM and antibiotics) and incubated at 37°C, 5% CO2. After 3 days, the immature human epidermis was elevated at the air-liquid interface to allow keratinocytes differentiation. The proliferation medium was removed and differentiation medium (DMEM/HAM:F12, fetal calf serum 1%, hydrocortisone 0.4 µg/ml, insulin 0.12 UI/ml, ascorbyl glucoside 300 µM and antibiotics) was added under the culture inserts. Argan oil was applied at the surface of RHE model at day 7, 9 and 12.

Lucifer yellow: Skin barrier permeability assay

On day 14, Lucifer yellow (100 mM in phosphate buffered saline (PBS)) were applied on the top of each RHE and incubated at 37°C, 5% CO2 for 2 hours. Culture medium under the RHE was collected for Lucifer Yellow quantification by reading the fluorescence on the VarioskanTM microplate reader (λexcitation 428 nm, λemission 540 nm).

Immunofluorescence analysis

RHE frozen sections (6 µM) were fixed with 100% cold methanol for 10 min prior PBS wash. After one-hour blocking with PBS/BSA 1%, slides were incubated with primary antibodies 2h at room temperature. The primary antibodies used were anti-FLG (Abcam) at 1/500, anti-

TGM1 (Abcam) at 1/200 and anti-IVL (Abcam) at 1/500. After rinsing in PBS, slides were treated with the corresponding secondary antibody: Alexa fluor 633-labeled anti-mouse IgG or Alexa fluor 633-labeled anti-rabbit IgG (Life Technologies; dilution, 1/1000) diluted in PBS/BSA 1% for 1 h at room temperature. Images were taken with a confocal microscope (Zeiss, LSM 800) using the 405 nm and 488 nm lasers. All images for a same protein were taken at the same laser intensity and image settings.

Clinical test: Evaluation of aspect of skin – Digital dermoscope (C-Cube)

15 healthy female volunteers (from 33 to 67 years old, mean 56 years old), with dry to very dry skin on half-legs, applied daily argan oil (standardized distribution) by gentle massage until full penetration into the skin. A clinical visible-light dermatological camera (C-Cube, Pixience Inc, France) was used to capture high-resolution close-up in-face images of the skin and is imaging the region of interest under controlled lighting conditions. Each 2D C-Cube image covers an area of approximately 16×12 mm. Standardised image acquisitions were performed before the first application of argan oil at day 0 and day 7. Acquisition of high-definition images permits to determine the microrelief and the quantity of scales on skin. Dry skin is characterised by an accumulation of scales in and between the furrows, and hydrated skin has a less marked microrelief and quantity of scales. Differences under untreated and argan-oil treated area were considered to be statistically significant at $p < 0.05$.

Results

Argan oil is rich in triglycerides

Its composition is characterized by triglycerides (95% of constituent). FAs are composed of UFA represents by oleic acid (46%), linoleic acid (34%), α-linolenic (0.09%) and non-identified FAs (2.41%). Small amounts of saturated FAs are also identified with stearic acid (5.51%) and palmitic acid (12%). Unsaponifiable represents about 1% of argan oil. Major unsaponifiable families are squalene (30%), sterols (22%) represented by α-spinasterol and shottenol which are characteristic of argan oil, triterpene alcohols (9%) with β-amyrin (22%), butyrospermol (23%), lupeol (9%) and tirucallol (45%), and Vitamin E (7%).

RHE model: Argan oil reinforces skin barrier

Because of richness in triglycerides, we hypothesise that argan oil treatment could alter epidermis permeability and differentiation marker proteins. First, a Lucifer yellow assay was used to test the outside-in permeability of stratum corneum in RHE model. Argan oil-treated RHE were compared to untreated control after 5-day application. The hydrophilic fluorescent dye Lucifer Yellow was retained in the SC and higher diffused staining was seen in untreated control than those argan oil-treated RHE. Indeed, less significant Lucifer yellow dye was detected in the culture medium of Argan oil-treated (-48.1±13.6%) than in untreated control medium suggesting an improvement of the outside-in skin barrier (Fig. 1).

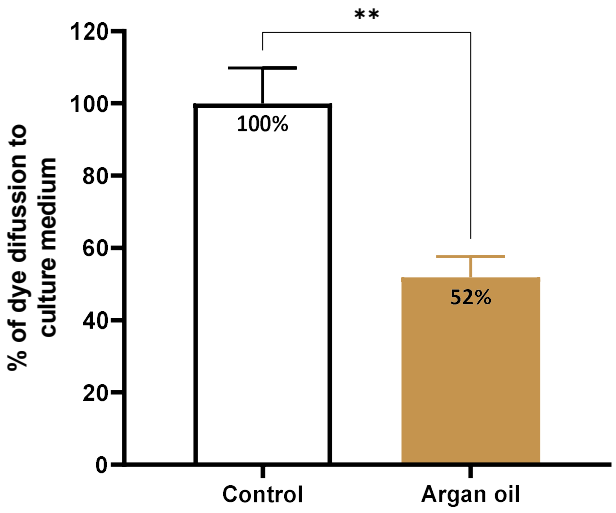

Fig 1. Argan oil decrease skin permeability

Permeability to Lucifer yellow was tested at day 14 and was applicated at the surface area of RHE model. Means are reported ± SEM (n=7) and untreated control were normalized to 100%. Statistical significance between untreated control and argan oil treated RHE were determined using the non-parametric Mann-Whitney test, $^{**}P<0.01$.

Moreover, immunofluorescence study showed that the argan oil treatment increased the expression of the cornified envelope proteins IVL, TGM1 and FLG (Fig. 2).

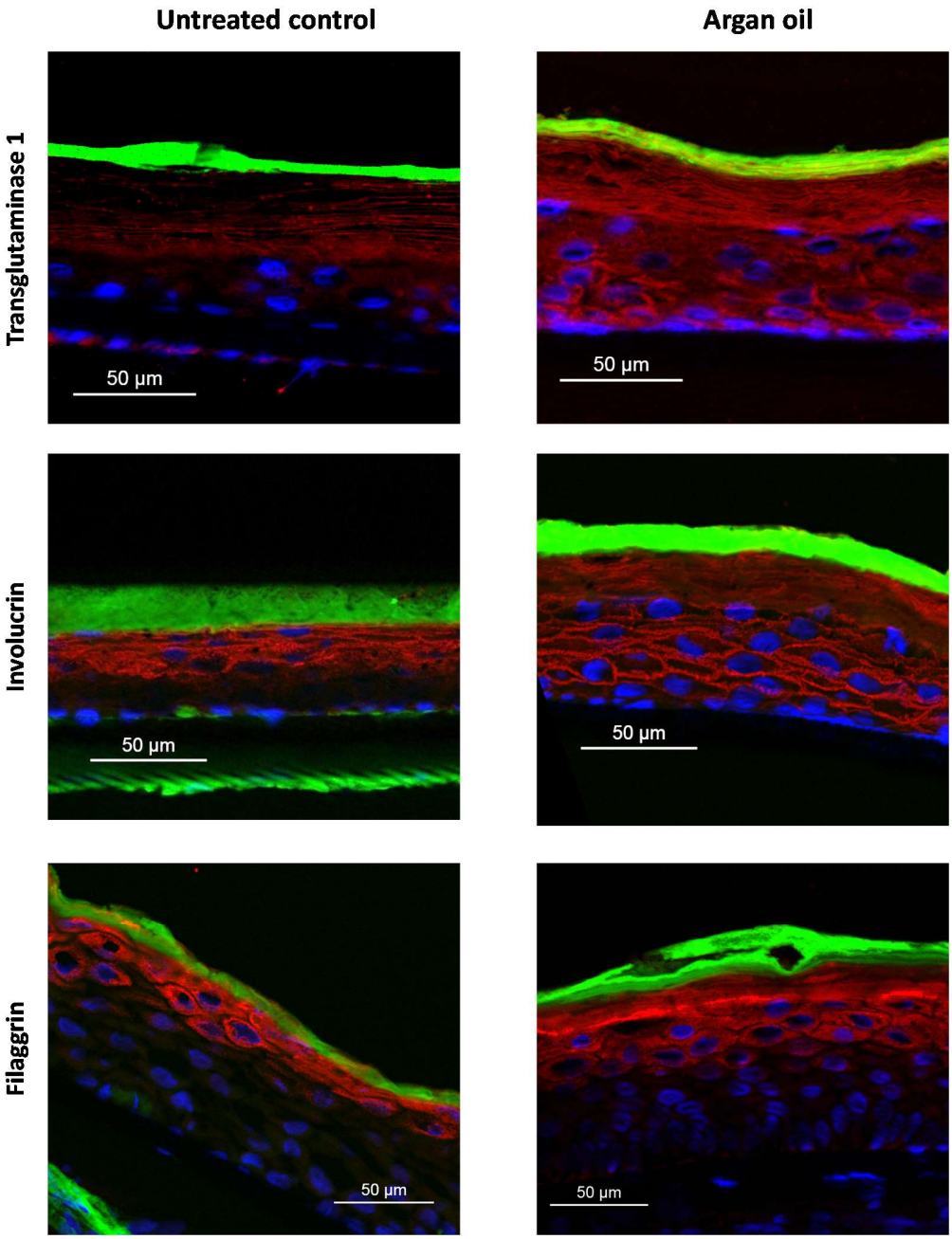

Fig 2. Epidermal proteins detection. Immunofluorescence was conducted on control and argan oil treated RHE. Involucrin (IVL), transglutaminase 1 (TGM1) and filaggrin (FLG) appear in red, nuclei in blue and Lucifer yellow in green.

While in the control RHE, IVL localization seemed mainly intracellular, in the argan-treated RHE, it was clearly associated to the cell membrane, suggesting a better incorporation into the cornified envelope. This observation was correlated with a higher expression of TGM1

that is responsible for the crosslinking of IVL and other proteins to the cell membrane and participate in the formation of the cornified envelope.

C-cube acquisitions: Argan oil visibly improves skin aspect

As shown in Fig. 3, the effect of the Argan oil on the superficial aspect of the skin was assessed using a digital dermoscope (C-Cube, Pixience Inc, France).

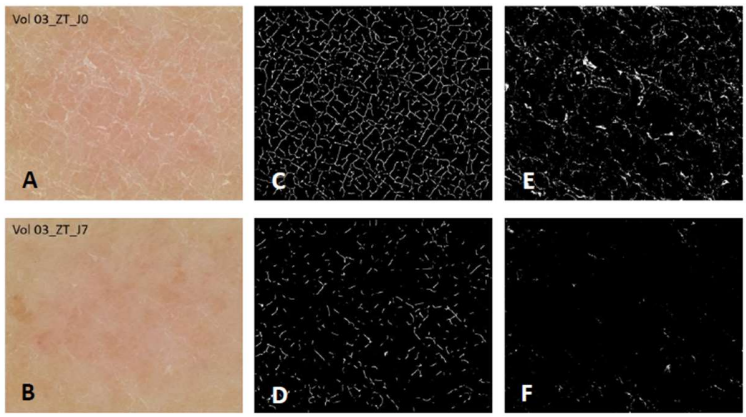

Fig 3. Argan oil visibly improves skin aspect
C-Cube test acquisitions of the volunteer #3 who took part in the clinical study. Images A, C and E correspond to untreated area on day 0. Images B, D and F correspond to area treated with argan oil on D7. Images C and D correspond to the visualization of the cutaneous microrelief. Images E and F correspond to the visualization of scales on the surface of the skin.

After one week, the improvement of skin hydrated aspect was visible, with a 74% reduction of the quantity of the scales (Table 1) and -45% of the sharpness of the microrelief (Table 2) on the whole panel. Statistical significance between untreated control and argan oil treated area were determined using Fisher test, with a threshold of 5%.

Table 1. Quantity of scale

	Untreated		Argan oil-treated	
	D0	D7	D0	D7
Score mean	0.013	0.011	0.015	0.004
n	15		15	
p (Fisher 5%) between D0 and D7	0.746		0.039	
Variation (%)	-10		-74	
p (Fisher 5%) between untreated and argan oil-treated	0.045			

Table 2. Sharpness of the microrelief

	Untreated		Argan oil-treated	
	D0	D7	D0	D7
Score mean	28.387	24.658	28.397	15.639
n	15		15	
p (Fisher 5%) between D0 and D7	0.425		0.006	
Variation (%)	-13		-45	
p (Fisher 5%) between untreated and argan oil-treated	0.007			

Discussion.

Argan oil is known in the literature to have skin repair barrier action, anti-inflammatory effect, and wound healing [4, 5, 17, 18] capacity.

In this study, a daily application of argan oil during a 7 days' period have a beneficial effect on skin relief and visibly improved the skin, that was related with a decrease the permeability of the epidermis by increasing terminal differentiation markers in RHE. We have demonstrated that IVL and TGM1 proteins are increased by argan oil treatment, and also FLG that is degraded in NMF components within the corneocytes and contribute to the hydration of the SC. Those proteins contribute to the maintaining of skin barrier function and by the way influencing the hydration status of the SC [12]. The treatment of RHE with Argan oil decrease the permeability of the epidermis, demonstrated by the measurement of Lucifer yellow penetration, and probably maintained the hydration of the epidermis [12]. Indeed, a reduction in the quantity of scales and microrelief of the skin were observed in our clinical study, that may correlate with the increase of terminal differentiation markers in RHE and maintenance of hydration status of the epidermis.

By its composition rich in triglycerides with a mixture of UFA plus small amounts of SFA and unsaponifiable fraction, argan oil used in this study modulate skin barrier homeostasis by increasing terminal differentiation markers. Research has suggested that oils containing mixture of both UFA and SFA decreased skin permeability compared to monounsaturated oleic acid and the ratio between oleic acid and TAG determines molecular interactions with SC lipids and the extent of their penetration within the epidermis [16]. In addition, the other components such unsaponifiable, sterols and triterpenes alcohols or tocopherols may have an anti-inflammation and antioxidant effect as demonstrated previously by Silva *et al.* [19].

Conclusion. Argan oil is a moisturizer agent increasing FLG, IVL, TGM1 proteins involved in the maintenance of skin barrier and by the way, decreases the penetration of harmful compounds. As FLG is a molecule involved in the production of the NMF that allows hydration of the skin, argan oil could help maintain skin moisture biologically through this mechanism. These results demonstrate that Argan oil helps to reduce the symptoms of dry skin, and visibly improve the skin.

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Conflict of Interest Statement. NONE.

References.

1. Charrouf Z, Guillaume D (2010) The rebirth of the argan tree or how to give a future to Amazigh women. In: Harpelle RL, Muirhead B, eds. Long-Term Solutions for a Short-Term World, Canada and Research Development Waterloo, WLU Press
2. Charrouf Z (2002) The argan tree, an asset for Morocco. Biofutur 220: 54–57
3. Boucetta KQ, Charrouf Z, Aguenaou H, Derouiche A, Bensouda Y (2015) The effect of dietary and/or cosmetic argan oil on postmenopausal skin elasticity. Clin Interv Aging10:339–349.
4. Boucetta KQ, Charrouf Z, Derouiche A, Rahali Y, Bensouda Y (2014) Skin hydration in postmenopausal women: Argan oil benefit with oral and/or topical use. Prz. Menopauzalny 13:280–288.
5. Manca ML, Matricardi P, Cencetti C, Peris JE, Melis V, Carbone C, Escribano E, Zaru M, Fadda AM, Manconi M (2016) Combination of argan oil and phospholipids for the development of an effective liposome-like formulation able to improve skin hydration and allantoin dermal delivery. Int J Pharm 505:204–211.
6. Fadda AM, Manconi M (2016) Combination of argan oil and phospholipids for the development of an effective liposome-like formulation able to improve skin hydration and allantoin dermal delivery. Int J Pharm 505:204–211
7. Elias PM (1983) Epidermal lipids, barrier function, and desquamation. J Investig Dermatol Suppl 80:44s–49s
8. Kang L, Ho PC, Chan SY (2006) Interactions between a skin penetration enhancer and the main components of human stratum corneum lipids. J Therm Anal Calorim 83:27–30
9. Ansari MN, Nicolaides N, Fu HC (1970) Fatty acid composition of the living layer and stratum corneum lipids of human sole skin epidermis. Lipids 5:838–845.
10. Raney SG, Hope MJ (2006) The effect of bilayer and hexagonal HII phase lipid films on transepidermal water loss. Exp Dermatol 15:493–500.
11. Breternitz M, Kowatzki D, Langenauer M, et al (2008) Placebo-controlled, double-blind, randomized, prospective study of a glycerol-based emollient on eczematous skin in atopic dermatitis: biophysical and clinical evaluation. Skin Pharmacol Physiol 21:39–45.

12. Nakagawa N, Sakai S, Matsumoto M, et al (2004) Relationship between NMF (lactate and potassium) content and the physical properties of the stratum corneum in healthy subjects. J Invest Dermatol 122:755-763
13. Tanojo H, Boelsma E, Junginger HE, et al (1998) In vivo human skin barrier modulation by topical application of fatty acids. Skin Pharmacol Appl Skin Physiol 11:87–97
14. Elias PM, Brown BE, Ziboh VA (1980) The permeability barrier in essential fatty acid deficiency: Evidence for a direct role for linoleic acid in barrier function. J Investig Dermatol 74:230–233.
15. Hansen HS, Jensen B (1985) Essential function of linoleic acid esterified in acylglucosylceramide and acylceramide in maintaining the epidermal water permeability barrier. Evidence from feeding studies with oleate, linoleate, arachidonate, columbinate and alpha-linolenate. Biochim Biophys Acta 834:357–363.
16. Mack Correa MC, Mao G, Saad P et al (2014) Molecular interactions of plant oil components with stratum corneum lipids correlate with clinical measures of skin barrier function. Exp Dermatol 23:39–44.
17. Jordan M, Nayel A, Brownlow B, Elbayoumi (2012) Development and evaluation of tocopherol-rich argan oil-based nanoemulsions as vehicles possessing anticancer activity. J Biomed Nanotechnol 8:944–956.
18. Avsar U, Halici Z, Akpinar E et al (2016) The Effects of Argan Oil in Second-degree Burn Wound Healing in Rats. Ostomy Wound Manag 62:26–34.
19. Silva S, Michniak-Kohn B, Leonardi GR (2017) An overview about oxidation in clinical practice of skin aging. An Bras Dermatol 92:367–374.