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Phytochemical Characterization and *in vivo* Anti-inflammatory and Wound-healing Activities of *Argania spinosa* (L.) Skeels Seed Oil

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Abstract: The extracted oil of *Argania spinosa* (L.) was investigated in regard to its fatty acid composition and polyphenols by Gas Chromatography-Mass Spectrometry (GC–MS) and Ultra-high Performance Liquid Chromatography-Electro Spray Ionization-Quadruple Time Of Flight-Mass Spectrometry (UPLC-ESI-QTOF-MS), respectively. The reduction rate of topical inflammation of extracted oil was calculated using a mouse model. The skin toxicity of argan oil on intact and damaged skin was assessed using a rabbit model. The findings revealed a rich content of monounsaturated and polyunsaturated fatty acids and presence of phenolic acids. The oil exhibited a reduction of inflammation and facilitated a healing process without any irritation. The experimental study revealed that *A. spinosa* seed oil displays remarkable wound-healing and anti-inflammatory activities related to its chemical composition. Argan oil has positive potential for skin medicinal application.

Keywords: *Argania spinosa* (L.) Skeels; oil; chemical composition; anti-inflammatory; wound-healing.

1. Introduction

Argan tree, *Argania spinosa* (L.) Skeels, belongs to the *Sapotaceae* family and represents only the species of the genus *Argania* [1].

The species *A. spinosa* is the most original tree of North Africa, and endemic in southwestern Morocco, where it grows over about 828 000 ha [**2**]. In Algeria, the argan tree can be found in the southwest of the province of Tindouf between Jebel Ouarkziz and Hamada.Unfortunately, it has been ignored by the local population its range is now limited to an area of 3 000 hectares [3, 4]. Some years ago, successful attempts to cultivate this tree were made in many places such as Stidia (a region in Mostaganem province), Chlef and Mascara.

The most valuable part of argan tree is its fruit, which is highly sought after its oil is extracted from its seeds. Argan oil is eaten raw in the southwest of Morocco [4, 5]. Recent studies suggested that dietary argan oil from fruits could protect against cancer [6, 7] and atherosclerosis, and improve plasma lipid profiles, paraoxonase activities and LDL peroxidation in healthy Moroccan men [8]. It is traditionally used for treatment of rheumatism, constipation, diabetes and respiratory difficulties. Moreover, argan oil is used externally for skin diseases, against juvenile acne, flaking and wrinkled or scaly dry skin, as well as for nourishing hair and as a moisturizing oil [9]. This oil has been widely used in traditional medicine for centuries to cure wounds and burns [10, 11, 12].

The multiple virtues of argan oil regarding human health are due to its chemical composition, such as the glyceride fraction (99%), which is rich in polyunsaturated fatty acids like oleic (47.7%) and

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linoleic acid (29.3%) [13, 14], the unsaponifiable fraction, rich in tocopherol, particularly α and γ-tocopherol, and the phenolic compounds [15].

The aim of this study is to provide scientific proof to validate the traditional use of Algerian argan oil for treatment of damaged skin.

2.Material and methods

2.1. Extraction of Argan oil

The fruits of *Argania spinosa* (L.) Skeels were collected during June and July from the Stidia region, Mostaghanem, in the northwest of Algeria, located at latitude 35°48'N, longitude 0°03'O at an altitude of 35 m. The identification was performed following Quézel and Santa (1963) [3] and confirmed by a comparison with an *Argania spinosa* (L.) Skeels specimen (054 Med) from the botanical garden herbarium at Hamma, Algiers, Algeria. A voucher specimen was deposited in the laboratory of protection and valorization of agro-biological resources, University Saad DAHLEB Blida, Algeria (Herbarium number must be provided).

The study was related to the oil extracted from the seeds of *A. spinosa* fruits. The mature fruits were harvested, washed thoroughly and dried in shade. The dried pulp was separated from the nut by simple manual crushing. The removed nuts were crushed in the traditional way, and the resulting seeds were dried in an oven at 40 °C for 72 h. The study was performed on the seeds ground directly after drying. The extraction was performed with hexane in an ultrasonic bath for 30 minutes and the solvent was evaporated to obtain the oil ready for use.

2.2. Chemical analysis of A. spinosa seeds oil

2.2.1. Fatty acids composition

The argan oil was used for transesterification reactions according to the ISO draft standard [16]. Fatty acids in argan oil were transesterified in 5 mL of methanolic solution of BF_3 and 1 mL of 10 N KOH in a 90 °C water bath for 5 min. The mixture was cooled and 1 mL of n-hexane and 2 mL of saturated solution of NaCl were then added and mixed. The apolar layer was separated and injected into GC-MS.

2.2.2. *Fatty acids methyl ester analysis by the GC-MS method:*

GC-MS analysis for fatty acid methyl esters (FAME) of argan oil was performed on an Agilent 7890 A System gas chromatography coupled with an Agilent MSD-5975 CVL. A HP-5MS capillary column (5% phenyl methylsiloxane) with dimensions of 30 m × 0.25 mM i.d. × 0.25 μM film thickness (Agilent Technologies, Palo Alto, CA, USA) was used for the separation of FAMEs. The initial temperature of 150 °C was maintained for 2 min then raised to 240 °C at a rate of 4 °C/min, and kept at 240 °C for 10 min. Helium was used as a carrier gas with a flow rate of 1 ml/min. Injection was performed in splitless mode; injector and detector temperatures were 240 and 260 °C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in a scan range of 50–550 m/z. Identification of the fatty acids was carried out by comparison of their relative retention times and MS fragmentation with those of standard fatty acid methyl esters (C14–C22) (Sigma-Aldrich). The GC-MS chromatogram was also compared with two libraries (NIST & Wily), which provided the best information about the identification of the fatty acids.

2.2.3. Determination of Total polyphenols

The liquid–liquid extraction method was used to extract the phenolic compounds from the argan oil. One gram of sample was added to 1 mL of n-hexane and 2 mL of methanol 60 % (v/v). The mixture was subjected to agitation for 30 min at room temperature in darkand then centrifuged. The supernatants (n-hexane) underwent three successive extractions repeating the centrifugation process. The residual

portions were washed with n-hexane and the solvent was evaporated to dryness using a rotary evaporator and finally the remaining was reconstituted in 1 mL of pure methanol and stored at −20 °C.

Total polyphenols were determined using Folin–Ciocalteu reagent, following the literature procedure [17]. Gallic acid was used as a standard for the calibration curve and the measurements were carried out at 725 nm using a UV–Vis spectrophotometer (Shimadzu UV 1650, Italy). The results were expressed as milligrams of gallic acid equivalent per gram of oil (mg GAE/g of oil).

2.2.4. Polyphenols analysis by the UPLC – ESI – QTOF–MS method:

Identification of the polyphenolic acids in the extract was performed using an Acquity Ultra Performance LC system (UPLC) with binary solvent manager (Water Corporation, USA) and a Micromass Q-Tof spectrometer (Waters, UK), equipped with an electrospray ionization (ESI) source operating in positive mode.

Chromatographic separation was carried out on a UPLC HSS T3, C18 column (1.8μM, 2.1×100 mM, Waters Corporation) at 30 °C. The mobile phase was composed of Solvent (A) (0.1% formic acid, v/v) and Solvent (B) (100% methanol). The gradient used was 0–5 min, 0–10% B; 5–10 min, 10–15% B; 10–15 min, 15–20% B; 15–20 min, 20–30% B; 20–30 min, 30–40% B; 30–40 min, 40–0% B. The solvent flow rate was 0.4 mL/min. The injection volume was 2μL. Analysis was performed at 280 nm and MS scanning was from *m/z* 50 to 1500. Analysis was achieved at positive ion mode, having an electrospray source with a block temperature of 120 °C and a desolvation temperature of 500 °C, a capillary voltage of 1 kV and a cone voltage of 20 eV. Nitrogen was used as a nebulizing and collision gas.

For instrument control, MassLynx software (Version 4.1) was used for data acquisition. The experimental exact masses and MS fragmentation data were compared to metabolomics databanks (ReSpect for Phytochemicals and Mass Bank) and the available literature in order to identify the nature of the phenolic compounds.

2.3. Animal Experiments

NMRI mice and New Zealand white rabbits of both sexes were used in this study. All the animals were housed at the animal care center of the pharmaco-toxicology laboratory, antibiotic complex of SAIDAL, Medea, Algeria, having standard environmental conditions of 22 ± 3 °C temperature, 60 ± 5% humidity and a 12 hours light/dark cycle.

All the experimental protocols were performed with the approval of a veterinary doctor. Food pellets and water were provided *ad libitum* throughout the experiment. No topical or systematic therapy was given to the animals. The experiments were performed in accordance with the guidelines of the Federation of European Laboratory Animal Science Associations (FELASA), required by Directive 2010/63/EU for animal experiments.

2.4. Topic anti-inflammatory test

The mice model (n = 15, 22.5±2.5 g, 2 months old, both sexes) were equally assigned to three groups, group I, group II and group III. A dose of 0.5 mg Indometacine® dissolved in 1 mL acetone was applied to the right ear of the mice in group I. Those in group II received argan seed oil on the right ear and those in group III received physiological saline solution on the right ear. Each mouse received 10 μL of croton oil 5% (croton oil 1 mL, acetone 19 mL), applied to the right and left ears, and 20 μL of Indometacine®, argan oil and physiological saline solution were applied to the right ear of each mouse from groups I, II and III, respectively. The left ear of each mouse was used as a control [18, 19]. After 4 hours, mice were sacrificed using ether, and a 6 mm-diameter sections of the right and left ears were cut and weighed. Topical anti-inflammatory activity (*TA*) was evaluated according to the following equation:

$$\%TA = [(W_L - W_R)/W_L] \times 100$$

W_L: Value of the weight of the left ear (control untreated)

W_R: Value of the weight of the right ear (treated)

2.5. Skin toxicity test

Rabbit models (*n* = 3, 2.5±0.4 kg, 4 months -old, both sexes) were used to evaluate skin toxicity. Rabbits are suitable test animals for this study since the results could be of value in predicting the likely skin irritancy potential of the test material for humans [20].

For skin preparation, back legs of each rabbit was shaved (5 cm×5 cm) without causing irritation to the skin. The animals were left for 24 hours and then only those with healthy and glabrous skin were selected for the tests. The location of all the treatment sites on the two members' hindquarters was ensured that all the materials were applied to skin of identical histological structure. The right flank was scarified by three superficial parallel incisions, 2.5 cm long and 0.5 cm apart, using a sterile vaccination lancet. The incisions were restricted to the epidermis and if the dermis was damaged and bleeding occurred, the animal was discarded. The left flank skin was intact.

The rabbits were treated with 1 mL of argan oil on the right flank (scarified) and 1 mL on the left flank (nonscarified). The argan oil was applied once a day for 3 days. Twenty-four and 72 hours after the first application, the primary skin irritation was assessed in the group.

Each treated area was scored for erythema and edema using a numerical system (O–4 in each case according to severity) described by Draize et *al*. (1944) [21]. The scores obtained for erythema and edema at both of the treated sites in all three animals at the two reading times were totaled. The sum obtained was then divided by the total number of readings to provide a mean score termed the "primary cutaneous irritation" (PCI) index. For this purpose, the total number of readings (erythema + edema) corresponding to the two application sites on three animals at 24 and 72 h were evaluated. The irritancy of each test substance was then defined on the basis of the PCI, using classification of irritancy on the following scale: PC1 below 0.5, nonirritant; 0.5–2, slightly irritant; 2–5, moderately irritant; 5–8, severely irritant.

2.6. Wound healing test

2.6.1. SPORTSs Circular excisions creation and the treatment

Back sites of the mouse models (*n* = 15, 22.5±2.5 g, 2 months old, both sexes) were shaved (5 cm×5 cm) and anesthetized by intramuscular injection of Calmivet® (Acepromazine 0.5%; 0.5 mg/kg, i.m.). The shaven surfaces were cleaned with 70% ethanol. A 2 cm circle was drawn on the shaved area, the mice were anesthetized by cutaneous injection of Xylocaïne and the tissue of the drawn circle was excised using a sterile surgical blade [22]. Next, the mice were equally assigned to three groups, group I, group II and group III. The mice in group I were treated topically with 1g of the reference drug Madecassol® (Bayer) [23], which is indicated for the treatment of various kinds of wounds, containing 1% plant extract of *Centalla asiatica*. Those in group II were treated with 1ml of argan oil, while those in group III were treated with 1 mL of physiological saline solution and used as a negative control. The treatment was applied immediately after the excision and continued every 24 h for 12 days. The animals were housed individually in sterile cages. Wound surface area was measured after the wound excision (day 0) and on days 6 and 12 by tracing its contour using a transparent paper before the treatment application. To determine wound closure kinetics, the area (mm^2) within the boundary was measured planimetrically. The percentage wound closure was calculated according to the following equation [22]:

$$\% \text{ wound closure} = [(S_0 - S_n) / S_1] \times 100$$

S_1: Surface wound on day 0 of treatment.

S_n: Surface wound after *n* days of treatment, *n* = 6 and 12.

2.6.2. Linear incision wounds creation and the treatment

The mouse models (*n* = 15, 22.5±2.5 g, 2months old, both sexes) were anesthetized by intramuscular injection of Calmivet® (Acepromazine 0.5%;0.5 mg/kg, i.m.). The 5x5 cm shaven surfaces were cleaned with 70% ethanol.

At the shaved area, the mice were anaesthetized by coetaneous injection of Xylocaïne, the "linear incisions" were incised on the back site of the mice following the protocol [22]. Wounds, 3 cm×0.5cm (length × depth), were induced using a surgical scalpel and closed with stitches. The mice with "linear

incision" wounds were equally assigned to three groups, group I, group II and group III. The mice in group I were treated topically with 0.5 g of the reference drug Madecassol® (Bayer), which is indicated for the treatment of various kinds of wounds, containing 1% plant extract of *Centalla asiatica* [23]. Those in group II were treated with 0.5 ml of argan oil, while those in group III were treated with 0.5 mL of physiological saline solution and used as a negative control. The treatment was applied immediately after the incision and continued every 24 h for 12 days. The animals were housed individually in sterile cages.

The evolution of the linear incision healing is followed by using the tool for evaluation of the wounds of Bates-Jensen (BWAT, Bates Jensen Wound Assessment Tool) (Table 1) [24]. The eight item descriptors used to mark the wound features are summarized in Table 1. Each feature was scored on a five-point scale. A total wound severity score can be derived by adding the individual item scores. The wound was visually examined and scored after the wound excision (day 0) and on days 6 and 12 by a single observer.

Table 1. Item descriptors, according to the Bates–Jensen Wound Assessment Tool (BWAT), used to assess the wound features and their score scheme were summarized [24].

Item descriptor	1	2	3	4	5
Edges	Indistinct, diffuse, no clearly visible outlines	Distinct, outline clearly visible, attached, even with wound base	Well-defined, not attached to wound base	Well-defined, not attached to base, rolled under, thickened	Well-defined, fibrotic, scarred, or hyperkeratotic
Undermining	None present	Undermining < 2 cm in any area	Undermining 2–4 cm involving <50% wound margins	Undermining 2–4cm involving > 50% wound margins	Undermining > 4 cm or tunneling in any area
Exudate type	None	Bloody	Serosanguineous: thin, watery, pale red/pink	Serous: thin, watery, clear.	Purulent: thin or thick, opaque, tan/yellow, with or without odor
Exudate amount	None, dry wound	Scant, wound moist but no observable exudates	Small	Moderate	Large
Skin color surrounding Wound	Pink or normal for ethnic group	Bright red and/or blanches to touch	White or grey pallor or hypopigmented	Dark red or purple and/or non-blanchable	Black or hyperpigmented
Peripheral tissue edema	No swelling or edema	Non-pitting edema extends < 4 cm around wound	Non-pitting edema extends > 4 cm around wound	Pitting edema extends < 4 cm around wound	Crepitus and/or pitting edema extends > 4 cm
Peripheral tissue Induration	None present	Induration, < 2 cm around wound	Induration 2–4cm extending < 50% around wound	Induration 2–4cm extending > 50% around wound	Induration > 4 cm in any area around wound
Epithelialization	100% wound covered, surface intact	75–100% wound covered and/or epithelial tissue extends > 0.5cm into wound bed	50–75%wound covered and/or epithelial tissue extends to 0.5cm into wound bed	25–50% wound covered	< 25% wound covered

Each feature was scored on a five-point scale. A score of 5 represents the most severe outcome for a particular feature, with 1 representing the least severe. A total wound severity score can be derived by adding the individual item scores.

2.6.3. Histological examinations

Skin specimens on the 12th day post-wounding from wound-healing tests on each mouse were excised and fixed in 10% neutral buffered formalin, processed and blocked with paraffin. Six-micrometer sections were cut, stained with hematoxylin-eosin and observed for histopathological changes under a microscope. A representative area was selected for descriptive light microscopic analysis (Nikon, Tokyo, Japan) at 100x and 400x magnifications.

2.7. Statistical analysis

Results were shown as mean ±S.D. The data on percentage anti-inflammatory and wound-healing were statistically analyzed using a variance analysis (ANOVA) and the paired Student's *t*-test. The values of $P \leq 0.05$ were considered statistically significant.

Histopathologic data were considered to be nonparametric; therefore, no statistical tests were performed.

3. Results

3.1. Extraction

The proportions of argan oil extracted as described previously were show that the seeds contained about 37.84% of oil (w / w).

3.2. Chemical analysis of Argania spinosa seed oil fatty acids and polyphenols

The results of the *GC-MS* analysis are shown in Table 2. The main components were found to be omega-9 monounsaturated (oleic acid) and omega-6 polyunsaturated (linoleic acid), which constituted respectively 44% and 35% of the argan seed oil. Other significant components were palmitic acid (14.16%) and stearic acid (4.16%) (Supporting information).

Table 2. Analysis of argan seed oil fatty acids.

Fatty acid	***C:D***	***%***
Myristic	14:0	00.42
Palmitic	16:0	14.17
Stearic	18:0	04.16
Oleic	18:1	44.00
Linoleic	18:2	35.69
Arachidonic	20:4	00.20
Behenic	22:0	00.07
Total		**98.71**
Unidentified compound (UC)	***m/z***	***%***
UC 1	149	00.56
UC 2	74	00.62

(C:D = Carbon number: Double bond)

The total phenolic content in argan oil, measured by the Folin- Ciocalteu method, had an estimated level of 58.57 mg / kg of oil.

Examination of the chromatograms in *TOF-MS* mode of phenolic extract of argan oil and comparison of the experimental exact masses and MS fragmentation of each compound with metabolomics data banks and available literature revealed the presence of coumaric (*m/z* 164.98) [25], caffeic (*m/z* 180. 98) [26] and vanillic (*m/z* 168) [27] acids. MS of these ions showed the protonated molecule $[M\_H]^+$ as a characteristic ion. The analysis in TOF-MS mode also indicated the presence of hydroxybenzoic acid (*m/z* 139.12) [28] and demethyloleuropein (*m/z* 527.18) [28].

3.3. Anti-inflammatory test

The argan oil and the reference product Indometacine® caused a reduction of the inflammation (Figure1) through an inhibition of edema formation in the ears of treated mice compared to the control mice (untreated). The percentage of edema reduction was calculated to have a rate of 39.68% for the group treated with the argan oil and 38.31% for the group treated with the reference product (Indometacine®), whose anti-inflammatory activity is already known. However, the negative control group showed a rate of 13.3%. The statistical Student's *t*-test revealed no significant difference between

the effect of the oil tested and that of the reference product. According to these results, we deduced that argan oil has an excellent anti-inflammatory activity, whose efficiency is similar to marketed anti-inflammatory drugs.

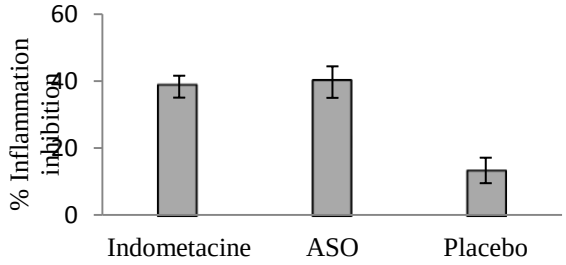

Figure 1. Percent reduction of inflammations treated with Indometacine®, argan seed oil (ASO) and saline solution (Placebo)

3.4. Skin toxicity test

The dermal toxicity test of argan oil did not show significant signs of skin irritation. This was confirmed by the calculated primary cutaneous irritation CPI index, which is equal to 0.

From the CPI values obtained, argan oil was classified as nonirritant according to the conventional DRAIZE standards. Accordingly, the studied oil could be used in topical treatments.

3.5. Wound-healing

3.5.1. Circular excision healing test

The figures showed that the reduction of the surfaces of the wounds after 6 days of treatment with argan oil and Madecassol® is much more significant than that of group III treated with physiological saline solution. It was noted that the latter developed very serious edemas in contrast to the former one. After 12 days of treatment, the wounds treated with the argan oil were almost closed, followed by those treated with Madecassol®, compared to those treated with the physiological saline solution (Figure 2).

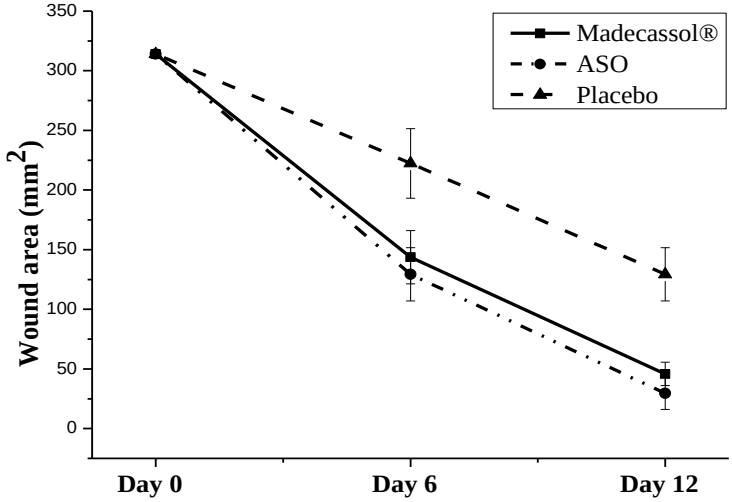

Figure 2. Evolution of wound area excisions treated with Madecassol®, argan seed oil (ASO) and saline solution at days 0, 6 and 12

According to Figure 3 and Figure 4, the group treated with the argan oil presented a high surface reduction on the 6th and 12th days with rates of 63.6% and 90.6%, respectively, followed by the commercial product with surface reduction rates of 56.2% for the 6th day and 85.4% on the 12th day and the physiological saline water, which showed highly reduced rates (6th day: 29.2%, 12th day: 58.8%).

The comparative Student's *t*-test between the group treated with the argan oil and the group treated with Madecassol® proved a similar effect for the two substances.

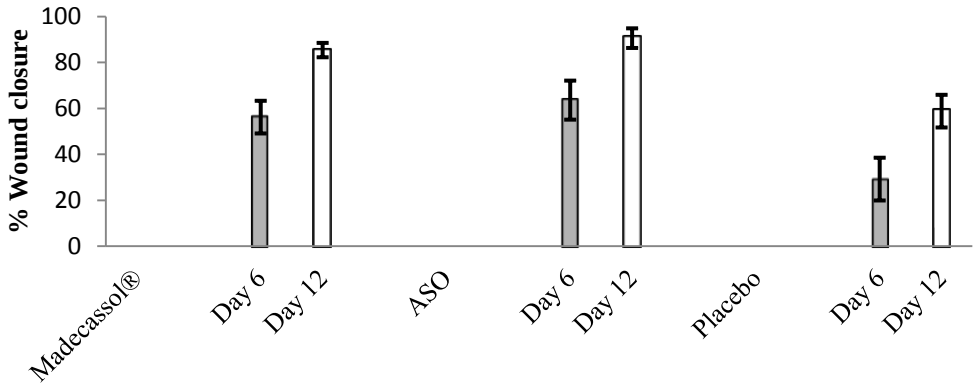

Figure 3. Percent closure of excisions treated with Madecassol®, argan seed oil (ASO) and saline solution at 6th and 12th day

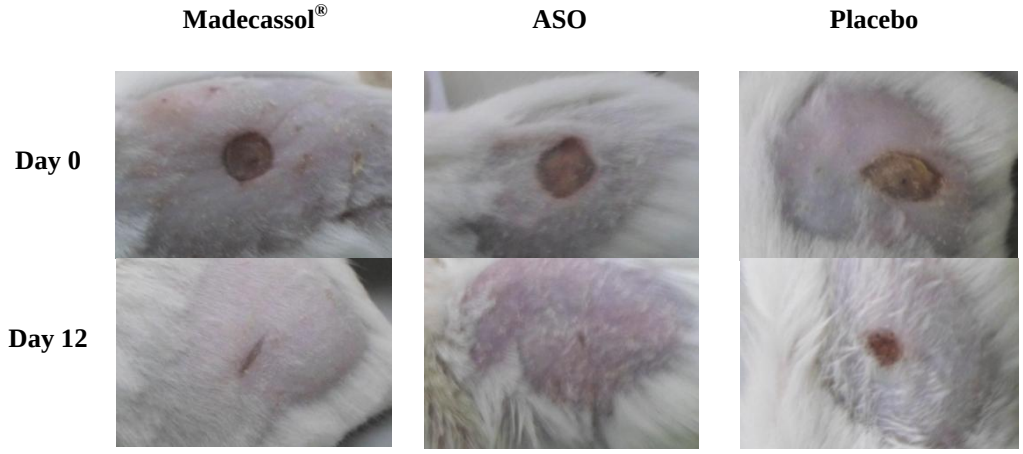

Figure 4. Evolution of wound-healing of excisions treated with Madecassol®, argan seed oil (ASO) and saline solution (Placebo) at days 0 and 12

3.5.2. Linear incision healing test

Effects of the argan oil and the Madecassol® presented by the scores according to the tool for the evaluation of wounds of Bates-Jensen (BWAT) for the days 0, 6 and 12 were almost identical (Figure 5) (no significant difference according to the Student's *t*-test). Moreover, no bleeding symptom of exudates or serious swelling was observed.

The evolution of the healing of incisions (Figure 6) was clearly better in the group treated with argan oil and the reference product than in the negative control group, in which the wounds were characterized by the presence of signs of cure delay, i.e. a light bleeding, a significant edema and granulous and thick crust (significant difference between the three groups according to the ANOVA test).

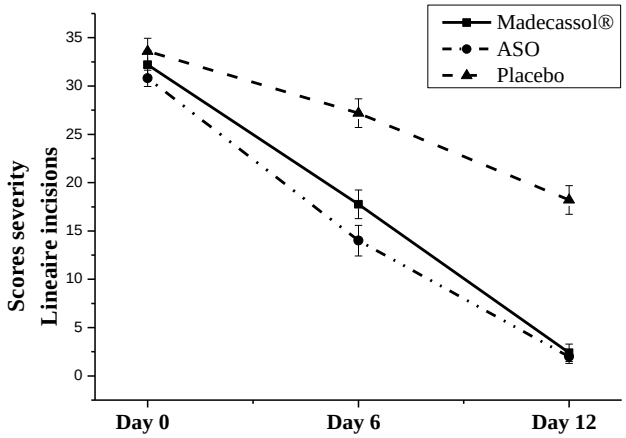

Figure 5. Score severity of incisions treated with Madecassol®, argan seed oil (ASO) and saline solution (Placebo) according to Bates–Jensen Wound Assessment Tool at days 0, 6 and 12.

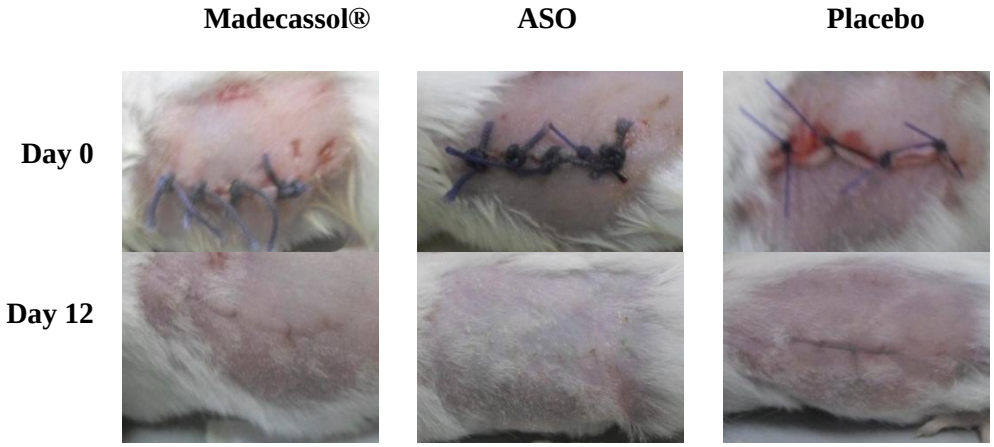

Figure 6. Evolution of wound healing of incisions treated with Madecassol®, argan seed oil (ASO) and saline solution (Placebo) at days 0 and 12.

3.5.3. Histological examinations

After 12 days of the treatment, a histology study of the skin specimens with incision wounds revealed (Figure 7) cutaneous coating with atrophic skin and a discrete collagenous fibrosis on the derma surface without inflammatory infiltrate for the groups treated with the argan oil and the Madecassol®, while the negative control group showed a collagenous fibrosis site on the derma surface, a scar home and a discrete nonspecific inflammation to the hypoderm.

The wounds excision test of the group treated with the argan oil and Madecassol® (Figure 8) showed the presence of collagenous fibrosis sites in the derma, which is a small scar home extending from the derma surface to the hypoderm consisting of discreetly congestive collagenous fibrous fabrics and sites of a lymphocytary inflammatory infiltrate with the presence of some polynuclear neutrophils. The negative control group showed a derma site of collagenous fibrosis with a broad scar extending to the hypoderm with lymphocytary inflammatory infiltrate in the deep derma and the hypoderm. This was edematous and contained dilated capillary vessels and a micro-abscess.

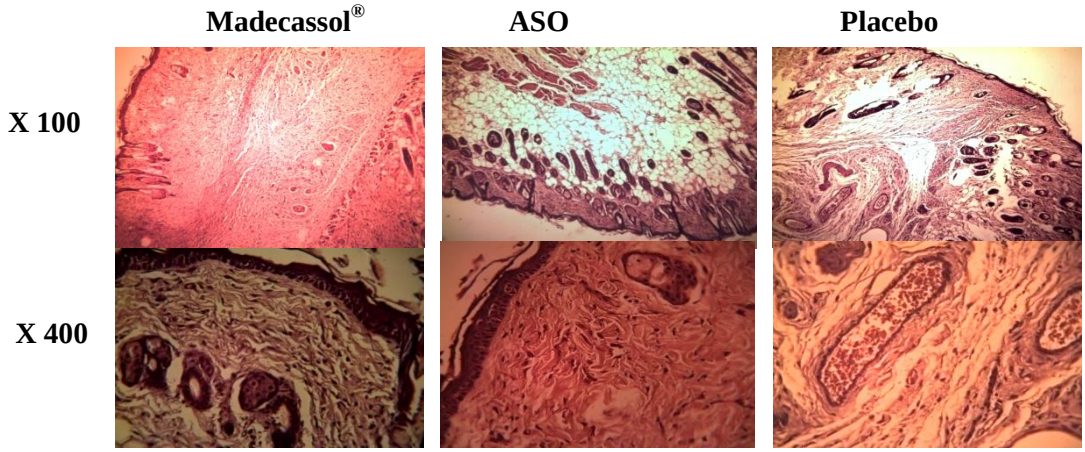

Figure 7. Histological view of Hematoxylin and Eosin (HE) stained skin sections of excision wound healing test of mice treated with argan seed oil (ASO), Madecassol® and saline solution (Placebo) at x100 and x400 magnification.

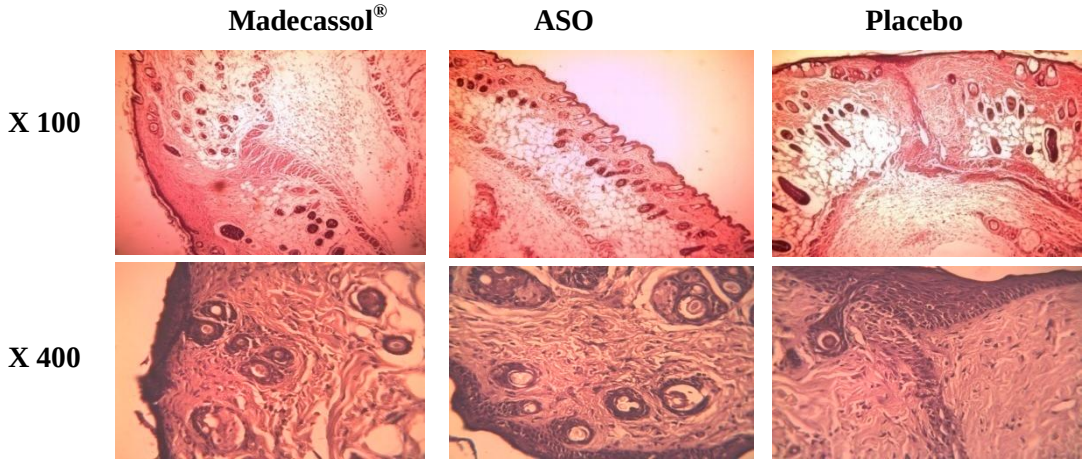

Figure 8. Histological view of Hematoxylin and Eosin (HE) stained skin sections of incision wound healing test of mice treated with argan seed oil (ASO), Madecassol® and saline solution (Placebo) at x100 and x400 magnification.

4. Discussion

Wound-healing is a complex alignment of different dynamic processes that are not yet fully understood. It is characterized by a complex multicomponent cascade of biosynthetic and degradative reactions, and this natural phenomenon included some molecular and cellular events in an ordered and well-coordinated process, involving inflammation, which plays an important role in fighting infection and inducing the proliferation phase necessary for healing [29]. Inflammation, which requires the migration of neutrophils and macrophages to wound areas, should occur rapidly to allow the development of subsequent phases, which are granulation tissue formation, fibrogenesis, neovascularization, wound contraction and resurfacing of the wound defect with the epithelium in the later stage of healing [30, 31].

The use of natural drugs has increased throughout the world, especially in underdeveloped countries, where more sophisticated and expensive therapies are not always available. This therapy has a great appeal for the maintenance and improvement of health and for treating various human conditions and diseases.

Many natural drugs have been found to be useful in treatment of wounds since ancient times. These drugs work in several ways. Some have anti-inflammatory and antiseptic properties. Others are astringent and help tissues contract to control the bleeding [32].

This study consists of preliminary tests to determine, for the first time, the effects of the oil extracted from the seeds of *Argania spinosa* (L.) Skeels in reducing inflammation and wound-healing.

In the present study, no experimental rabbits, administered with argan oil, showed any adverse effects in skin toxicity studies; accordingly argan oil can be used in topical treatments.

In the anti-inflammatory test, untreated mice ears showed an important edema reflecting persistent inflammation, whereas ears treated with argan oil and Indometacine® showed reduced edema. However, the tests of wound incisions and excisions showed that the seeds used for argan oil were found to possess excellent wound-healing activity comparable to the Madecassol® effect.

It should be noted that argan oil is increasingly used for skin care. it possesses many characteristics that make it an appealing target for investigation in wound therapy. Recently, a study [33] confirmed that this oil healed more expediently second-degree burns in rats than silver sulfadiazine, characterized by a high TGF-β1 expression and wound closure. Moreover, it can potentially act as an antimicrobial agent due to its abundant content of monounsaturated and polyunsaturated fatty acids with a rate exceeding 80% [34], such as oleic acid (50%) and linoleic acid (30%) [35], having antimicrobial properties [36] and as argan oil can isolate the wound area from the exterior environment. However, none of the wounds in the current study became infected.

The main fatty acids identified by gas chromatography in our study are omega-9 (44%) and omega-6 (35%), known to play a role at wound sites and, thus, reported to have therapeutic potential in cutaneous wound healing [37, 38].

Some studies proposed that administration of these fatty acids accelerates and reduces the response of the inflammatory phase of wound healing through improving cutaneous wound healing by decreasing nuclear factor B (NFB), downregulating cyclooxygenase-2 expression, reducing inflammatory response [39, 40], decreasing mononuclear cell number and tumor necrosis factor (TNF) levels in wound fluid [41] and activating immunity system cells like neutrophils and macrophages[42, 43].

Phenolic compounds were also found in argan oil with a rate of 58.57 mg/kg, identified by liquid chromatography. The findings showed the presence of phenolic acids (coumaric, caffeic, hydroxybenzoic and vanillic acids) and demethyloleuropein, which are known as biologically active ingredients in treatment of wounds, particularly due to their antimicrobial, anti-inflammatory and very strong antioxidant actions. In fact, during wound healing, the wound site is rich in oxidants, such as hydrogen peroxide, mostly contributed by neutrophils and macrophages. The wound-healing effect of phenolic compounds has been attributed to their antioxidant activity. They are able to reduce oxidative stress as they have capacity to donate hydrogen or electrons and scavenge free radicals, reduce lipid peroxidation and inflammatory damages, promote the proliferation of fibroblast adhesion during wound-healing and stimulate the re-epithelialization, neovascularization and maturation of extracellular matrices [44-47,].

Tocopherols, b-carotene and carotenoids are the other compounds present in argan oil that could accelerate the wound-healing process [48, 49].

5. Conclusion

The present study indicates that argan seed oil has significant anti-inflammatory and wound-healing activities in full-thickness wounds and is found to be safe for use.

The wound-healing potential of argan seed oil could be due to the presence of omega-9 and omega-6 fatty acids and polyphenolic compounds like coumaric, caffeic, benzoic and vanillic acids and demethyloleuropein. This appears to be related to their antioxidant and antibacterial properties and their ability to stimulate the immune system while reducing the inflammatory response.

These results provide support for the promising candidacy of *Argania spinosa* L. seed oil for application as a therapeutic agent for dermal wound-healing. Nevertheless, this study is the first to report on the beneficial effects of argan oil in topical applications such as wound-healing, using an animal

model. Future studies are warranted to explore the underlying mechanisms of the salutary effects of argan oil treatment.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

[1] J. F. Morton and G.L. Voss (1987). The argan tree (*Argania sideroxylon, sapotaceae*). A desert source of edible oil, Econ. Bot. **41**, 221-233.

[2] M. Alifriqui (2004). L'écosystème de l'Arganier, Etude réalisée à la demande du Programme des Nations Unies pour le développement, PNUD, Maroc.

[3] P. Quezel and S. Santa (1963). Nouvelle flore de l'Algérie et des régions désertiques méridionales, Vol. II, Ed. Centre National de la Recherche Scientifique, Paris.

[4] O. Mhirit and P. Blero (1999). Le grand livre de la forêt marocaine, Mardaga, Sprimont, Belgique.

[5] Z. Charrouf and D. Guillaume (1998). Ethnoeconomical, ethnomedical, and phytochemical study of *Argania spinosa* (L.) Skeels : A Review, J. *Ethnopharmacol.* **67**, 7-14.

[6] H. Bennani, A. Drissi, F. Giton, L. Kheuang, J. Fiet and A. Adlouni (2007). Antiproliferative effect of polyphenols and sterols of virgin argan oil on human prostate cancer cell lines, *Cancer Detect. Prev.* **31**, 64–69.

[7] F. Khallouki, C. Younos, R. Soulimani, T. Oster, Z. Charrouf, B. Spiegelhalder, H. Bartsch and R.W. Owen (2003). Consumption of argan oil (Morocco) with its unique profile of fatty acids, tocopherols, squalene, sterols and phenolic compounds should confer valuable cancer chemopreventive effects, *Europ. J. Cancer Prev.* **12**, 67-75.

[8] H. Berrougui, M. Cloutier, M. Isabelle and A. Khalil (2006). Phenolic-extract from argan oil (*Argania spinosa* L.) inhibits human low-density lipoprotein (LDL) oxidation and enhances cholesterol efflux from human THP-1 macrophages, *Atherosclerosis* **184**, 389–396.

[9] A. Moukal (2004). L'arganier, *Argania spinosa* L. (skeels), usage thérapeutique, cosmétique et alimentaire, *Phytothérapie* **2**, 135-141.

[10] A. Adlouni (2010). L'huile d'argan, de la nutrition à la santé, Phytothérapie **8**, 89-97.

[11] N. El Alaoui (1999). Paysages, usages et voyages *d'Argania spinosa* (L.) Skeels (IXe-Xe siècles), *J.A.T.B.A*. **41**,45-79.

[12] J. Belakhdar (1997). La pharmacopée marocaine traditionnelle, Médecine arabe ancienne et savoirs populaires, Ibis Press, France.

[13] M. Berrada (1972). Etude de la composition de l'huile d'argan, *Al Awamia* **42**, 1–14.

[14] T. Rezanka and H. Rezanková (1999). Characterization of fatty acids and triacylglycerols in vegetable oils by gas chromatography and statistical analysis, *Anal. Chim. Acta*. **398**, 253–26.

[15] M. Farines, J. Soulier, M. Charrouf and R. Soulier (1984). Etude de l'huile des graines d'*Argania spinosa* (L.); Sapotaceae. I. La fraction glycéridique, *Rev. Fr. Corps Gras* **31**, 283–286.

[16] International standard organization ISO 5509 (2000). Animal and vegetable fats and oils – Preparation of Methyl Esters of Fatty Acids, International standard organization, Geneva.

[17] L.Yu, J. Perret, M. Harris, J. Wilson and S. Haley (2003). Antioxidant properties of bran extracts from ''Akron'' wheat grown at different locations, *J. Agric. Food. Chem*. **51**, 1566–1570.

[18] C. Delporte, N. Backhouse, P. Salinas, A. San-Martín, J. Bórquez and A. Loyola (2003). Pharmaco-toxicological study of new diterpenoids, *Bioorgan. Med. Chem*. **11**, 1187–1190.

[19] G. Venzuela-Barra, C. Castro, C. Figueroa, A. Barriga, X. Silva, B. de las Heras, S. Hortelano and C. Delport (2015). Anti-inflammatory activity and phenolic profile of propolis from two locations in Región Metropolitana de Santiago Chile, *J. Ethnopharmacol*. **168**, 37–44.

[20] Organization for Economic Cooperation and Development OECD (2002). Guidelines for the testing of chemicals, No.404 acute dermal irritation / corrosion, Organization for Economic Cooperation and Development, Paris.

[21] J.H. Draize, G. Woodard and H.O. Calvery (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes, *Pharmacol. Exp. Therap*. **82**, 377-390.

[22] A. Mekonnen, T. Sidamo , K. Asres and E. Engidawork (2013). In vivo wound healing activity and phytochemical screeningof the crude extract and various fractions of Kalanchoe petitiana A. Rich (Crassulaceae) leaves in mice, *J. Ethnopharmacol*. **145**, 638–646.

[23] N. Eruygur, G. Yılmaz, O. Kutsal, G. Yücel and O. Üstün (2016). Bioassay-guided isolation of wound healing active compounds from *Echium* species growing in Turkey, *J. Ethnopharmacol*. **185**, 370-376

[24] Y. Jia, G. Zhao and J. Jia (2008). Preliminary evaluation: The effects of *Aloe ferox Miller* and *Aloe arborescens Miller* on wound healing, *J. Ethnopharmacol*. **120**,181–189.

[25] T. Aikaterini, K. Panagiotis and K. Eugene (2008). Analytical, Nutritional and Clinical Methods LC–DAD–MS (ESI+) analysis of the phenolic content of *Sorbus domestica* fruits in relation to their maturity stage, *Food Chem*. **106**, 1234–1245.

[26] H.X. Cheng, Y.C. Zeng, B.Y. Jia, C.F. Fang and W.M. Cheng (2012). Screening and identification of various components in Thalictrum fortunei using a combination of liquid chromatography/time-of-flight tandem mass spectrometry, *Pharmazie* **67**, 106–110.

[27] L.L. Saldanha, W. Vilegas and A.L. Dokkedal (2013). Characterization of flavonoids and phenolic acids in *Myrcia bella* Cambess. using FIA-ESI-IT-MSn and HPLC-PAD-ESI-IT-MS combined with NMR, *Molecules* **18**, 8402-8416.

[28] T. Jerman, P. Trebše and B. M. Vodopivec (2010). Ultrasound-assisted solid liquid extraction (USLE) of olive fruit (*Olea europaea*) phenolic compounds, *Food Chem*. **123**, 175–182.

[29] P. Beldon (2010). Basic science of wound healing, Surgery **28**, 409–412.

[30] R.N. Wysocki and B. Annette (1989). Surgical Wound Healing, A review for perioperative nurses, *A.O.R.N. Journal* **49**, 502-524.

[31] T. Wild, A. Rahbarnia, M. Kellner, L. Sobotka and T. Eberlein (2010). Basics in nutrition and wound healing, *Nutrition* **26**, 862–866.

[32] M. Raj and T. Luc (2011). The Basic Needs to Achieve Wound Healing, First ed. Jaypee Brothers Medical Publishers, India.

[33] U. Avsar, Z. Halici , E. Akpinar , M. Yayla , U. Avsar , U. Harun, U. Harun , A. Hasan Tarik and Z. Bayraktutan (2016). The Effects of Argan Oil in Second-degree Burn Wound Healing in Rats, *Ostomy Wound Manage*. **62**, 26-34.

[34] H. Chimi, J. Cillard and P. Cillard (1994). Autoxydation de l'huile d'argan (*Argania spinosa*) sapotacée *du Maroc, Sci. Aliment*. **14**, 117–124.

[35] H. Harhar, S. Gharby, B. Kartah, D. Pioch, D. Guillaume and Z. Charrouf (2014). Effect of harvest date of *Argania spinosa* fruits on Argan oil quality, *Ind. Crop. Prod*. **56**, 156–159.

[36] J.J. Kabara, D.M. Swieczkowski, A.J. Conley and J.P. Truant (1972). Fatty acids and derivatives as antimicrobial agents, *Antimicrob. Agents Chemother*. **2**, 23-8.

[37] C.R. Cardoso, M.A. Souza, E.A. Ferro, S. J. Favoreto and J.D. Pena (2004). Influence of topical administration of n-3 and n-6 essential and n-9 nonessential fatty acids on the healing of cutaneous wounds, *Wound Repair Regen*. **12**, 235–243.

[38] H.G. Rodrigues, M.A. Vinolo, J. Magdalon, K. Vitzel, R.T. Nachbar and A.F. Pessoa (2012). Oral administration of oleic or linoleic acid accelerates the inflammatory phase of wound healing, *J. Invest. Dermatol.* **132**, 208–215.

[39] C.R. Cardoso, S. J. Favoreto, L.L. Oliveira, J.O. Vancim, G.B. Barban and D.B. Ferraz (2011). Oleic acid modulation of the immune response in wound healing: a new approach for skin repair, *Immunobiology* **216**, 409–415.

[40] N.Y. Park, G. Valacchi and Y. Lim (2010). Effect of dietary conjugated linoleic acid supplementation on early inflammatory responses during cutaneous wound healing, *Mediators Inflamm*. **17**, 1–8.

[41] D.N. Linz, V.F. Garcia, G. Arya and M.M. Ziegler (1994). Prostaglandin and tumor necrosis factor levels in early wound inflammatory fluid: effects of parenteral omega-3 and omega-6 fatty acid administration, *J. Pediatr. Surg*. **29**, 1065–1069.

[42] H.G. Rodrigues, M.A. Vinolo, J. Magdalon, H. Fujiwara, D.M. Cavalcanti and S.H. Farsky (2010). Dietary free oleic and linoleic acid enhances neutrophil function and modulates the inflammatory response in rats, *Lipids* **45**, 809–819.

[43] P. Yaqoob and P. Calder (1995). Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages, *Cell Immunol*. **163**, 120–128.

[44] I. Ammar , S. Bardaa, M. Mzid, Z. Sahnoun, T. Rebaii, H. Attia and M. Ennouri (2015). Antioxidant, antibacterial and *in vivo* dermal wound healing effects of *Opuntia* flower extracts, *Int. J. Biol. Macromol.* **81**, 483–490.

[45] B.S. Weeks and P.P. Perez (2007). A novel vitamin C preparation enhances neurite formation and fibroblast adhesion and reduces xenobiotic-induced T-cell hyperactivation, *Med. Sci. Monit*. **13**, 51–58.

[46] Y. Ponnusamy, N. J. Chear, S. Ramanathan and C.S. Lai (2015). Polyphenols rich fraction of *Dicranopteris linearis* promotes fibroblast cell migration and proliferation *in vitro, J. Ethnopharmacol.* **168**, 305–314.

[47] E. Küpeli-Akkol, I.Süntar, H. Keleş, E. Sezik and G. Gürler (2015). Bioassay-guided isolation and characterization of wound healer compounds from *Morus nigra* L. (Moraceae), *Rec. Nat. Prod*. **9:4,**484-495.

[48] R. Shivan, S. Ramandeep, T. Preeti, K. Satinder and S. Alok (2012). Wound healing Agents from Medicinal Plants, *Asian Pac. J. Trop. Biomed*. **2**, 1910-1917.

[49] N. Zampieri, V. Zuin, R. Burro, A. Ottolenghi and F.S. Camoglio (2010). A prospective study in children: Pre- and post-surgery use of vitamin E in surgical incisions, *J. Plast. Reconstr. Aesthet. Surg*. **63**, 1474–1478.