Journal of Ethnopharmacology 176 (2015) 327–335

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Safety and efficacy of *Sclerocarya birrea* (A.Rich.) Hochst (Marula) oil: A clinical perspective

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ARTICLE INFO

Article history:
Received 29 July 2015
Received in revised form
29 September 2015
Accepted 25 October 2015
Available online 31 October 2015

Keywords:
Cosmetic
Efficacy
GC × GC–MS
Marula
Sclerocarya birrea
Safety

ABSTRACT

Ethnopharmacological relevance: Sclerocarya birrea (A.Rich.) Hochst (Marula) nut oil is a popular ingredient in cosmetics such as skin lotions, lipsticks and foundations. The demand for this African oil increased tremendously such that in 2008 almost US$ 20 million was spent on Marula oil for cosmetic product manufacturing. The ethnobotanical literature states that the Zulu people in South Africa amongst others applied the oil to maintain a healthy skin. Scientific studies to support the traditional use as well as the inclusion of Marula oil in cosmetic products is lacking. This study evaluated the irritancy potential (safety), the moisturising and hydrating effects as well occlusivity properties (efficacy) of Marula oil after topical application. In addition, the Marula oil used in this study was comprehensively characterised using two-dimensional gas chromatography coupled to mass spectrometry.

Methods and materials: Quantification of the fatty acid methyl esters (FAMEs) was done using a LECO Pegasus 4D GC × GC–MS. To determine the safety and efficacy of Marula oil healthy caucasian adult female volunteers ($n=20$) who complied with the inclusion and exclusion criteria for the irritancy patch, moisture efficacy, hydrating and occlusivity tests were recruited for each study. A 2 × magnifying lamp (visual observation), Chromameter®, Aquaflux® and Corneometer® instruments were used to evaluate and monitor the irritancy level, skin barrier function, transepidermal water loss, hydrating and occlusive effects of topically applied Marula oil.

Results: The GC × GC–MS analysis identified several saturated as well as unsaturated fatty acids. Oleic acid was the major fatty acid constituting 69.0% of the oil followed by palmitic acid (15.3%), linoleic acid (9.2%), palmitoleic acid (4.1%) and stearic acid (1.5%). The clinical study revealed that Marula oil is non-irritant ($p<0.001$), with moisturising and hydrating properties ($p<0.001$) when applied to a lipid-dry (xerosis) skin. Additionally the oil exhibited occlusive effects ($p<0.001$) when applied to normal skin. These findings may be linked to the absorption of the oil into the skin due to the high percentage of oleic acid and the presence of palmitic acid which are known to disturb the stratum corneum intercellular lipids. These fatty acids present in Marula oil are very similar to those present in the epidermis, and can be considered biomimetic.

Conclusions: Marula oil rich in fatty acids exhibits moisturising, hydrating and occlusive properties. As the oil is non-irritating and provides a moisturising effect with moderate prevention of transepidermal water loss, average moisture retention properties and noteworthy occlusive effects, its inclusion in cosmetic products based on its traditional use may be justified depending on the application.

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1. Introduction

Sclerocarya birrea (A.Rich.) Hochst (Marula), also referred to as the tree of life, is a well-known member of the Anacardiaceae family. The genus name *Sclerocarya* is derived from the Greek words for hard (*skleros*) and wallnut (*karyon*) while the specific epithet *birrea* is derived from *birr*, a common name used by the Senegalese when referring to a tree (Palmer and Pitman, 1972). The tree also forms an integral part of African cultural beliefs and practices (Wynberg et al., 2002) and as early as 1951, the Marula

Abbreviations: ANOVA, analysis of variance; CIR, cosmetic ingredient review; DNA, deoxyribonucleic acid; FAMEs, fatty acid methyl esters; GC-FID-MS, gas chromatography with flame ionisation detection coupled to mass spectrometry; GC × GC–MS, two-dimensional gas chromatography coupled to mass spectrometry; SD, standard deviation; SLS, sodium lauryl sulphate; TEWL, transepidermal water loss; ToF, time of flight

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http://dx.doi.org/10.1016/j.jep.2015.10.037
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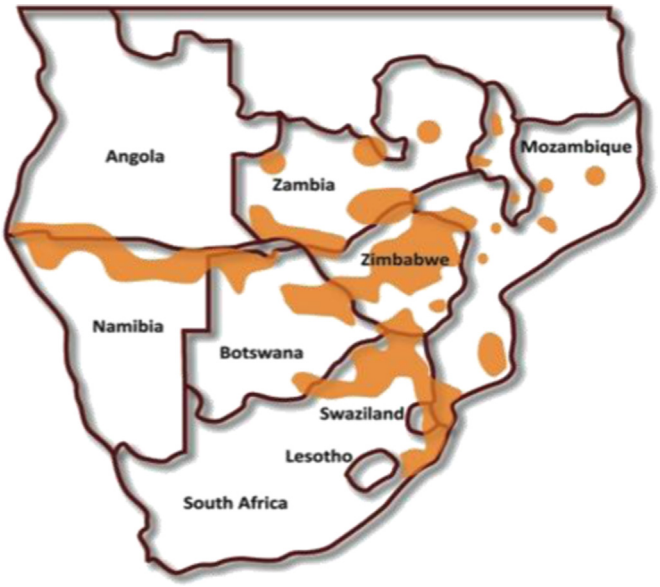


Fig. 1. Distribution map of *Sclerocarya birrea* in southern Africa.

tree was declared a protected tree of South Africa (Wehmeyer, 1966). Using pair-wise ranking, *S. birrea* emerged after *Trichilia emetica* Vahl. as one of the most preferred fruit species (Bandeira et al., 1999). This deciduous tree reaches 7–17 m in height and is commonly distributed in southern African regions namely South Africa, Zimbabwe, Botswana, Mozambique, Zambia and Namibia as illustrated in Fig. 1 (Palmer and Pitman, 1972; Johnson and Johnson, 1993; Shackleton et al., 2002; Street and Prinsloo, 2013). The white, translucent, sweet sour flesh of the fruit adheres to the hardy woody nut which contains kernels and produces quality crude oil of approximately 11% and 63.3% respectively (Salama, 1973; van Wyk, 1974; Ogbobe, 1992; Zharare and Dhlamini, 2000; Shackleton et al., 2002). The composition of Marula oil has been reported by several authors using one-dimensional gas chromatography coupled to flame ionisation detection and a mass spectrometer in some cases (GC-FID-MS). The major fatty acid was shown to be oleic acid (C18:1; 70.0–78.0%) followed by lesser amounts of palmitic (C16:0; 9.0–12.7%), stearic (C18:0; 5.0–8.0%), linoleic (C18:2; 4.0–9.5%) and linolenic (C18:3; trace − 1.1%) acids amongst others (Ogbobe, 1992; Zharare and Dhlamini, 2000; Kleiman et al., 2008; Vermaak et al., 2011). In this study, two-dimensional gas chromatograpy was used to determine the fatty acid content.

Marula is considered a multipurpose tree as the fruits are eaten fresh or fermented to make a beer, the kernels are eaten or the oil is extracted for meat preservation and for skin care, the leaves are browsed by livestock and it is used medicinally (van Wyk, 1974; Shackleton et al., 2002; Palgrave, 1972; Cunningham et al., 1992; Mabogo, 1990). A plethora of biological activities has been attributed to various plant parts of Marula including anti-diarrhoeal, anti-diabetic, anti-inflammatory, antimicrobial, antiplasmodial, antihypertensive, anticonvulsant, antinociceptive and antioxidant properties, thus lending pharmacological support to some of the traditional medicinal uses of the plant (Ojewole, 2003, 2006; Ojewole et al., 2010).

Marula is one of the many plants that have recently gained popularity as a natural ingredient in cosmetic formulations based on ethnomedicinal reports. Palgrave (1972) stated that traditionally, the Zulu people crushed the nuts and boiled them in water, thereafter the oil was skimmed off and massaged into the skin as a cosmetic. The Tsonga people of South Africa and Mozambique use Marula oil for moisturising and hydrating the skin (Botelle, 2001). Den Adel (2002) reported after a survey that in Central North Namibia, 28% of the participants stated that the household use of Marula oil was for skin moisturisation (Shackleton et al., 2002). In Botswana, Marula fruits are processed into a range of cosmetic products such as soap and oil (Motlhanka and Makhabu, 2011). In a survey conducted by Maroyi (2013) it was noted that approximately 8.6% of the participants in south central Zimbabwe used Marula oil for cosmetic benefits. It is this indigenous knowledge upon which current *S. birrea* oil commercialisation initiatives were developed (Hutchings et al., 1996; Shackleton et al., 2002). Marula oil is commonly used in cosmetics by the European industry (Poulton and Poole, 2001; Leakey et al., 2005). Additionally, an *S. birrea* cosmetic extract has been patented by PhytoTrade® Africa in partnership with Aldivia® (France) (Street and Prinsloo, 2013; Gurib-Fakim et al., 2010). Currently, community fair-trade companies supply The Body Shop® with Marula oil for cosmetic purposes (Schreckenberg, 2004; Belcher and Schreckenberg, 2007). However, to date there is insufficient published clinical evidence regarding the traditional use of Marula oil and its moisturing, hydrating and occlusivity properties (efficacy) as well as potential irritant effect (safety) when applied topically.

2. Materials and methods

2.1. Materials and sample preparation

Commercially available Marula oil (Batch number: MAR03: OCK; Product code: PCMARLB01) was purchased from Scatters Oils CC (Johannesburg, South Africa). A retention sample (TUT0017) was retained in the Department of Pharmaceutical Sciences. A fatty acid methyl esters (FAMEs) 37-component standard mix as well as pure reference standards (linoleic, oleic, palmitic, stearic, arachidic and myristic acid) were obtained from Sigma-Aldrich® (Johannesburg, South Africa). The FAMEs were prepared using the modified method of Ross and Harynuk (2010). Briefly, 310 μl of Marula oil or standards were mixed with 500 μl of 2.8 g potassium hydroxide in 100 ml of methanol solution (stock solution) and sonicated for 30 min in a water bath at 60 °C. Boron-triflouride (1000 μl) in methanol was used as a catalyst and the mixture was sonicated again for 30 min at 60 °C. Petroleum ether (1000 μl) and saturated sodium chloride was added to the mixture. Finally, the mixture was centrifuged for 5 min at 10,000 rpm at 5 °C. The supernatant was collected and injected into the GC × GC–MS (Kothiyal et al., 2010).

For the clinical component of the study, the irritancy patch test was conducted using 1% sodium lauryl sulphate (SLS) solution as a positive control (irritant) and de-ionised water as a negative control. For the moisture efficacy, hydration and occlusivity tests, liquid paraffin, Vaseline® intensive care lotion and Vaseline® petroleum jelly were used as positive controls while untreated skin was used as a negative control.

2.2. GC × GC–MS analysis

The GC × GC–MS (LECO Pegasus 4D, LECO Africa, Pretoria) was equipped with an Agilent GC (7890), Gerstel Autosampler (MPS2), a secondary oven and a dual stage modulator. Liquid nitrogen cooling was used for the cold jets and synthetic air for the hot jets. Separation of target compounds was achieved on a polar Stabilwax® polyethylene glycol column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Restek, USA) in the first dimension coupled with a non-polar Rxi®-5Sil MS column (0.79 m × 0.25 mm i.d. × 0.25 μm) film thickness (Restek, USA) in the second dimension. Primary and secondary columns were connected using a press-tight connector. Helium (99.9999% purity) was used as carrier gas at a constant

flow rate of 1.4 ml/min and the split injector was set to 1:200. The main oven temperature was initially set to 40 °C for 1 min, and then ramped to 260 °C at 10 °C/min, with a final isothermal period of 2 min at 260 °C. The secondary oven was programmed with a +15 °C offset above the primary oven. The GC temperature programme and MS method was developed to utilise the added selectivity of GC × GC and the full range mass spectra generated by ToF (time of flight)-MS for separating and identifying components, even at low concentrations. Different modulation periods (2 s, 4 s, 5 s, 6 s and 8 s) were tested during the optimisation stage to determine the best separation. The chosen modulation period was 2 s and the hot pulse duration was set at 0.5 s. The mass spectrometer was operated at an acquisition rate of 100 spectra/s. A solvent acquisition delay of 180 s was used to protect the MS analyser from excessive solvent exposure. The ion source temperature and the transfer line to the ToF-MS were set to 200 °C and 280 °C, respectively. The detector voltage used was 1650 V and electron ionisation at 70 eV was used. Mass spectra were acquired from 30–450 *m/z* and 1 μl (1:9 dilution) of the sample was injected in duplicate using the Gerstel multipurpose sampler.

2.3. Clinical study

2.3.1. Patient selection and study design

Three single-blinded quantitative clinical trials (irritancy patch test, moisture efficacy and occlusivity studies) were conducted between July and September 2014. For each study, twenty ($n=20$) healthy caucasian adult female volunteers (18–65 years old) who complied with the inclusion and exclusion criteria were recruited. Exclusion criteria comprised the following: known allergy to moisturisers, creams, lotions or cleansing products; use of medication which may influence the interpretation of the data such as topical/systemic corticosteroids, chronic antihistamines and/or anti-inflammatories; use of any topical medications on the test areas; clinically significant skin diseases which may contraindicate participation, including psoriasis, eczema, skin cancer or other skin pathology; damaged skin in or around the test areas such as sunburn, excessive suntan, uneven skin tones, scars, cuts, scratches, varicose veins, tattoos, active dermal lesions, or other disfiguration of the test area that would interfere with visual evalutions; immunological disorders such as rheumatoid arthritis, HIV positive status, AIDS or systemic lupus erythematosus; insulin-dependent diabetes; any condition, which in the opinion of the investigator may affect the results or place the subject at undue risk; be currently pregnant, planning a pregnancy, lactating, or have given birth in the last 4 weeks; peripheral vascular disease; and/or participated in a study involving the same test area within the three weeks prior to the dry-down period.

Signed informed consent was obtained from all participants prior to the commencement of the clinical trials. All studies were conducted at the Photobiology Laboratory according to Standard Operating Procedures and carried out in accordance with the Declaration of Helsinki and the Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participation in South Africa. Permission to conduct the study was granted by the Research, Ethics and Publications Committee of the Medunsa Campus, Sefako Makgatho Health Sciences University (MREC/H/48/2014: CR) and the study was approved by the Senate Committee for Research Ethics at Tshwane University of Technology (SCRE/2014/06/008).

2.3.2. Irritancy patch test

The irritancy patch test was conducted on the volar forearm where a template was used to draw out patch test sites. Baseline measurements were taken pre-application using a Minolta Cr400 Chromameter® to assess the surface colour based on the tristimulus analysis of the reflected xenon light pulse using the L^* a^* b system. The L^* value is the luminance variable while a^* and b^* values are the chromaticity co-ordinates; red–green (a^*) and blue–yellow (b^*). The values are used to define a point in a three-dimensional colour space that characterises a colour (Abuagla and Al-Deeb, 2012). In this study, a^* value (redness) readings were recorded. The Chromameter® was calibrated against a D65 white plate at the start of the study. Marula oil (20 μl), negative (de-ionised water) and positive (1% sodium lauryl sulphate) controls were applied on individual filter discs in a randomised blinded pattern at 0 h and repeated at the same position at 24 h. The sites were occluded with aluminium Finn Chambers® on Scanpore® tape for the first 2 × 23 h, thereafter the chambers were removed. The visual assessment was carried out using a 2 × magnifying lamp for visual scoring using the following grading system: 0=no reaction, 0.5=slight reaction, 1.0=weak reaction, 1.5 moderate reaction, 2.0=strong reaction within the test site, 3.0=strong reaction that spreads beyond the test site. The maximum irritancy was calculated using the mean visual score+one standard deviation for Marula oil and the controls – a standard approach for this test. Irritancy levels for the sample were classified as follows: mean visual score+SD > 1.5=sample is an irritant; mean visual score+SD < =1.5 and > negative control=sample has low irritancy potential and a mean visual score+SD < =negative control=sample is non-irritant. To quantify the skin response for each test site compared to baseline, the a^* value readings were calculated at 0, 24, 48, 72 and 96 h post-application; using the equation:

$$\textit{Delta } a^* = (\textit{product } a^* \textit{ time } t - \textit{product } a^* \textit{ time } 0) - (\textit{untreated } a^* \textit{ time } t - \textit{untreated } a^* \textit{ time } 0) \quad (1)$$

The Delta a^* values for all participants for a given product at a given time point were averaged and plotted (Adams and Singh, 1995; Spiewak, 2008).

2.3.3. Moisture efficacy study

In this study a visual assessment of the skin was conducted to evaluate the skin barrier function, followed by the transepidermal water loss test using the Aquaflux® (Biox Systems Limited, London) instrument. Thereafter, a capacitance (skin moisture content) test was conducted using a Corneometer® (Courage and Khazaka, Electronic GmbH, Khöln, Germany).

Participants were initially exposed to a 7-day dry-down period prior to commencement of the study where the calf area was washed in a standardised way 2–4 times daily using soap and water only. Participants were not allowed to apply any products to the legs during this period.

The participants applied the test products (Marula oil, liquid paraffin, Vaseline® intensive care lotion and Vaseline® (petroleum jelly) in the morning and approximately 8 hours post-assessment at home after washing.

2.3.3.1. Visual assessment. Five test sites (5.7x3.7 cm^2) on the calf area were rotationally randomised to all test sites (Untreated, Marula oil, liquid paraffin, Vaseline® intensive care lotion and Vaseline®). Baseline readings were taken at 0 h using a 2 × magnifying lamp for visual scoring. Visual assessment of skin dryness was based on the following grading; 0.0=no evidence of dryness, 1.0=slightly dry skin, 2.0=moderately dry skin, 3.0=severely dry skin and 4.0=extremely dry skin. An amount of 0.1 ml of each sample was applied in circular movements on the skin surface over a diameter of 20 mm. Visual assessments were performed on days 1, 2, 3, 4, 5, 8, 10 and 12.

2.3.3.2. Aquaflux® transepidermal water loss (TEWL). The Aquaflux® instrument (Biox, UK) was used to measure water

evaporation (TEWL) on the calf area based on the vapour pressure gradient (Imhof, 2007). Readings were recorded on days 1, 2, 3, 4, 5, 8, 10 and 12 post-application. Aquaflux® assessments for each test site compared to baseline, was calculated at time 0 using the same approach as in Eq. (1).

2.3.3.3. Capacitance – Corneometer® moisture retention (hydration). The moisture retention test was conducted using a Corneometer®. The Corneometer® instrument records the concentration gradient of water in the stratum corneum based on the capacitive measurement of di-electrical constant of the stratum corneum (Alanen et al., 2004). The hydration of the stratum corneum is related to the suppleness, softness and smoothness as well as the youthful and healthy appearance of the skin (Jiang and Delacruz, 2011). The readings range from 0 (no moisture/water) up to 120 (high level of moisture/water) (Clarys et al., 2011). The values are generally classified under different categories with extreme dry being rated <30, dry being between 30 and 40 and normal >40 (Heinrich et al., 2003).

The same methodology that was used in (Sections 2.3.3.1 and 2.3.3.2) was applied to obtain data. The raw data obtained from visual assessment and Corneometer® readings for each test site compared to baseline, was calculated at time 0 using the same approach as in Eq. (1).

2.3.4. Occlusivity study

A wipe off test was conducted on the volar forearm for five consecutive days using Aquaflux® and Corneometer® instruments. Five test sites (Untreated, Marula oil, liquid paraffin, Vaseline® intensive care lotion and Vaseline® petroleum jelly) were rotationally randomised and 0.1 ml of the each sample was applied to the skin surface (20 mm diameter) using circular movements. Readings were recorded at 0 min and 30 min post-wipe off period and Eq. (1) was used for calculation of the Delta a^* value.

2.4. Data analysis

Statistical analyses were performed using Stata® 10 software. The Pearson's chi-square test was used to test for associations between the samples and the reaction categories. The Kruskal–Wallis test was used to compare samples over measured outcomes in cases where the outcome is not normally distributed. Otherwise, two-sample independent *t*-tests or one-way analysis of variance (ANOVA) tests were employed for continuous and normally distributed outcomes. Where a significant difference was observed; a Sidek post-hoc test was performed (Kohler and Kreuter, 2012). All the interpretations are performed at a $\alpha=0.05$ error rate.

3. Results and discussion

3.1. GC × GC–MS analysis

Table 1 shows the fatty acid composition of Marula oil as determined using two-dimensional gas chromatography coupled to mass spectrometry (GC × GC–MS). Several saturated fatty acids including myristic, palmitic, margaric, stearic and arachidic acids were detected together with the unsaturated fatty acids palmitoleic, oleic, linoleic, linolenic and gondoic acids. Oleic acid was the major fatty acid detected constituting 69.0% of the oil with lower quantities of palmitic acid (15.3%), linoleic acid (9.2%), palmitoleic acid (4.1%) and stearic acid (1.5%). The nut oil composition reported by Kleiman et al. (2008) was similar regarding the major fatty acids detected as well as the percentage composition in most cases; oleic acid = 71.3%, palmitic acid = 12.7%, stearic acid = 7.4% and linoleic acid = 6.7%. A review on African seed oils showed that the composition range of selected fatty acids was 70.0–78.0% for oleic acid, 9.0–12.0% for palmitic acid, 5.0–8.0% for stearic acid and 4.0–7.0% for linoleic acid (Vermaak et al., 2011).

3.2. Irritancy patch test

An irritancy patch test is used in clinical practice as an investigative and diagnostic method to recognise contact hypersensitivity (Geier et al., 2003; Ale and Maibach, 2010). The possible irritancy as assessed by the presence of erythema (redness) was determined using two methods; visual and Chromameter® reading-based assessments. The study was conducted on 20 participants but one participant withdrew, therefore only the data of 19 participants were analysed.

The results of the visual erythema reaction are presented in Table 2. Sodium lauryl sulphate is an anionic surfactant mostly used in the pharmaceutical and cosmetic industries as an emulsifier in soap manufacture. The primary side-effect of sodium lauryl sulphate is skin irritation, depending on the percentage included in the formulation as well as the duration of exposure. It alters the stratum corneum to enhance the penetration of products into the skin and is recognised as a suitable non-invasive method for quantifying skin response due to its ability to influence the skin barrier function and cause inflammation (Agner and Serup, 1990; Zhai and Maibach, 2004). In this study, sodium lauryl sulphate exhibited a slight to strong statistically significant skin reaction in all the participants after 72 h as compared to the negative control (de-ionised water) and Marula oil (test sample). The application of de-ionised water produced no reaction to slight reaction in some participants. One incidence of a weak reaction was recorded for one participant but this effect dissipated after 48 h. There were significant differences between the skin reactions for Marula oil and de-ionised water. No visual skin reaction was

Table 1
Fatty acid composition of *Sclerocarya birrea* commercial oil as determined by GC × GC–MS.

Fatty acids detected	Chemical name	Molecular formula	Carbon bonds	Percentage detected (%)	Retention time 1st Dimension (min)	Retention time 2nd Dimension (s)
Myristic acid	Tetradecanoic acid	$C_{14}H_{28}O_2$	14:0	0.1	912	0.68
Palmitic acid	Hexadecanoic acid	$C_{16}H_{32}O_2$	16:0	15.3	990	0.94
Palmitoleic acid	9-Hexadecenoic acid(*Z*)	$C_{16}H_{30}O_2$	16:1	4.1	1002	0.88
Margaric acid	Heptadecanoic acid	$C_{17}H_{34}O_2$	17:0	nd*	1046	0.92
Stearic acid	Octadecanoic acid	$C_{18}H_{36}O_2$	18:0	1.5	1106	0.95
Oleic acid (*Z*)	9-Octadecenoic acid(*Z*)	$C_{18}H_{34}O_2$	18:1	69.0	1114	0.96
Linoleic acid (*E,E*)	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	18:2	9.2	1132	0.87
Linolenic acid (*Z,Z,Z*)	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	18:3	0.1	1162	0.82
Arachidic acid	Eicosanoic acid	$C_{20}H_{40}O_2$	20:0	0.4	1202	0.99
Gondoic acid	*cis*-11-Eicosenoic acid	$C_{20}H_{38}O_2$	20:1	0.3	1210	0.94

* nd: not detected.

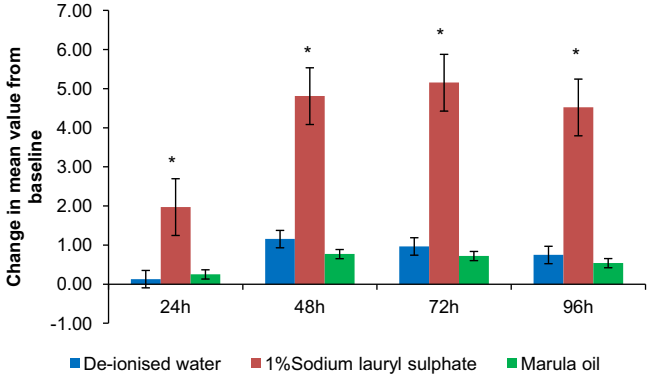
Table 2
Visual erythema skin reaction results obtained with the application of test samples to the volar forearm area of participants ($n=19$).

		Reaction categories				
		No reaction 0	Slight reaction 0.5	Weak reaction 1	Moderate reaction 1.5	Strong reaction 2
24 h	De-ionised water	78.9% ($n=15$)	15.8% ($n=3$)	5.3% ($n=1$)	0% ($n=0$)	0% ($n=0$)
	1% Sodium lauryl sulphate	26.4% ($n=5$)[a]	36.8% ($n=7$)[a]	36.8% ($n=7$)[a]	0% ($n=0$)	0% ($n=0$)
	Marula oil	100% ($n=19$)	0% ($n=0$)	0% ($n=0$)	0% ($n=0$)	0% ($n=0$)
48 h	De-ionised water	73.7% ($n=14$)	26.3% ($n=5$)	0% ($n=0$)	0% ($n=0$)	0% ($n=0$)
	1% Sodium lauryl sulphate	5.3% ($n=1$)[a]	0% ($n=0$)[a]	42.1% ($n=8$)[a]	47.3% ($n=9$)[a]	5.3% ($n=1$)[a]
	Marula oil	100% ($n=19$)	0% ($n=0$)[b]	0% ($n=0$)	0% ($n=0$)	0% ($n=0$)
72 h	De-ionised water	84.2% ($n=16$)	15.8% ($n=3$)	0% ($n=0$)	0% ($n=0$)	0% ($n=0$)
	1% Sodium lauryl sulphate	0% ($n=0$)[a]	5.3% ($n=1$)[a]	42.1% ($n=8$)[a]	42.1% ($n=8$)[a]	10.5% ($n=2$)[a]
	Marula oil	100% ($n=19$)	0% ($n=0$)[b]	0% ($n=0$)	0% ($n=0$)	0% ($n=0$)
96 h	De-ionised water	100% ($n=19$)	0% ($n=0$)	0% ($n=0$)	0% ($n=0$)	0% ($n=0$)
	1% Sodium lauryl sulphate	0% ($n=0$)[a]	10.5% ($n=2$)[a]	52.6% ($n=10$)[a]	21.0% ($n=4$)[a]	15.9% ($n=3$)[a]
	Marula oil	100% ($n=19$)	0% ($n=0$)	0% ($n=0$)	0% ($n=0$)	0% ($n=0$)

[a] *Significant difference ($p<0.001$) between de-ionised water and 1% sodium lauryl sulphate.
[b] Significant difference ($p<0.001$) between de-ionised water and Marula oil.

recorded for Marula oil in any of the participants irrespective of time and it can therefore be classified as non-irritating. It has been reported in literature that Marula oil improves skin hydration and smoothness and reduces skin redness (Gruenwald, 2006; Lall and Kishore, 2014).

Furthermore, the degree of erythema was determined based on the a^*. Fig. 2 indicates that all the participants reacted clinically positive to 1% sodium lauryl sulphate with statistically significant erythema values of 1.97 at 24 h, 4.81 at 48 h, 5.15 at 72 h and 4.52 at 96 h compared to de-ionised water with erythema values of 0.13 at 24 h, 1.16 at 48 h, 0.97 at 72 h and 0.75 at 96 h. Low erythema values of 0.25 at 24 h, 0.77 at 48 h, 0.72 at 72 h and 0.54 at 96 h was recorded for Marula oil. The calculated differences of 0.12 ($p=0.996$), 0.39 ($p=0.905$), 0.25 ($p=0.970$) and 0.21 ($p=0.991$) between Marula oil and de-ionised water were not significant at 24, 48, 72 and 96 h, respectively. However, significant differences were observed when 1% sodium lauryl sulphate and Marula oil were compared with mean differences of 1.72 ($p=0.015$) at 24 h; 4.04 ($p<0.001$) at 48 h; 4.43 ($p<0.001$) at 72 h and 3.98 ($p<0.001$) at 96 h. The reaction between de-ionised water and 1% sodium lauryl sulphate was significantly different at all time points



*significant difference (p=0.05) between de-ionised water and 1% Sodium lauryl sulphate

Fig. 2. Calculated erythema values of de-ionised water (negative control), 1% sodium lauryl sulphate (positive control) and Marula oil (test sample) on the forearm area at 24, 48, 72 and 96 h time intervals ($n=19$).

with mean differences of 1.84 ($p=0.008$); 3.65 ($p=0.015$); 4.18 ($p<0.001$) and 3.77 ($p<0.001$), respectively.

Both Marula oil and de-ionised water were non-irritating to the skin; at 48 h, the irritancy score of de-ionised water was 1.16 while the irritancy score of Marula oil was even lower with a value of 0.77. According to Botelle (2001), in the past, Namibian women used Marula oil rather than water to clean themselves. A study conducted by de Groot (1988) stated that no compelling evidence exists to show that high quality edible lipids cause adverse reactions on the skin, except for potential comedogenicity. Very few reports of adverse reactions to the cosmetic application of edible fatty acid oils have been reported. It was also stated that most irritant allergic reactions caused by plant-derived fatty acid-containing oils are thought to be as a result of proteins present in food. This view was corroborated by a report published by the Cosmetic Ingredient Review (2011) which stated that an individual that is allergic to a food product with allergens is likely to not react to the refined oil, because the major concern associated with allergic reactions is the presence of the proteins. These proteins are mostly absent in the refined oil; therefore, the oil is likely to be safe for use in cosmetics.

Currently, Marula oil is one of the 244 plant-derived oils included in the Cosmetic Ingredient Review (CIR) expert panel list as a safe cosmetic ingredient for dermal applications, ingestion, inhalation and hair care at a concentration of 1% (Cosmetic Ingredient Review, 2011). The panel also noted that arachidonic acid was previously published in the CIR evaluation as an unsafe cosmetic ingredient due to insufficient data. The panel concluded that the concentration of arachidonic acid ($\pm 8\%$) present in Marula oil was sufficiently low and therefore does not warrant concern (Cosmetic Ingredient Review, 2011). In this study, no arachidonic acid was detected, but arichidic acid, formed through the hydrogenation of arachidonic acid, was present at 0.4%. Previous reports indicated arachidonic acid percentages of 0.3–0.7% in Marula oil (Vermaak et al., 2011). Presently, 23 reports of frequent use of Marula oil as a dermal topical product and 6 reports of use as a hair care product have been documented but not reviewed by the CIR (Cosmetic Ingredient Review, 2011).

According to the European Union (2007), if a cosmetic product/oil is intended to be topically applied, an in depth understanding of the cosmetic product/oil toxicity level should be documented to

minimise systemic exposure. A study by Rügheimer (2001) suggested that Marula oil is prone to hydrolytic rancidity although it is a relatively stable oil. During hydrolytic rancidity the oil may destroy the biological integrity of essential fatty acids and damage DNA. As a result the skin may be prone to premature visible aging and inflammatory disorders. It is therefore crucial to monitor and record the shelf life of the oil with proper labelling indicating the life span. In another study by Muhammad et al. (2011), an aqueous extract of edible kernels of the Marula fruit were found to be relatively toxic when fed to rats ($n=10$) at a dosage of 3000 and 4000 mg/kg for a period of 28 days. Significant weight-loss ($p<0.05$) was observed in the experimental group compared to the control group as well as an increase in serum total protein, albumin, bilirubin, trans-aminases, creatinine, urea, uric acid and electrolytes suggesting that the aqueous extract of the kernel might induce liver and kidney toxicity. Although the oil is intended for topical use and the doses used in Muhammad et al. (2011) study were high, consumers should be advised to exercise caution when using the oil on the mucous membranes to minimise systemic effects.

3.3. Moisture efficacy study

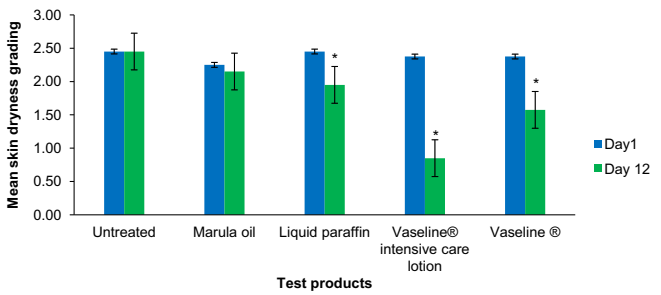
The results of the visual assessment, transepidermal water loss and moisture retention tests are as follows:

3.3.1. Visual assessment

Results depicted on Fig. 3 indicates that Vaseline® intensive care lotion caused pronounced skin recovery while Marula oil resulted in marginal skin recovery from initial severe-moderate dryness observed on day 1 after the 7-day dry-down period to moderate dry skin noted on day 12. A statistically significant difference $p<0.001$ was observed when liquid paraffin, Vaseline® intensive care lotion and Vaseline® were applied.

These findings confirm those of the article by Lynde (2014), stating that Vaseline intensive care lotion® offers triple-action therapy through a moisturising effect that blocks transepidermal water loss (occlusive); the product attracts water to the stratum corneum (humectant) and additionally, it smooths out the dry skin by filling in flaking spaces with oil droplets (emollient). This oil-in-water emulsion improves barrier repair, alters cutaneous moisture partition co-efficient, promotes moisture diffusion from the dermal capillary bed across dermal–epidermal junction into the epidermis and restores functional intercellular lipids to attract, hold and redistribute the body's own natural moisture effectively (Jackson, 1992; Abeck et al., 2003).

It has been claimed that Marula oil was applied topically to moisturise and hydrate the skin, though these claims were not scientifically substantiated (van Wyk and Gericke, 2000). Very few scientific studies to substantiate the choice of Marula oil as a skin



* Significant difference ($p<0.001$) observed when liquid paraffin, Vaseline® intensive care lotion and Vaseline® were compared on days 1 and 12.

Fig. 3. Skin recovery level based on visual assessments observed at day 1 and day 12 ($n=20$).

hydration and smoothing product through the prevention of transepidermal water loss have been conducted. Clinical tests results on Marula oil as a potential cosmetic formulation yielded moderate success (Houghton, 1999; Kleiman et al., 2008; Lall and Kishore, 2014). When applied topically, the oil creates a smooth layer on the skin through filling of open spaces between flaking skin (Lynde, 2014). These smoothing effects could be due to the oleic acid content. Oleic acid is an emollient which is easily absorbed into the skin. This mechanism of action provides credibility to Marula oil to decrease skin roughness. In South Africa, Marula oil has been used for several years to protect against dry and cracking skin (Hein et al., 2009).

3.3.2. Aquaflux® transepidermal water loss (TEWL)

Condensed water which diffuses through the skin, known as transepidermal water loss (TEWL), was determined by measuring the rate of water evaporation from the skin surface using a closed-chambered Aquaflux® instrument. The instrument disturbs the microclimate of uncovered skin dominated by air movements and measures the quantity of water that passes from the inside of a body through the epidermal layer to the surrounding atmosphere via diffusion and evaporation processes (Imhof, 2007).

The results in Table 3 show the mean difference observed at day 1 and day 12. Day 12 indicates that Vaseline® intensive care lotion (10.31), Vaseline® petroleum jelly (10.67) and liquid paraffin (11.69), prevents TEWL significantly to a greater extent than Marula oil (12.14). A negative value observed on all test products at the change from baseline ($\Delta D_{12}–D_1$) corroborates improved TEWL barrier function. Vaseline® intensive care lotion is known to improve skin barrier function due to its emollient, humectant and occlusive properties (Lynde, 2014). Table 4 indicates that a significant difference was observed between the untreated and Vaseline® (difference=1.40; $p<0.001$), liquid paraffin and Vaseline® (difference=1.48; $p=0.02$) and Vaseline® and Marula oil groups (difference=−1.29; $p<0.001$). These findings could be linked to the mechanisms of action observed when Vaseline® is applied. The slip velocity of Vaseline® petroleum jelly when topically applied makes it the preferred occlusive moisturising ingredient which prevents transepidermal water loss when the skin is exposed to dry air or wind (Lynde, 2014). Marula oil performed better with a negative mean value indicating improved TEWL barrier and this could be due to the impact that Marula oil has on the triglyceride balance of the skin. Marula oil is easily recognised by the skin due to the presence of palmitic acid in the skin barrier layer and the relatively fast absorption of oleic acid present in Marula oil allows enzymatic hydrolisation of the Marula oil into glycerine and fatty acids when applied topically. This mechanism of action presents the oil with the ability to smooth out dry flaking skin (Weichers and Barlow, 1999).

It is however, crucial to understand that TEWL is regarded as flux density, which is the quantity of water per unit area of skin per unit time. Evaporation into the surrounding atmosphere is not essential since TEWL does not stop immediately when the skin is occluded. Conversely, evaporation into the surrounding atmosphere is necessary for the measurement of TEWL, because the measurement takes place in the air above the skin (Imhof, 2007).

3.3.3. Capacitance – Corneometer® moisture retention test (hydration)

A Corneometer® is an instrument that is commonly used to provide reproducible and accurate hydration levels of the skin surface by evaluating the capacitance of the skin superficial layers (Faria et al., 2014). After a 7-day dry-down period, a Corneometer® was used to obtain hydration moisture readings on the calf area over a period of 12 days to observe whether the products could increase skin hydration and modify the physical and chemical

Table 3
Moisture efficacy test: transepidermal water loss mean readings at day 1 and day 12 ($n=20$).

Test products	Day 1: mean value (Standard deviation)	Day 12: mean value (Standard deviation)	Change from baseline $\Delta D_{12}–D_1$
Untreated	16.77; (2.86)	11.86; (2.26)*	−4.91
Marula oil	17.18; (3.40)	12.14; (2.72)*	−5.04
Liquid paraffin	16.53; (3.20)	11.69; (2.16)*	−4.84
Vaseline® intensive care lotion	15.62; (3.97)	10.31; (2.23)*	−5.31
Vaseline®	17.00; (3.10)	10.67; (2.74)*	−6.33

* Significant difference ($p<0.001$) observed on all test products when day 1 mean readings were compared to day 12.

Table 4
Moisture efficacy test: transepidermal water loss mean difference readings using pair-wise comparisons between the test products ($n=20$).

Test products	Mean difference($\Delta D_{12}–D_1$)	p-Value
Untreated vs Marula oil	0.11	0.80
Untreated vs liquid paraffin	−0.08	0.81
Untreated vs Vaseline® intensive care lotion	−0.50	0.67
Untreated vs Vaseline®	1.40	0.00
Liquid Paraffin vs Marula oil	0.19	0.75
Liquid paraffin vs Vaseline® intensive care lotion	−0.42	0.66
Liquid paraffin vs Vaseline®	1.48	0.02
Vaseline® intensive care lotion vs Marula oil	0.61	0.61
Vaseline® vs Marula oil	**−1.29**	**0.00**
Vaseline® vs Vaseline® intensive care lotion	−1.90	0.12

nature of skin surface (Bonté, 2011; Walters and Roberts, 2002). Table 5 indicates that all the products hydrated the skin as compared to the untreated control. Vaseline® intensive care lotion significantly hydrated the skin with a moisture efficacy value of 30.56 on day 12 compared to untreated skin with 16.24. The positive mean difference observed indicates the moisture/hydration efficacy of all test products. The hydration level of the stratum corneum is a valuable parameter for different cosmetic applications. Moisturisers are used to improve the elasticity of the skin and the hydration level determines the elasticity. Increasing hydration increases skin elasticity which decreases as age increases. It is therefore rational to consider that the elasticity of skin is improved by applying a skin moisturiser (Weichers and Barlow, 1999; Clarys et al., 2011). Although the influence of the other products, including Marula oil, was not significant, they did slightly hydrate the dried-down skin. These changes in the amount of water in skin caused alterations in the local dielectric constant and consequently the capacitance (Wiedersberg et al., 2009). According to Lodén (2004), lipids in moisturisers can increase skin hydration by occlusive and humectant mechanisms and since Marula oil is lipophilic it could be possible that an occlusive effect might cause the moisture content increase in the stratum corneum. This could corroborate the traditional use of Marula oil by the Tsonga people of South Africa and Mozambique for moisturising and hydrating the skin (Junod, 1978; Botelle, 2001; Venter, 2012; Lall and Kishore, 2014).

3.4. Occlusivity test

A wipe off study using an Aquaflux® instrument indicated the moisture level of the skin was increased significantly in all cases. The application of Vaseline® petroleum jelly caused the highest short-term increase followed by liquid paraffin, Marula oil and Vaseline® intensive care lotion. Vaseline® is a well-known purified mixture of semi-solid, saturated paraffinic hydrocarbons. The results (Fig. 4) show that both liquid paraffin and Marula oil caused an increase in the moisture content of the skin due to their occlusive effects. Marula oil performed better than Vaseline® intensive care lotion presumably due to oleic acid present in the oil which has excellent absorption properties (Houghton, 1999; Lynde, 2014; Hein et al., 2009).

On the other hand the results yielded from the Corneometer® instrument, a widely used non-invasive biometrical method used to measure skin hydration by evaluating the capacitance of the skin's superficial layers (Fluhr et al., 1999a, 1999b; Darlenski et al., 2009; Byrne, 2010; Sotoodian and Maibach, 2012) indicated a statistically significant difference ($p<0.001$) for all the test products indicating an immediate moisture-retention effect (Fig. 5). This shows that Marula oil has a significant occlusive effect which causes hydration. This may be due to the presence of oleic acid and palmitic acid in the composition. Vaseline® intensive care lotion has been shown to have a physical block property that prevents transepidermal water loss when topically applied (Lynde, 2014).

4. Conclusions

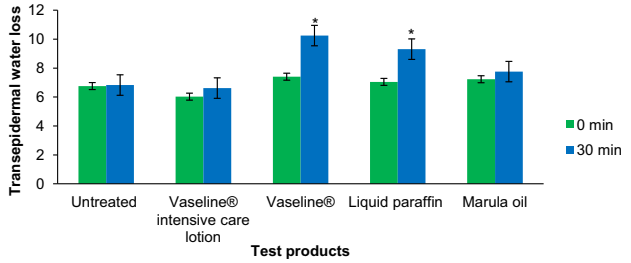
There is growing interest in the inclusion of Marula oil use in cosmetic products even though the efficacy and safety of the Marula oil have not previously been determined. This study serves as a foundation in which Marula oil irritancy potential, moisture efficacy, hydrating property and occlusive effects were evaluated. The major fatty acid constituent was confirmed to be oleic acid, an emollient which is absorbed rapidly into the skin.

Marula oil affects the moisture level of the skin by causing some hydration and may be beneficial in cosmetic products depending upon its application. Most importantly, Marula oil was non-irritating to the skin and can therefore be regarded as safe for topical application.

Table 5
Moisture efficacy test: capacitance moisture retention mean readings at day 1 and day 12 ($n=20$).

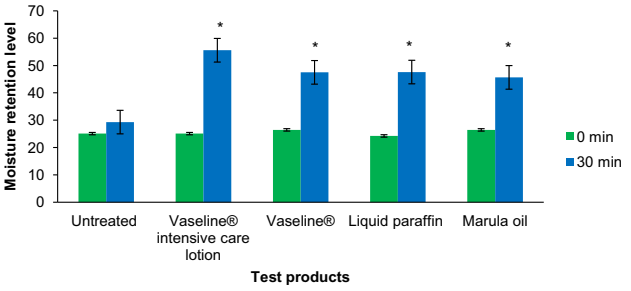
Test products	Day 1: mean value (standard deviation)	Day 12: mean value (standard deviation)	Change from baseline $\Delta D_{12}–D_1$
Untreated	17.15, (4.95)	16.24, (4.83)	−0.91
Marula oil	16.78, (5.26)	17.27, (4.08)	0.49
Liquid paraffin	16.96, (5.39)	17.14, (5.03)	0.18
Vaseline® intensive care lotion	19.80, (5.91)	30.56, (5.90)*	10.76
Vaseline®	19.24, (6.32)	19.70, (5.18)	0.46

* Significant difference ($p<0.001$) observed when day 1 mean readings were compared to day 12.



* Significant difference ($p<0.001$) was observed when Vaseline® and liquid paraffin were evaluated for transepidermal water loss at time 0 min and 30 min post application.

Fig. 4. Occlusivity test: transepidermal water loss readings of all test products at 0 min and 30 min ($n=20$).



* Significant difference ($p<0.001$) observed when Vaseline® intensive care lotion, Vaseline®, liquid paraffin and Marula oil were evaluated for moisture retention level at time 0 min and 30 min post application.

Fig. 5. Occlusivity test: moisture retention readings of all test products at 0 min and 30 min ($n=20$).

With reference to Nohynek et al. (2010), the safety and toxicity of cosmetic products is of significance as once a cosmetic product (natural or synthetic) is applied to the skin, there is a risk of systemic exposure which may lead to adverse localised effects such as irritation, sensitisation or photoreactions. Therefore, evaluation of the safety is required prior to release of the product onto the market. However, global challenges such as a "legal definition" of a cosmetic still remains unclear and the regulations differ from country to country. As South Africa adopts the European cosmetic safety regulations, Marula oil can be classified safe for use in cosmetic products where minimal absorption occurs.

Acknowledgements

The authors thank and acknowledge the participants and technicians at the Photobiology Laboratory (Sefako Makgatho Health Sciences University); Mr Maupi Letsoalo (Statistician; Tshwane University of Technology) for his assistance in data analysis; and Dr. Guy Kamatou (GC × GC–MS analysis; Tshwane University of Technology). The National Research Foundation, Tshwane University of Technology and Restek are acknowledged for financial support. Phytotrade Africa is thanked for the use of the marula kernel image.

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