Revista Brasileira de Farmacognosia 27 (2017) 1–8

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Beauty in Baobab: a pilot study of the safety and efficacy of *Adansonia digitata* seed oil

Baatile M. Komane[a], Ilze Vermaak[a,b], Guy P.P. Kamatou[a], Beverley Summers[c], Alvaro M. Viljoen[a,b,*]

[a] *Department of Pharmaceutical Sciences, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa*
[b] *South African Medical Research Council Herbal Drugs Research Unit, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa*
[c] *Department of Pharmacy, Photobiology Laboratory, Sefako Makgatho Health Sciences University, Pretoria, South Africa*

ARTICLE INFO

Article history:
Received 31 May 2016
Accepted 14 July 2016
Available online 12 August 2016

Keywords:
Adansonia digitata
Baobab
Cosmetic
Efficacy
Safety
Seed oil

ABSTRACT

Recently there has been a renewed impetus in the search for novel ingredients to be used in the cosmetic industry and Baobab (*Adansonia digitata* L., Malvaceae) seed oil has received high interest. In this study, a commercial Baobab seed oil sample was characterised (fatty acid content) using GCxGC-ToF-MS and a pilot study on the safety and efficacy of the seed oil was performed. The safety and efficacy of Baobab seed oil after topical application was determined using healthy adult female caucasian participants ($n = 20$). A 2× magnifying lamp was used for visual analysis, while for monitoring and evaluation of the irritancy level, transepidermal water loss (TEWL) and hydration level of the skin, Chromameter®, Aquaflux® and Corneometer® instruments, respectively, were used. In addition, Aquaflux® and Corneometer® instruments were used to assess occlusive effects. Thirteen methyl esters were identified using GCxGC-ToF-MS. The major fatty acids included 36.0% linoleic acid, 25.1% oleic acid and 28.8% palmitic acid with 10.1% constituting trace fatty acids. The irritancy of sodium lauryl sulphate (SLS) in the patch test differed significantly compared to both de-ionised water ($p < 0.001$) and Baobab seed oil ($p < 0.001$) but the difference between the irritancy of Baobab seed oil and de-ionised water was not significant ($p = 0.850$). The moisture efficacy test indicated a reduced TEWL ($p = 0.048$) and an improved capacitance moisture retention ($p < 0.001$) for all the test products (Baobab oil, liquid paraffin, Vaseline® intensive care lotion and Vaseline®). The occlusivity wipe-off test indicated an increased moisture hydration ($p < 0.001$) and decreased TEWL particularly when Baobab oil was applied. Baobab possesses hydrating, moisturising and occlusive properties when topically applied to the skin. Baobab seed oil could be a valuable functional ingredient for cosmeceutical applications.

Introduction

The Baobab (*Adansonia digitata* L., Malvaceae) tree, which has been used extensively as a source of food, fibre and medicine, is commonly referred to as "arbre a palabre", meaning the place in the village where the elders meet to resolve problems (Kamatou et al., 2011). This deciduous tree is approximately 23 m tall and has a smooth, reddish-brown, greyish-brown or purplish-grey bark with green simple or digitate leaves that alternate at the ends of the branches followed by progressively 2–3 foliolate leaves. The flowers are pendulous with five white petals while the pulp-containing fruits are apex-pointed, covered by velvety pale yellow-brown hairs with smooth dark brown to blackish seeds covered by cream-coloured kernels (Fig. 1) (Palgrave, 1983; Wickens and Lowe, 2008; Kamatou et al., 2011). The Baobab tree belongs to a pan-tropical family with six of the eight species spanning Madagascar, the seventh species is endemic to north-western Australia and the eighth species is widely spread in sub-Saharan Africa. In southern Africa (Fig. 2), Baobab is found in Angola, Zambia, Mozambique, Zimbabwe and South Africa (Limpopo region) (Wickens and Lowe, 2008; Rahul et al., 2015).

In South Africa, the Baobab and Marula are two of the most popular indigenous tree species used for seed oil production (Venter, 2012). The seed oil extracted from the baobab fruit pulp is popularly used in the cosmetics industry and sold internationally (Munthali et al., 2012). About 33% of the seed content is oil with oleic and linoleic acids as the major fatty acids followed by palmitic and α-linolenic acids. The high content of linoleic and oleic acids are known to soften the skin and to restore and moisturise the epidermis. In addition, the fatty acids regenerate epithelial tissues which renders the seed oil a very good carrier oil of value to the cosmetic industry (Glew et al., 1997; Chindo et al., 2010).

* Corresponding author.
E-mail: viljoenam@tut.ac.za (A.M. Viljoen).

http://dx.doi.org/10.1016/j.bjp.2016.07.001

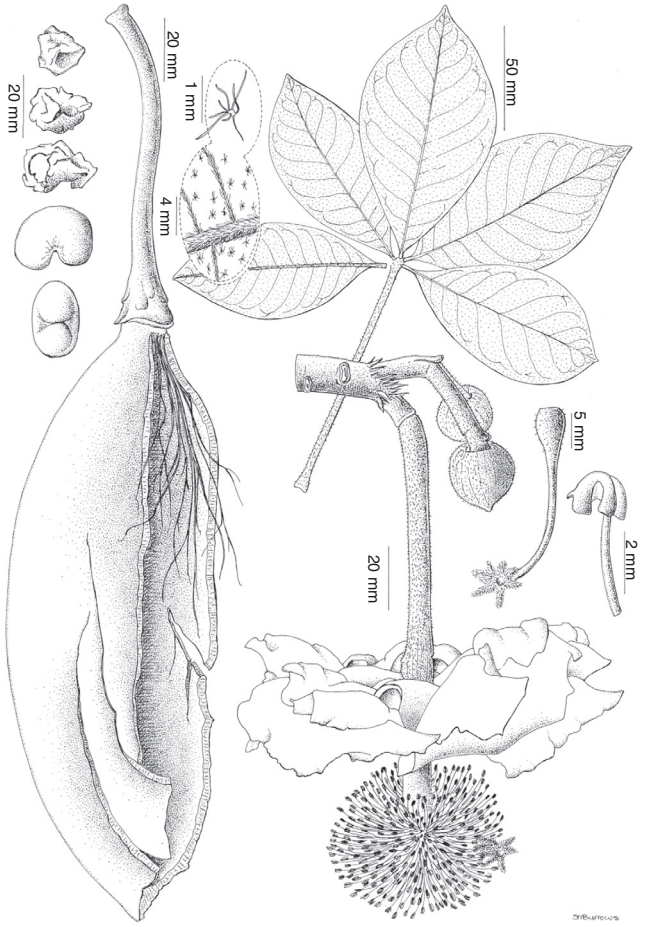


Fig. 1. Botanical line drawing illustrating the diagnostic features of *Adansonia digitata* (Baobab).

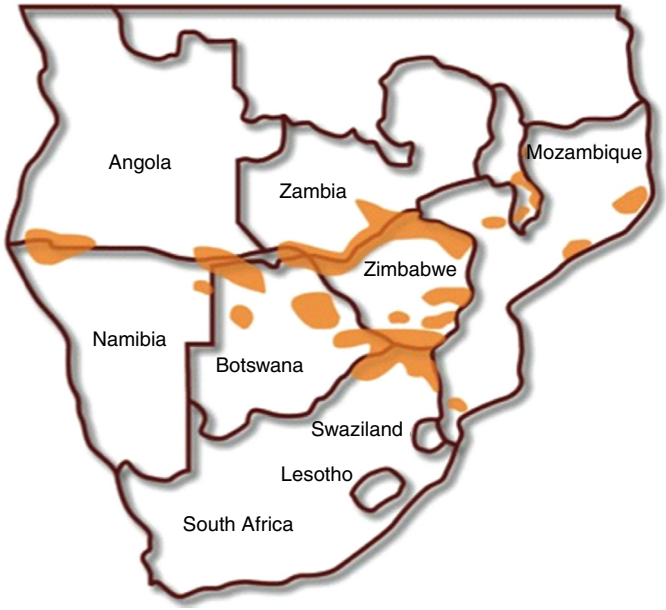


Fig. 2. Distribution map of *Adansonia digitata* (Baobab) in southern Africa.

The seed oil has been used for centuries by local communities for food, medicine and cosmetic applications. The nutritional seeds are rich in protein and the oil is generally used in food preparation for sauce/paste or eaten raw/roasted (Osman, 2004). It has been used to produce lubricants, soaps and toothpaste, in the topical treatment of various conditions such as muscle spasms, varicose veins and wounds and applied as a moisturiser for skin hydration, for hair and nail conditioning and to treat dandruff (Zimba et al., 2005; Nkafamiya et al., 2007; Chindo et al., 2010). Sidibe and Williams (2002) reported that the Baobab seed oil is used for cosmetic applications to treat skin ailments. The Baobab tree has been referred to as a small pharmacy or chemist tree and many authors have reported that all Baobab plant parts are valuable (Gebauer et al., 2002; De Caluwé et al., 2010).

Baobab oil, extracted from the seed, is used in the cosmetics industry and is also sold internationally even though there is a lack of clinical studies to confirm its traditional use (Gruenwald and Galizia, 2005). The global demand for Baobab oil has grown substantially with exports to Europe, Asia and North American markets. In Zimbabwe, approximately 20,000 litres of Baobab oil worth $100,000 is produced annually (Kamatou et al., 2011; Vermaak et al., 2011; Venter, 2012). In South Africa, commercialisation of the seed oil started as early as 2005 at the Vhembe Municipal District in the Northern region of Venda where seeds were sold at local markets and the oils extracted from the seeds were sold to the cosmetic market (Venter and Witkowski, 2010).

There have been several reports on the physico-chemical properties, nutritional content and fatty acid profile of the seed oil using conventional methods such as gas-chromatography coupled to mass spectrometry (GC–MS) (Sidibe and Williams, 2002; De Caluwé et al., 2010). Comprehensive two-dimensional analysis which provides better separation could give further insight into the composition as it can identify fatty acids present in trace-level concentrations. This study characterised the fatty acid composition of commercially available Baobab seed oil subsequently used in a pilot study to determine its safety and efficacy.

Materials and methods

Materials and sample preparation

Refined Baobab seed oil (Batch number: BAO0311EP; Product code: PDBAOA DO1) was purchased from a reputable commercial supplier (Scatters® Oils). Scatters® Oils (South Africa) is a supplier and exporter of natural, indigenous organic oils to the local and international market (http://www.scattersoils.com/). The odourless yellow seed oil was stored at 2–8 °C and allowed to warm up to room temperature before commencement of the pilot study. The certificate of analysis indicated a refractive index of 1.476 (1.474–1.485) and a relative density of 0.894 g/ml (0.892–0.950 g/ml). A retention sample (BAOB005) is stored in the Department of Pharmaceutical Sciences, Tshwane University of Technology. A fatty acid methyl esters (FAMEs) 37-component standard mixture as well as pure (≥99.0%) reference standards (linoleic, oleic, palmitic, stearic, arachidic, linolenic and myristic acid) were obtained from Sigma–Aldrich® (Johannesburg, South Africa). The FAMEs were prepared using the modified method of Rossé and Harynuk (2010). A volume of 310 μl of the oil sample and the standards ($n = 7$) were separately prepared in a vial and 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 (Kothiyal et al., 2010). The supernatant was collected and analysed using the GCxGC-ToF-MS system.

The irritancy patch test was conducted using 1% sodium lauryl sulphate (SLS) (≥99.0% purity; Sigma–Aldrich®, Johannesburg, South Africa) solution as a positive control (irritant) and de-ionised water as a negative control. For the moisture efficacy, hydration and occlusivity tests, liquid paraffin (≥95%), Vaseline® intensive care lotion (MSDS #4059) and Vaseline® petroleum jelly (MSDS #4056) obtained from Unilever© Pty Ltd (Durban, South Africa) were used as positive controls while untreated skin was regarded as the negative control.

GCxGC-ToF-MS analysis

The GCxGC-ToF-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 GCxGC and the full range mass spectra generated by ToF (time of flight)-MS for separating and identifying components. 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 2.0 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 to 450 *m/z* and 1 μl (1:50 dilution) of the sample was injected in duplicate using the Gerstel multipurpose sampler. The percentage area was automatically calculated by the software taking into account peak height, area, width and the noise level. Identification of peaks was based on retention times of reference compounds (linoleic, oleic, palmitic, stearic, arachidic, linolenic and myristic acid as well as the 37-component mixture) and mass spectra library matching using NIST® Mass Spectral Library (NIST® 11) and NIST® 08 (Adams Library). Library similarity factors were determined for both forward and reverse searches.

Pilot study

Patient selection and study design

Three single-blind quantitative pilot studies (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 evaluations; 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 study. 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 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).

Irritancy patch test

The irritancy patch test was conducted on the volar forearm. Baobab oil (20 μl), de-ionised water (negative control) and 1% sodium lauryl sulphate (positive control) were applied topically using white litmus paper (5 mm × 10 mm) and placed on the skin in Finn chambers and kept in position for 2 × 23 h until assessment. Reassessment was conducted for any irritant (not an allergic) reaction after a further 48 h. Reactions were graded; 0.0 = no reaction, 0.5 = slight reaction, 1.0 = weak reaction, 1.5 = moderate reaction, 2.0 = strong reaction) for each irritant. The visual assessment was carried out using a 2× magnifying lamp for visual scoring and a Minolta Cr400 Chromameter® was used 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.

Irritancy levels were classified as follows: mean visual score + one standard deviation (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:

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

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

Moisture efficacy study

Baobab oil and the well-known skin protectants liquid paraffin, Vaseline® intensive care lotion and Vaseline® petroleum jelly were tested for their effect on TEWL, moisture retention (hydration) and skin barrier function (occlusivity) properties. A 2× magnifying

lamp was used for visual assessment; an Aquaflux® (Biox Systems Limited, London) instrument was used to determine the TEWL and skin barrier efficacy; and a Corneometer® (Courage and Khazaka, Electronic GmbH, Khöln, Germany) was used to record moisture retention readings and determine skin barrier function. Prior to the commencement of the study, participants were exposed to a 7-day dry-down period 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. After the 7-day dry-down period an amount of 0.1 ml of each sample was applied twice daily on 5.7 cm × 3.7 cm randomised test sites (Untreated, Baobab oil, liquid paraffin, Vaseline® intensive care lotion and Vaseline® petroleum jelly) in circular movements on the calf area over a diameter of 20 mm. The laboratory technician applied the test products in the morning and Aquaflux® and Corneometer® readings were recorded. The participants re-applied the products at home after bathing approximately 8 h later. Assessments were performed on days 1, 2, 3, 4, 5, 8, 10 and 12.

Visual assessment

Before application, 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. Visual assessments were performed on days 1, 2, 3, 4, 5, 8, 10 and 12. The raw data obtained from visual assessment readings for each test site compared to baseline, was calculated using Eq. (1).

Aquaflux® transepidermal water loss (TEWL)

The Aquaflux® instrument was used to measure water evaporation from the calf area based on the vapour pressure i.e. transepidermal water loss (TEWL) (Imhof, 2007; Farahmand et al., 2009). In most cases, if the skin water barrier is damaged the TEWL reading will be increased. The test products were applied on the five test sites and readings were recorded on days 1, 2, 3, 4, 5, 8, 10 and 12. Aquaflux® assessments for each test site compared to baseline, was calculated using Eq. (1).

Capacitance – Corneometer® moisture retention (hydration)

The Corneometer® instrument records the concentration gradient of water in the stratum corneum based on the capacitive measurement of the di-electrical constant of the stratum corneum. 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 dryness being rated < 30, dry being between 30 and 40 and normal > 40 (Heinrich et al., 2003). The test products were applied to the five test sites and readings were recorded on days 1, 2, 3, 4, 5, 8, 10 and 12. Corneometer® assessments for each test site compared to baseline, was calculated using Eq. (1).

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, Baobab oil, liquid paraffin, Vaseline® intensive care lotion and Vaseline® petroleum jelly) were rotationally randomised and 0.1 ml of 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 (Spiewak, 2008).

Data analysis

Statistical analyses were performed using Stata® 10 data 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. All the interpretations are performed at $\alpha = 0.05$ error rate.

Results and discussion

GCxGC-ToF-MS analysis

Table 1 shows the thirteen (13) methyl esters identified through comparison to the mass spectroscopy data of the standards and standards mixture from the refined Baobab seed oil characterised using two-dimensional gas chromatography. The average percentage of saturated fatty acids present in the oil sample was 34.6% with 28.7% monounsaturated and 36.7% polyunsaturated fatty acids. Major saturated fatty acids identified in the oil sample included palmitic (28.8%) and stearic (4.4%) acids. Unsaturated fatty acids including monousaturated oleic acid (25.1%) and a high quantity of polyunsaturated linoleic acid (36.0%) was detected. Other studies reporting the fatty acid content of baobab seed oil used one-dimensional GC–MS for quantification. De Caluwé et al. (2010) reported that the major fatty acids present in baobab seed oil

Table 1
Fatty acid composition of *Adansonia digitata* commercial oil as determined by GCxGC–MS.

Fatty acids detected	Chemical name	Molecular formula	Carbon bonds	Percentage detected (%) ± standard deviation	Retention time 1st Dimension (s)	Retention time 2nd Dimension (s)
Pelargonic acid	Nonanoic acid, methyl ester	$C_9H_{18}O_2$	C9:0	0.05 ± 0.07	502	0.74
Myristic acid	Tetradecanoic acid, methyl ester	$C_{18}H_{34}O_2$	C14:0	0.1 ± 0.14	852	0.82
Palmitic acid	Hexadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	C16:0	28.5 ± 0.42	972	0.85
Palmitoleic acid	7-Hexadecenoic acid, methyl ester, (*Z*)-	$C_{16}H_{30}O_2$	C16:1	0.25 ± 0.07	984	0.8
Heptadecanoic acid	Heptadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	C17:0	0.17 ± 0.04	1026	0.86
cis-10-Heptadecenoic acid	*cis*-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	C17:1	0.58 ± 0.02	1036	0.82
Linolelaidic acid	9,12-Octadecadienoic acid, methyl ester, (*E*,*E*)-	$C_{18}H_{32}O_2$	C18:2n6t	0.18 ± 0.03	1058	0.77
Stearic acid	Octadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	C18:0	5.85 ± 2.05	1080	0.88
Oleic acid	9-Octadecenoic acid, methyl ester, (*Z*)-	$C_{18}H_{34}O_2$	C18:1n9c	25.66 ± 0.95	1090	0.83
Linoleic acid	9,12-Octadecadienoic acid, methyl ester, (*Z*,*Z*)-	$C_{18}H_{32}O_2$	C18:2n6t	35.75 ± 0.35	1110	0.8
Linolenic acid	9,12,15-Octadecatrienoic acid, methyl ester, (*Z*,*Z*,*Z*)-	$C_{18}H_{30}O_2$	C18:3n3	0.5 ± 0.00	1142	0.75
Elaidic acid	9-Octadecenoic acid methyl ester (*E*)-,	$C_{18}H_{34}O_2$	C18:1n9t	2.8 ± 0.00	1144	0.86
Arachidic acid	Eicosanoic acid, methyl ester	$C_{20}H_{40}O_2$	C20:0	0.7 ± 0.28	1182	0.92

were palmitic (27%), linoleic acid (27%) and oleic (25%) acids while Namratha and Sahithi (2015) reported 36% oleic acid, 33% palmitic acid and 31% linolenic acid. A review article further reported that oleic acid in baobab seed oil samples ranged from 30 to 42%, linoleic acid from 20 to 35% and palmitic acid from 18 to 30%, (Vermaak et al., 2011). Fatty acids are considered to be a combination of triglycerides of higher saturated and unsaturated fatty acids. These compounds are esters of glycerol and higher fatty acids containing long aliphatic carbon chains. Depending on their fatty acid percentages they may exhibit a variety of properties which could be beneficial to the skin during daily cosmetic use (Zielińska and Nowak, 2014). With high percentages of palmitic (unsaturated), oleic (monounsaturated) and linoleic (polyunsaturated) acids as detected in the Baobab seed oil, the findings could suggest that the seed oil is of great importance as a cosmetic base to prevent transepidermal water loss by creating a protective layer on the epidermis (Zielińska and Nowak, 2014). In addition, linoleic acid (polyunsaturated fatty acid) is a natural component of sebum and plays a significant role in strengthening the lipid barrier of the epidermis and normalises the skin metabolism (Zielińska and Nowak, 2014). Furthermore, the presence of linoleic acid in the ceramides of the stratum corneum directly correlates with the permeability barrier function of the skin (Stages, 2012). Kanlayavattanakul and Lourith (2011) reported that Baobab seed oil could be regarded as a potential therapeutic topical application for acne treatment due to the high percentage of linoleic acid present. The oil sample contained 36% omega-6 (linoleic acid) which has been shown to contribute towards maintaining a healthy skin particularly when topically applied in cases of acne. The application of linoleic acid for oily and problematic skin may result in improved sebaceous gland function and the prevention of comedo-acne formation (Zielińska and Nowak, 2014).

Irritancy patch test

Irritancy patch testing is the principle test demonstrating irritant contact dermatitis. An irritant is a substance that would cause inflammation in almost every individual if applied in high concentration for a long period and will result in an irritant reaction but will not influence the immune system (Lewis, 2014). Visual erythema assessment (Fig. 3) revealed that sodium lauryl sulphate (positive control) was highly irritating to the skin, as expected, at all time intervals while de-ionised water and Baobab oil were non-irritating with low erythema scores. The visual grading scores of de-ionised water (negative control) were 0.13 (24 h), 0.13 (48 h), 0.08 (72 h) and 0.00 (96 h) while the results yielded after application of Baobab oil were 0.08 (24 h), 0.05 (48 h), 0.05 (72 h) and 0.06 (96 h). De-ionised water is chemically processed water using ion-exchange resin and it is considered suitable for the manufacturing of cosmetics. In recent years, Baobab oil has been added to the list of fixed oils commonly included in cosmetic products due to its high content of palmitic and oleic acids (Bazongo et al., 2014).

The visual erythema assessment results were corroborated through the Chromameter® results. Sodium lauryl sulphate (1%) was highly irritating, with average increased chroma a^* readings of 1.97 (24 h) 4.81 (48 h), 5.15 (72 h) and 4.52 (96 h), respectively (Fig. 4) compared to baseline readings of 0.64 (24 h) 1.05 (48 h), 0.11 (72 h) and 0.73 (96 h). Baobab oil was non-irritating to the skin with readings of 0.33 (24 h), 0.67 (48 h), 0.51 (72 h) and 0.51 (96 h). Although de-ionised water was also non-irritating at all time points, the observed readings at 48, 72 and 96 h were slightly higher than Baobab oil with values of 1.15, 0.96 and 0.74 respectively. These findings correlated to a report published by Burnett (2010) in the Cosmetic Ingredient Review (CIR, 2011) during which 100% Baobab oil was tested for dermal irritation potential using the MatTek EpiDerm™ MTT viability assay where tissue viability indicates

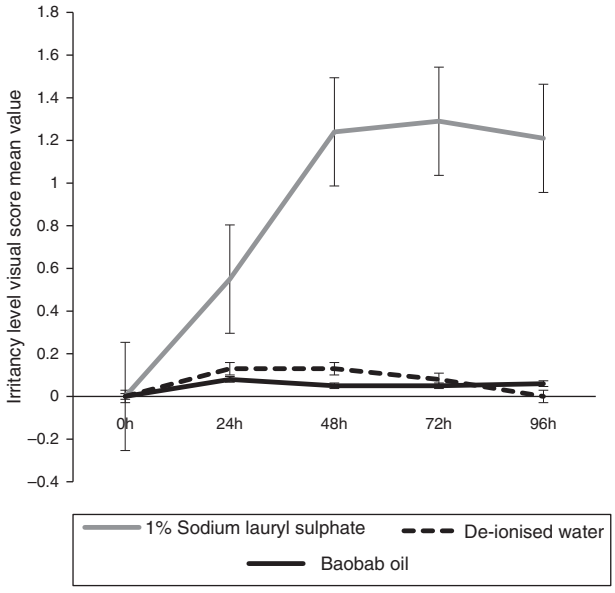


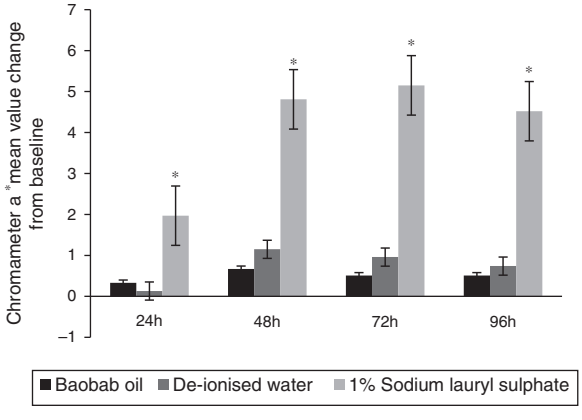
Fig. 3. Irritancy level visual score mean value when 1% sodium lauryl sulphate (positive control), de-ionised water (negative control) and Baobab oil (test product) was applied on the volar forearm area at 24, 48, 72 and 96 h time interval ($n = 19$).

skin irritation potential of test materials. The MatTek EpiDerm™ tissue samples were incubated with 100 μl of the test material for 1, 4 and 24 h together with the positive control (1% Triton X-100) which was incubated for 4 or 9 h, and the negative control (undosed tissues incubated for 4 h). The positive control caused expected irritation while Baobab oil caused no irritation (Burnett, 2010).

Moisture efficacy test

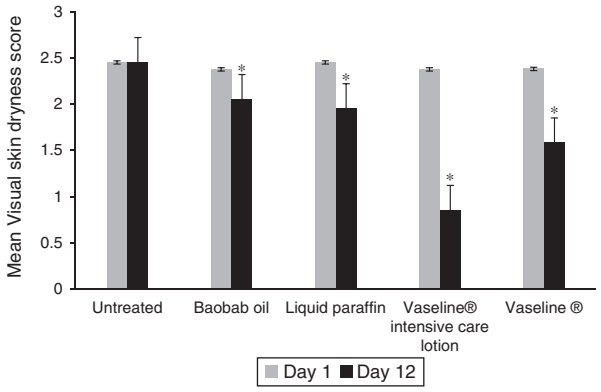
Visual assessment (2× magnifying lamp)

The results in Fig. 5 indicated that skin dryness recovery was significant for all test products compared to untreated skin with Vaseline® intensive care lotion showing the highest skin recovery potential. Baobab oil and liquid paraffin exhibited similar skin recovery properties. Baobab oil is well known for its non-siccative (non-drying) property and contains palmitic, oleic and linoleic acids which renders it a suitable cosmetic oil for the prevention of skin dryness (PhytoTrade Africa, 2012). The small molecular structure results in rapid absorption and palmitic and oleic acids have



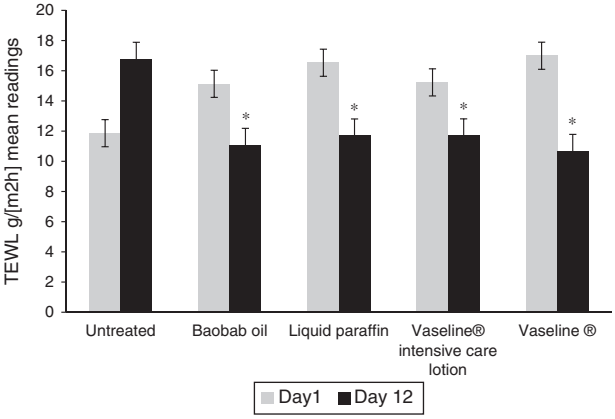
* Significant difference (P=.005) between Baobab oil and 1% sodium lauryl sulphate

Fig. 4. Chromameter® a^* mean change from baseline at 24, 48, 72 and 96 h time interval ($n = 19$) after de-ionised water (negative control), 1% sodium lauryl sulphate (positive control) Baobab oil (test product) were applied on the volar forearm area.



* Significant difference (P=.005) observed when baobab oil, liquid paraffin, Vaseline® intensive care lotion and Vaseline® were compared at Day 1 and 12.

Fig. 5. Visual skin dryness score readings at Day 1 and 12 (n = 20).



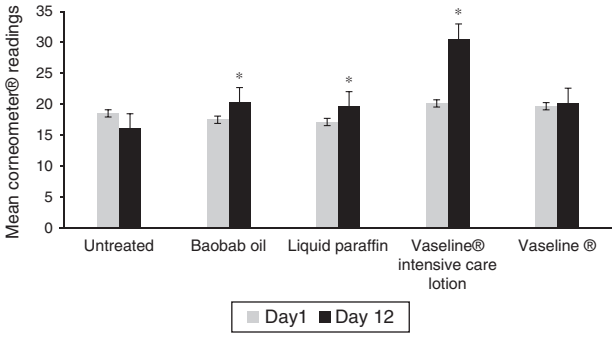
* Significant difference (P=.005) observed when baobab oil, liquid paraffin, Vaseline® intensive care lotion and Vaseline® were compared at Day 1 and 12.

Fig. 6. Mean TEWL g/[m^2 h] readings at Day 1 and 12 (n = 20).

been reported to be effective percutaneous absorption enhancers. It was further reported that linoleic acid (36.0% in the test sample) is the most frequently used fatty acid in cosmetic products as it moisturises the skin and aids in the healing process of dermatoses and sunburns (Bazongo et al., 2014).

Aquaflux® transepidermal waterloss (TEWL)

All of the test products significantly reduced TEWL over a test period of 12 days (Fig. 6). Vaseline® was particularly effective with a high negative value of −6.32 observed at change from baseline ($\Delta D_{12} - D_1$). Liquid paraffin (−4.84) and Baobab oil (−4.07) exhibited similar effectivity while Vaseline® intensive care lotion was the least effective at −3.53 (Table 2). Negative values confirm that skin barrier properties of the test products had an efficient impact on TEWL. A healthy skin has a healthy barrier function which depends on lipids that are present in the corneocytes. Any cause of lipid content reduction will lead to a compromised barrier and an increased



* Significant difference (P=.005) observed when baobab oil, liquid paraffin and Vaseline® intensive care lotion were compared at Day 1 and 12.

Fig. 7. Mean Corneometer® readings at Day 1 and 12 (n = 20).

TEWL (Bouwstra and Ponec, 2006). Baobab oil is considered an excellent oil to improve skin barrier function by preventing reduced TEWL that occurs as a result of an impaired barrier, due to its high saturated and unsaturated fatty acid content (Kamatou et al., 2011). In 2005, Venter reported that Baobab oil is a good quality natural moisturiser as determined through a survey conducted in the Louis Trichardt (in the Limpopo Province of South Africa) VhaVhenda region where the use of Eco products© oils were included as ingredients in bath soaps, body lotions and face moisturisers (Ham et al., 2010). In a recent patent by Engels and Gladbach (2009), an additive from the Baobab plant was used in formulations of cosmetic and dermatological emulsions for skincare as a moisturiser that creates a feeling of smoothness on the skin.

Capacitance-Corneometer® moisture retention test (hydration)

Techniques for the assessment of skin hydration are mostly based on the electrical properties of the stratum corneum and the Corneometer® instrument is regarded as the most popular approach to measure skin moisture (Lodén et al., 1995). The results (Fig. 7) indicated that significantly higher mean value readings were recorded for three of the four test products on day 12 with Vaseline® intensive care lotion readings the highest at 30.56 followed by Baobab oil at 20.28 and liquid paraffin at 19.61. There was no significant difference recorded for Vaseline® with a mean reading on day 1 of 19.70 and 20.18 on day 12. Baobab oil was less hydrating than Vaseline® intensive care lotion. These findings could be attributed to the nature of Vaseline® intensive care lotion being a water in oil emulsion that significantly induce changes in skin capacitance compared to Baobab oil which is a lipid-rich occlusive product, rich in essential fatty acids (palmitic, oleic and linoleic) and has the ability to penetrate into the epidermis with benefits of restoring the integrity of the skin cell membrane (Johnson, 1981; Jemec and Wulf, 1999). The high concentration of the linoleic and oleic acids present in Baobab oil renders it an excellent skin regenerating seed oil that mimics the three skin mechanisms of hydration, reinforcing the functionality of the lipidic mantle with lipophilic substances thus increasing the capacity to link water through hydrophilic substances and improving skin defence which

Table 2
TEWL 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	11.86 ± 2.21	16.77 ± 2.79	4.91
Baobab oil	15.14 ± 4.22	11.07 ± 2.26	−4.07
Liquid paraffin	16.53 ± 3.13	11.69 ± 2.11	−4.84
Vaseline® intensive care lotion	15.23 ± 3.88	11.70 ± 2.18	−3.53
Vaseline®	17.00 ± 3.02	10.67 ± 2.67[a]	−6.32

[a] Vaseline® statistically significantly different when day 1 mean readings were compared to day 12.

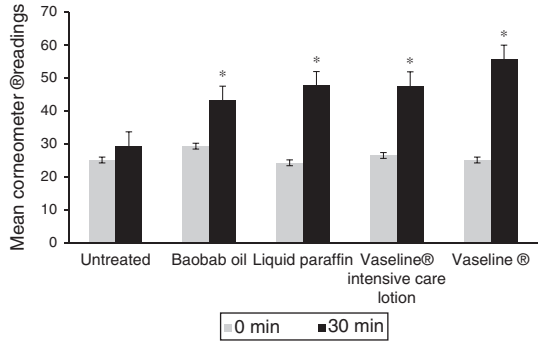


Fig. 8. Mean Corneometer® readings for all test products at time 0 min pre and immediate post wipe off at 30 min ($n = 20$).

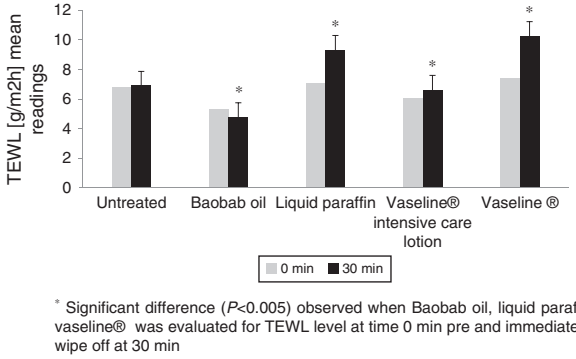


Fig. 9. Mean TEWL g/[m^2 h] readings of all test products at time 0 min pre and immediate post wipe off at 30 min ($n = 20$).

makes the seed oil a very good carrier oil and an essential ingredient in the cosmetic industry (Theodore, 1989; Darlenskì et al., 2011).

Fig. 8 reflects the occlusion potential in a 30-min wipe-off test. All test products improved moisture retention. Vaseline® demonstrated the best moisture retention property followed by liquid paraffin, Vaseline® intensive care lotion and Baobab oil. The occlusion properties are further illustrated in Fig. 9. Baobab oil application caused a reduction in the TEWL value with a mean reading of 4.77 g/[m^{2}h]. In contrast, Vaseline® intensive care at 6.62 g/[m^2 h], liquid paraffin at 9.32 g/[m^2 h] and Vaseline® at 0.25 g/[m^2 h] caused no reduction in TEWL. Vaseline® is a well-known petrolatum that has been used as an ingredient for over 50 years to smoothen out skin surface due to its occlusive property that causes moisture to accumulate in the stratum corneum thus preventing abnormal desquamating property of the skin (Kligman, 1978; Burton, 1983). Yet, according to Engels and Gladbach (2009), baobab is regarded as a preferred oil in skin care products due to its revitalising, moisturising and restructuring properties as a result of the balance in the saturated (palmitic) monounsaturated (oleic) and polyunsaturated (linoleic) acid composition (Engels and Gladbach, 2009).

Conclusion

Seed oils are an important component of many plants and currently play a significant role in the cosmetic and pharmaceutical industry. Baobab (*A. digitata* L.) seed oil is one such ingredient, which has rapidly gained popularity on global markets and is highly regarded by researchers in the field of ethnobotany and ethnopharmacology particularly for cosmetic benefit. This study aimed to elaborate on the scientific confirmation that Baobab oil has good cosmetic potential with its non-irritating, hydrating, moisturising and occluding benefit on the skin. In addition, the unique ratio of saturated and unsaturated fatty acid present in the oil renders it an oil which may have pharmacological properties that could be highly significant in the cosmetic industry when topically applied.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Authors' contributions

BK was involved in all the experimental work, analysed the data and drafted the paper. IV contributed to project planning and drafting of the manuscript. GK performed the fatty acid profiling using GCxGC-MS. BS planned and supervised the safety and efficacy studies and critically reviewed the manuscript. AV initiated the project and was involved in all aspects of planning in addition to critical reviewing of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank Tshwane University of Technology, the National Research Foundation and Restek for financial support. We further acknowledge participants and technicians at the photobiology Laboratory (Sefako Makgatho Health Sciences University) and Mr. Maupi Letsoalo (Tshwane University of Technology) for assistance with statistical analysis. We thank Sandie Burrows for preparing the botanical line diagram (Fig. 2).

References

Abuagla, A.M., Al-Deeb, M.A., 2012. Effect of bait quantity and trap color on the trapping efficacy of the, pheromone trap for the red palm weevil, *Rhynchophorus ferrugineus*. http://www.insectscience.org/12.120 (accessed September 2015).

Bazongo, P., Bassolé, I.H.N., Nielsen, S., Hilou, A., Dicko, M.H., Shukla, V.K.S., 2014. Characteristics, composition and oxidative stability of *Lannea microcarpa* seed and seed oil. Molecules 19, 2684–2693.

Bouwstra, J.A., Ponec, M., 2006. The skin barrier in healthy and diseased state. Biochim. Biophys. 1758, 2080–2095.

Burnett, C.L., 2010. Draft report of the vegetable oil group: Green Book 2 and 3, Cosmetic Ingredient Review. http://www.cir-safety.org/aug10.shtml (accessed January 2016).

Burton, C.K., 1983. Skin care composition, Patent Application Publication, Pub. No.: US 1982/4389418A.

Chindo, I.Y., Gushit, J.S., Olotu, P.N., Mugana, J., Takbal, D.N., 2010. Comparison of the quality parameters of the seed and condiment oil of *Adansonia digitata*. J. Am. Sci. 6, 990–994.

Clarys, P., Clijsen, R., Taeymans, J.O., Barel, A., 2011. Hydration measurements of the stratum corneum: comparison between the capacitance method (digital version of the Corneometer CM 825®) and the impedance method (Skicon-200EX®). Skin Res. Technol. 18, 316–323.

Cosmetic Ingredient Review, 2011. Final Report, Plant-derived Fatty Acid Oils as Used in Cosmetics. http://www.beauty-review.nl/wp-content/uploads/2014/05/plant-derived fatty-acid-oils as used in cosmetics.pdf (accessed September 2015).
Darlenskì, R., Kazandjieva, J., Tsankov, N., 2011. Skin barrier function: morphological basis and regulatory mechanisms. J. Clin. Med. Res. 4, 36–45.
De Caluwé, E., Halamová, K., Van Damme, P., 2010. *Adansonia digitata* L. A review of traditional uses, phytochemistry and pharmacology. Afrika Focus 23, 11–51.
Engels, P., Gladbach, B., 2009. Cosmetic preparations with an additive from the Baobab tree, Patent Application Publication, Pub. No.: US 2009/0324656 A1.
Farahmand, S., Tien, L., Hui, X., Maibach, H.I., 2009. Measuring transepidermal water loss: a comparative *in vivo* study of condenser-chamber, unventilated-chamber and open-chamber systems. Skin Res. Technol. 15, 392–398.
Gebauer, J., El-Siddig, K., Ebert, G., 2002. *Baobab* (*Adansonia digitata* L.): a review on a multipurpose tree with promising future in the Sudan. Gartenbauwissenschaft 67, 155–160.
Glew, R.H., Vanderjagt, D.J., Lockett, C., Grivetti, L.E., Smith, G.C., Pastuszyn, A., Millson, M., 1997. Amino acid, fatty acid and mineral composition of 24 indigenous plants of Burkina Faso. J. Food Compos. Anal. 10, 205–217.
Gruenwald, J., Galizia, M.D., 2005. Market brief in the European Union for selected natural ingredients derived from native species. *Adansonia digitata* L. Baobab. The United Nations Conference on Trade and Development (UNCTAD). Bio Trade Initiative/BioTrade Facilitation Programme (BTFP) Hardcastle.
Ham, C., Diederichs, N., Jacobson, M., Falcão, M., Howard, M., Mander, M., Manjoro, A., Dube, T., 2010. Increasing the rural livelihood benefits from natural plant product ventures in Southern Africa: Case Studies and Business Models, Commercial products from the wild research group (CPWild) Department of Forest and Wood Science, Stellenbosch University in collaboration with: The School of Forest Resources. Pennsylvania State University, Eduardo Mondlane University, Catholic University of Beira.
Heinrich, U., Koop, U., Leneveu-Duchemin, M.C., Osterrieder, K., Bielfeldt, S., Chkarnat, C., Degwert, J., Häntschel, D., Jaspers, S., Nissen, H.P., Rohr, M., Schneider, G., Tronnier, H., 2003. Multicentre comparison of skin hydration in terms of physical-physiological- and product-dependent parameters by the capacitive method (Corneometer CM 825). Int. J. Cosmet. Sci. 25, 45–53.
Imhof, R.E., 2007. *In-vivo* and *in-vitro* applications of closed-chamber TEWL measurements. Lecture Notes: Intensive course in Dermato-cosmetic Sciences.
Jemec, G.B.E., Wulf, H.C., 1999. Correlation between the greasiness and the plasticizing effect of moisturizers. Acta Derm. Venereol. 79, 115–117.
Jiang, Z.X., Delacruz, J., 2011. Appearance benefits of skin moisturization. Skin Res. Technol. 17, 51–55.
Johnson, Z.M., 1981. Method of treating psoriasis, Patent Application Publication, Pub. No.: US 1981/4,454,188.
Kamatou, G.P.P., Vermaak, I., Viljoen, A.M., 2011. An updated review of *Adansonia digitata*: a commercially important African tree. S. Afri. J. Bot. 77, 908–919.
Kanlayavattanakul, M., Lourith, N., 2011. Therapeutic agents and herbs in topical application for acne treatment. Int. J. Cosmet. Sci. 33, 289–297.
Kothiyal, S.K., Semwal, D.K., Badoni, R., Rawat, U., 2010. GC–MS analysis of fatty acids and the antimicrobial activity of *Ilex dipyrena* Wallich leaves. Asian J Tradit. Med. 5, 153–157.
Kligman, A.M., 1978. Regression methods for assessing the efficacy of moisturizers. Cosmet. Toilet. 93, 27–35.
Lewis, V., 2014. Patch Testing for Skin Allergies. http://www.netdoctor.co.uk/../examinations (accessed December 2015).
Lodén, M., Hagforsen, E., Lindberg, M., 1995. The presence of body hair influences the measurement of skin hydration with the Corneometer. Acta Derm. Venereol. 6, 449–450.
Munthali, C.R.Y., Chirwa, P.W., Akinnifesi, F.K., 2012. Phenotypic variation in fruit and seed morphology of *Adansonia digitata* L. (Baobab) in five selected wild populations in Malawi. Agrofor. Syst. 85, 271–290.
Namratha, V., Sahithi, P., 2015. Baobab: A review about "The Tree of Life". Int. J. Adv. Herbal Sci. Technol. 1, 20–26.
Nkafamiya, I., Osemeahon, S., Dahiru, D., Umaru, H., 2007. Studies on the chemical composition and physicochemical properties of the seeds of Baobab (*Adasonia digitata*). Afr. J. Biotechnol. 6, 756–759.
Osman, M.A., 2004. Chemical and nutrient analysis of Baobab (*Adansonia digitata*) fruit and seed protein solubility. Plant Foods Hum Nutr. 59, 29–33.
Palgrave, K.C., 1983. Trees of Southern Africa. Struik Publishers, South Africa.
PhytoTrade Africa, 2012. Baobab tree and its fruit. http://www.phytotrade.com/products/baobab/ (accessed January 2016).
Rahul, J., Jain, M.K., Singh, S.P., Kamal, R.K., Naz, A.A., Gupta, A.K., Mrityunjay, S.K., 2015. *Adansonia digitata* L. (Baobab): a review of traditional information and taxonomic description. Asian Pac. J. Trop. Biomed. 5, 79–84.
Rossé, A.D., Harynuk, J.J., 2010. Methodology for the derivatization and quantification of dialkyl phosphate esters in petroleum samples. Anal. Methods. 2, 1176–1179.
Sidibe, M., Williams, J.T., 2002. Baobab – *Adansonia digitata*. Fruits for the future 4. International Centre for Underutilised Crops, United Kingdom, Southampton.
Spiewak, R., 2008. Patch testing for contact allergy and allergic dermatitis. Open Allergy J. 1, 42–51.
Stages, L., 2012. Essential fatty acids and skin health. http://www.lpi.oregonstate.edu/mic/..health/skin-health/nutrient (accessed January 2016).
Theodore, J., 1989. Baobab encyclopaedia Americana Grolier incorporated 3, 207.
Venter, S.M., Witkowski, T.F., 2010. Baobab (*Adansonia digitata* L.) fruit production in communal and conservation land-use types in Southern Africa. For. Ecol. Manag. 259, 294–300.
Venter, S.M., (Ph.D. thesis) 2012. The ecology of Baobabs (*Adansonia digitata* L.) In relation to sustainable utilization in Northern Venda. University of the Witwatersrand, South Africa, pp. 200.
Vermaak, I., Kamatou, G.P.P., Komane-Mofokeng, B., Viljoen, A.M., Beckett, K., 2011. African seed oils of commercial importance – cosmetic applications. S. Afr. J. Bot. 77, 920–933.
Wickens, G.E., Lowe, P., 2008. The Baobabs: Pachycauls of Africa, Madagascar and Australia. Springer Science and Business Media, United Kingdom.
Zielińska, A., Nowak, I., 2014. Fatty acids in vegetable oils and their importance in cosmetic industry. Chemik 68, 103–110.
Zimba, N., Wren, S., Stucki, A., 2005. Three major tree nut oils of southern central Africa: their uses and future as commercial base oils. Int. J. Aromather. 15, 177–182.