In Vivo Skin Hydration And Anti-Erythema Effects Of *Aloe vera*, *Aloe ferox* And *Aloe marlothii* Gel Materials After Single And Multiple Applications

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ABSTRACT

Background: Although *Aloe vera* extracts have exhibited various effects on the skin, less is known about the biological activities of other aloe species on the skin. **Objective:** To investigate the skin hydrating and anti-erythema activity of gel materials from *Aloe marlothii* A. Berger and *A. ferox* Mill. in comparison to that of *Aloe barbadensis* Miller (*Aloe vera*) in healthy human volunteers. **Methods:** Aqueous solutions of the polisaccharidic fractions of the selected aloe leaf gel materials were applied to the volar forearm skin of female subjects. The hydration effect of the aloe gel materials were measured with a Corneometer® CM 825, Visioscan® VC 98 and Cutometer® dual MPA 580 after single and multiple applications. The Mexameter® MX 18 was used to determine the anti-erythema effects of the aloe material solutions on irritated skin areas. **Results:** The *A. vera* and *A. marlothii* gel materials hydrated the skin after a single application, whereas the *A. ferox* gel material showed dehydration effects compared to the placebo. After multiple applications all the aloe materials exhibited dehydration effects on the skin. Mexameter® readings showed that *A. vera* and *A. ferox* have anti-erythema activity similar to that of the positive control group (i.e. hydrocortisone gel) after 6 days of treatment. **Conclusion:** The polysaccharide component of the gel materials from selected aloe species has a dehydrating effect on the skin after multiple applications. Both *A. vera* and *A. ferox* gel materials showed potential to reduce erythema on the skin similar to that of hydrocortisone gel.

KEYWORDS: *Aloe ferox; Aloe marlothii; Aloe vera;* Anti-erythema, Herbs, Skin Hydration

INTRODUCTION

There are more than 360 species of aloe known worldwide[1], of which 160 are indigenous to South Africa.[2] Therapeutic uses of aloe are based almost exclusively on research obtained for *A. vera*, therefore it is vital for scientists to investigate and determine the medicinal uses and pharmaceutical applications of other aloe species.[3] The main polysaccharide of *A. vera* gel, acetylated mannan (Acemannan), which consists of a polydispersed β-1,4-linked mannan substituted with *O*-acetyl groups[4] is a proprietary substance covered by many patents.[5] Commercially available Carrisyn™ by Carrington Laboratories, Texas, is one example of an acemannan product.[5, 6] Acemannan possesses biological activity, particularly with regards to the skin as it appears to be a superb emollient with very important moisturizing capabilities.[4]

There is much controversy over the active ingredient(s) in *A. vera* and several mechanisms of action have been suggested.[7] Polysaccharides as well as miscellaneous bioactive constituents have been identified from the leaves and roots of the *A. vera* plant.[8] Polysaccharides can exhibit physiological as well as pharmacological activities, and therefore it can be assumed that the mucilaginous gel of the aloe consisting mainly of polysaccharides holds the secret to the medicinal properties of this family of plants.[7] However, it is believed that the phytoconstituents in the aloe plant encourage healing in a concerted action rather than acting alone.[8]

The retention of water and the hydration balance in the superficial skin layers ensures the skin's elasticity and flexibility[9] as dehydration of the skin causes a decrease in skin elasticity.[10] Freeze-dried *A. vera* extract showed a humectant mechanism when improving skin moisture as it significantly increased the water content of the stratum corneum (SC) although it did not alter the transepidermal water loss (TEWL).[11] In another study, *A. vera* gel did not show any anti-

inflammatory effects after 24 h; although a significant effect could be detected after 48 h. Onset of the effect was delayed, but was stronger than that of the 1% hydrocortisone in placebo gel although weaker compared to the commercially available corticosteroids.[12]

In the present study, the hydration and anti-erythema effects of *A. vera* (*Aloe barbadensis* Miller), *A. ferox* (*Aloe ferox* Mill.) and *A. marlothii* (*Aloe marlothii* A. Berger) gel materials were evaluated in human subjects. The instruments used (i.e. Corneometer® CM 825, Visioscan® VC 98, Cutometer® dual MPA 580 and Mexameter® MX 18) are considered to be non-invasive and therefore cause no harm or discomfort during the *in vivo* investigation of the skin parameters, while accurately measure different aspects of the skin.[13]

MATERIALS AND METHODS

Plant material preparation

Organic solvent insoluble residues (or polysaccharidic fractions) were isolated from the leaf gel materials of the three selected aloe species (i.e. *A. vera, A. ferox* and *A. marlothii*) as described below.

Ethanol insoluble residues were separated from *A. vera* and *A. marlothii* gel materials according to a method previously described.[14, 15] The starting material for *Aloe vera* was dehydrated gel powder (Daltonmax 700®) obtained from Improve USA, Inc. (Texas, United States of America). The starting material for *Aloe marlothii* was obtained from natural populations near Koster in the North West Province of South Africa for which a specimen voucher (PUC 11151) was deposited at the Herbarium of the North-West University, South Africa. The traditional hand-filleting method for processing of the *A. marlothii* leaves were used as it was developed to prevent

contamination of the gel with the yellow sap (latex/aloin).[16] The *A. ferox* 200:1 gel powder was obtained from Organic Aloe (Pty) Ltd which consists of the methanol insoluble gel polisaccharidic fraction obtained from the leaves of *A. ferox* natural populations near Albertinia in the Western Cape Province of South Africa.

Nuclear magnetic resonance (^{1}H-NMR) fingerprinting of aloe gel materials

Approximately 30 mg, 3mg and 1mg of the *A. ferox*, *A. vera* and *A. marlothii* precipitated, dried gel materials were dissolved in 1.5 ml deuterium oxide (D_2O; Merck, South Africa) respectively. These solutions were filtered and a small quantity of 3-(trimethylsilyl) propionic acid-D4 sodium salt (Merck, South Africa) was added. ^{1}H-NMR spectra of the solutions were obtained in an Avance III 600 Hz NMR spectrometer (Bruker, Germany). The resultant ^{1}H-NMR spectra were used to identify certain marker molecules (e.g. aloverose, glucose and malic acid) in the test solutions, which are known to be present in aloe leaf gel materials.

Aloe and hydrocortisone gel preparations for application to the skin

Each of the selected aloe gel materials (i.e. *A. vera, A. ferox and A. marlothii*) was dissolved in ultrapure deionized water to obtain a 3% w/v solution with a gel structure. The composition of the 1% w/v hydrocortisone gel used as the positive control during the erythema study is given in Table 1.

To prepare the 1% w/v hydrocortisone gel, Carbopol Ultrez 20 was homogenized with distilled water for approximately 30 min using a Heidolph® Diax 600 homogenizer (Heidolph, Germany) at approximately 536 rpm. The polyethylene glycol was melted and hydrocortisone acetate was slowly added together with ethanol. This mixture was slowly added to the homogenized

Carbopol Ultrez 20 dispersion in distilled water while continuously homogenizing the mixture. Tri-ethanol amine was used to adjust the pH of the gel to approximately 6.8.

[Insert Table 1 here]

Clinical study protocol

This study was carried out according to the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of the North-West University, South Africa under the title of "(*In vivo)* Cosmetic efficacy studies" (NWU-0097-10-A5). A flow diagram of the enrollment, allocation and follow-up of the volunteers (adjusted from and based on CONSORT flow diagram) is given in Fig. 1.[17, 18]

[Insert Figure 1 here]

Race, sex and age are considered to be important variables that can affect skin function and biophysical measurements, which should be controlled or standardized and it has therefore been suggested that studies should be designed within the same age range, ethnic group and sex.[19] Consequently, to obtain a homogenous population, volunteers were selected by strict inclusion/exclusion criteria. Female volunteers between the ages of 20 and 40 years with a good health state and Fitzpatrick skin types II and III (based on Mexameter® readings on untreated skin) were included in the study. Exclusion criteria included history of eczema, psoriasis within 6 months prior to study, allergic skin reaction 30 days prior to the study, pregnant or lactating woman, having undergone cosmetic surgery within previous 12 months, recent treatment with aloe containing products, uncontrolled systemic disease, dermatological illnesses or conditions that may interfere with neuromuscular function such as myasthenia gravis, treatment with topical

or systemic drugs that may influence the test results and recent history of intolerance to drugs and/or cosmetic products. The study population included a total number of 59 subjects with 19, 23 and 17 volunteers that participated in the short-term, long-term and erythema studies, respectively. In general, a minimum of 12 subjects is required to complete the study for statistical data evaluation.[9]

Prior to the study, informed consent was obtained from each volunteer. A qualified medical practitioner was on standby throughout the study in case of medical emergencies relating to activities of this study. A washout period was started 7 days prior to onset of the study.[9] During this time and for the remainder of the study, the volunteers were only allowed to wash with Dove® soap. The application of other skin products, moisturizers, body powders and perfume on or near the test areas were prohibited during the study. On the day of the measurements the use of alcohol, caffeine and vasoactive medications was prohibited as they may alter skin microcirculation which can indirectly influence the skin hydration profile.[9]

The volar forearm was chosen as the anatomical test site due to its relatively large available skin surface area, its hairlessness and the fact that it contains only a small number of sebaceous glands. It can also be used to assess the efficacy of facial products as it was found to be an excellent representation of the facial skin.[10] The wrist and cubital fossa (anatomic occlusion zone) were avoided.[9]

The temperature of the environment where measurements were made on the volunteers (i.e. the Cosmetic Efficacy Laboratory, North-West University, Potchefstroom Campus, South Africa) was controlled at 20 to 25°C and 50 ± 10% relative humidity.[9] Volunteers acclimatized in the Cosmetic Efficacy Laboratory for at least 30 min prior to measurements to allow full skin

adaptation. In order to exclude the effect of circadian rhythms, measurements were performed during the same time of day. Direct sun light and air flow was also avoided.[9]

Due to the differences in the hydration level of the stratum corneum between individuals, the baseline hydration levels (before the application of the aloe gels to the skin) were measured to function as an internal control.[13] One test field on the volar forearm was left untreated and measured at every time point so that each volunteer served as her own control. The test materials were applied with a glove-covered finger to avoid interference with sebum and sweat secretion.[9] All the test material solutions were freshly prepared every week, code named and neither the subjects nor the technical assistant knew the content of the treatment groups and therefore a double-blind study was conducted.

Single application (short-term) and multiple applications (longer-term) hydration study

During the short-term and longer-term studies, the guidelines for the assessment of SC hydration by The European Group for Efficacy Measurements on Cosmetics and Other Topical Products (EEMCO) were followed.[20] The volar forearm skin of the dominant arm (short-term study) and non-dominant arm (longer-term study) was divided into 5 sites of 6 cm^2 each bordered with a cosmetic pencil. Space was left open between the sites to prevent any cross-contamination. The first three sites on the forearm were treated with 0.5 ml of a test material (i.e. 3% w/v *A. vera, A. marlothii, A. ferox* gel solution) and the fourth site was treated with deionized ultrapure water (placebo). The fifth site was left as 'untreated skin' (control).

A single application study (short-term study) was performed before the longer-term multiple application study commenced[20] and to investigate the short-term hydration effects of the aloe

gel materials. A baseline reading (T_0) was taken followed by measurements at 30 (T_1), 90 (T_2) and 150 (T_3) min after application of the aloe gel materials.[20]

During the long-term study, the aloe gel solutions were each applied twice daily (i.e. in the morning and in the evening). A baseline reading (T_0) was taken, followed by measurements after 1 (T_1), 2 (T_2), 3 (T_3) and 4 (T_4) weeks after commencement of treatment. Measurements were performed 12-20 h after the last treatment was applied in the evening prior to the day of measurements.

The following instruments were used to measure the hydration effect of the test materials on the skin: a Corneometer® CM 825 and Visioscan® VC 98 (Courage + Khazaka Electronic GmbH, Germany) during the short- and long-term studies and a Cutometer® dual MPA 580 (Courage + Khazaka Electronic GmbH, Germany) during the long-term study.

The Corneometer® operates at a low frequency (40-75 Hz) and measures the electrical capacitance of the SC. Since water has the highest di-electrical constant in the skin, capacitance values will increase with an increase in water content/skin hydration. The mean of three measurements are displayed in arbitrary units ranging from 0-130.[9, 20, 21] The Corneometer® only measures the moisture of the upper layers of the epidermis to an approximate depth of 10 μm due to the very small penetration depth of the electrical scatterfield.[10, 21]

The Visioscan® was used to analyze the skin topography. An image (6 x 8 mm) of the skin was taken by a built in CCD-camera. The Visioscan® was connected to a computer by means of an image digitalization unit which configures the image in 256 grey levels pixel by pixel, where black was resembled by 0, and white by 255.[22]

Energy (NRJ), entropy (ENT) and homogeneity (HOM) texture parameters were used in this study. The aforementioned parameters analyze differences in colors of neighbored pixels. Energy is an indicator for the homogeneity of an image, entropy indicates the "mess" of an image and homogeneity indicates the uniformity of an image. An increase in these parameter values indicates an increase in skin hydration.[22]

In addition to the hydration measurements with the Corneometer®, the Cutometer® was used to assess the skin's viscoelastic properties, which indirectly relate to skin hydration.[19] Skin viscoelasticity is the ability of the skin to return to its original position, after a certain delay, once a force is removed.[23]

The principle by which the Cutometer® measures the elasticity of the upper skin layer is based on the suction method. The skin was drawn into the aperture of the probe due to a negative pressure that was created in the probe, and released again after a specific time. A non-contact optical measuring system determined the penetration depth. During the measurement, the skin's resistance to the negative pressure (firmness) and its capability to return to its original position (elasticity) were displayed as curves (penetration depth in mm/time). Measurement parameters were calculated from these curves of which the R-parameters were used.[23] The curves represented the viscoelastic properties of the skin and consisted of two phases, suction and relaxation phase, which each consist of two parts. Fig. 2 illustrates a typical skin deformation curve obtained by the Cutometer®.

[Insert Figure 2 here]

During the first part of the suction phase, the skin enters the probe straight and immediately. The immediate elastic deformation/distension of the skin is expressed in Ue. The second part

represents the viscoelastic suction part, Uv, when the skin "creeps" into the probe (delayed distension). Uf is the maximum penetration after suction time (final distension/skin distensibility). The complete relaxation (Ua) can be divided into two parts: the immediate elastic return/retraction (Ur) and the flat, viscoelastic part (Ua-Ur). The overall ability of the skin to return to its original shape is shown by Uf – Ua. R (resilient distension) is the residual deformation at the end of the measuring cycle.[23, 24]

During this study, Mode 1 was used, which is a measurement with constant negative pressure. A probe with a 2 mm diameter aperture was used with 350 mBar pressure applied to suck the skin into the probe. The measurement consisted of two cycles with 5 s suction followed by 5 s of relaxation. The total skin deformation (R0 = Uf), the gross-elasticity (R2 = Ua/Uf), the viscoelastic to elastic extension (R6 = Uv/Ue), the biological elasticity (R7 = Ur/Uf) and the complete relaxation (R8 = Ua) were determined.[23; 25]

The closer the values of the R2- and R7-parameters are to unity (i.e. 1), the more elastic the skin is (positive percentage change as a function of treatment). The smaller the value of R6 is, the higher the skin elasticity becomes (negative percentage change as a function of treatment). The R8-parameter indicates a greater ability of the skin to return to its original state when its value is closer to that of the R0 value (positive change as a function of treatment).[23]

Erythema study

The effect of the materials on skin erythema was performed according to the guidelines on sodium lauryl sulfate (SLS) exposure tests from the Standardization Group of the European Society of Contact Dermatitis.[26]

Baseline readings (T_0) were taken before application of the Finn Chambers® (with an internal diameter of 8 mm containing filter papers) on Scanpor® (SmartPractice®, Mednom, Cape Town, South Africa) to the volar forearm. Sodium lauryl sulfate (SLS, 99% purity, Merck, South Africa) was dissolved in distilled water to obtain a 1% w/v solution. One Finn Chamber® was attached without being filled with any solution to function as untreated skin (negative control). The rest of the Finn Chambers® were filled with 20 µl of the 1% w/v SLS solution and all chambers were applied on the volar forearm skin of the dominant arm under occlusion for a period of approximately 22.5 h to induce erythema. Similar studies where SLS was applied for 24 h under occlusion showed an initial exsiccation of the SC, followed by hyperhydration (swelling of corneocytes). Therefore, a certain time period after the skin has been irritated is required before the first measurement can be performed. This prevented false readings due to the occlusive effect of the Finn Chambers and the initial hyperhydrating effect of SLS.[9, 27] Thus, the first measurement (T_1) was performed 24 h after removal of the Finn Chambers®.[28] T_1 was compared to T_0 to ensure that erythema was induced. The test materials (i.e. 3% w/v *A. vera*, *A. marlothii*, *A. ferox* gel solutions), 1% w/v hydrocortisone gel (positive control) and deionized ultrapure water (placebo) were applied to the volunteers where erythema was induced. Thereafter the test materials as well as 1% w/v hydrocortisone gel and water (placebo) were applied twice daily, in the morning and in the evening, for the rest of the study period. The second measurement (T_2) was made on the 2nd day following 1 day of treatment, and the final measurement (T_3) was on the 7th day following 6 days of treatment.

The Mexameter® MX 18 (Courage + Khazaka Electronic GmbH, Germany) was used during the erythema study. The Mexameter® MX 18 measures the two components mainly responsible for skin color, the content of melanin and hemoglobin (erythema) in the skin. Two different

wavelengths are utilized to measure the absorption capacity of the skin when erythema is measured. The one wavelength was chosen to avoid other color influences (e.g. bilirubin); whereas the second wavelength corresponds to the spectral absorption peak of hemoglobin. Results obtained were shown as indices on a scale from 0-999 which will ensure that even the smallest changes in color were observed.[29] After irritation with SLS, the hemoglobin content values (T_1) are expected to be higher than the baseline readings (T_0) to indicate erythema. For the test materials to be effective as anti-erythema agents, the hemoglobin content values should decrease after treatment (T_2 and T_3).

Data analysis

The effect of the test material are presented as percentage change (as calculated by Equation 1) relative to the initial conditions (T_0) and to untreated values ($T_{0\ (untr)}$ and $T_{n\ (untr)}$) in terms of all the parameters measured in each part of the study.

$$\% \text{ Change} = \left[\frac{T_n - T_0}{T_0} \times 100\right] - \left[\frac{T_{n(untr)} - T_{0(untr)}}{T_{0(untr)}} \times 100\right] \qquad \text{Equation 1}$$

Where T_n represents the value for: n = 30, 90 and 150 min in the short-term hydration study; n = 1, 2, 3 and 4 weeks in the long-term hydration study.

Equation 2 was utilized in the erythema study.

$$\% \text{ Change} = \left[\frac{T_n - T_1}{T_1} \times 100\right] - \left[\frac{T_{n(untr)} - T_{1(untr)}}{T_{1(untr)}} \times 100\right] \qquad \text{Equation 2}$$

Where T_n represents the time of measurement after skin irritation and n = 1 at 24 h after removal of Finn chambers, n = 2 on the 2nd day (i.e. one day after application of test materials) and n = 3 on the 7th day (i.e. 6 days of application of test materials).

Statistical data analysis

Statistical analyses for the single application and multiple applications studies were carried out using IBM SPSS Statistics Version 20.[30] Graphs were drawn as visual aids in order to investigate the effects of the different treatments on the skin. A 2-Way Repeat Measure ANOVA (analysis of variance) Design was followed in this study as measurements were repeated over time and each subject was exposed to all of the different treatments. The basic method generally used for this type of design is Repeated Measure Analysis of Variance (ANOVA) which assumes independent data (compound symmetry). However, given the dependence structure in the data, this assumption was violated. Therefore, mixed models were used to assess the influence of treatment and time on the various measures observed. Mixed model analysis allows a variety of variance-covariance structures[31], in this study unstructured or first-order autoregressive (AR(1)) covariance structures were used. The two covariance structures were compared using -2Restricted Log likelihood and Akaike's Information Criterion (AIC) measures. Mixed models employ both fixed and random effects. Fixed effects (such as treatment and time) have levels that are of primary interest. Random effects (such as subjects) are not of primary interest.[31] In order to test for significant differences between the fixed effects, test statistics (F) and probability (p) values were obtained by the Type III Test for Fixed Effects.

Statistical analysis for the erythema study was carried out using Microsoft Excel 2010. The Student *t*-test was performed to test for statistical significant differences between the different treatments and the different times. Statistical significance was tested at a 10% (0.10) level of significance. A p-value < 0.1 indicates statistically significant differences between the values that were compared.

RESULTS AND DISCUSSION

Percentage yield of ethanol insoluble residue

After lyophilisation of the precipitated ethanol insoluble gel materials, the average percentage yield obtained for *A. vera* was 13.81% and for *A. marlothii* it was 4.41% of the total pulp material.

Nuclear Magnetic Resonance (^{1}H-NMR) fingerprinting

The ^{1}H-NMR spectra of *A. vera*, *A. marlothii* and *A. ferox* gel materials (i.e. the precipitated ethanol/methanol residues or also referred to as the polisaccharidic fraction) are given in Fig. 3a, 3b and 3c, respectively. Aloverose (partly acetylated polymannan or acemannan), glucose and malic acid, which serve as marker molecules for identification of fresh *A. vera* gel material, were detected by ^{1}H-NMR spectroscopy in the *A. vera* precipitated gel material. Aloverose was not detected in the *A. marlothii* (Fig. 3b) and *A. ferox* (Fig. 3c) precipitated gel materials, although glucose and malic acid were present as previously shown.[32]

[Insert Figure 3 here]

Short-term hydration study

A visual representation for the results obtained in this study with the Corneometer® and the Visioscan® (entropy, homogeneity, energy parameters) instruments are depicted in Fig. 4a to 4d.

[Insert Figure 4 here]

As can be seen from the Corneometer® values (Fig. 4a) obtained, *A. vera* and *A. marlothii* gel materials proved to have a larger hydrating effect than deionized water on the skin at 30 (T_1), 90 (T_2) and 150 (T_3) min after a single application. Both these aloe materials exhibited a higher percentage increase in skin hydration at 30 and 150 min after application and the lowest percentage increase was observed at 90 min after application. *Aloe marlothii* gel material caused a slightly higher hydration effect than *A. vera* gel material at 90 and 150 min after application. *Aloe ferox* gel material showed a dehydrating effect (negative percentage change) over the short-term study. This dehydrating effect of *A. ferox* gel material became less over time after application. Deionized water initially dehydrated the skin at 30 and 90 min after application, but showed a skin hydration effect at 150 min after application. This increase in skin hydration 150 min after application with deionized water was however less than that obtained with *A. vera* and *A. marlothii* gel materials.

Fig. 4b, 4c and 4d indicate that *A. marlothii* gel material improved skin entropy, homogeneity and energy to a larger extent than the *A. vera* and *A. ferox* gel materials as well as the deionized water at 30 and 90 min after application. *Aloe vera* gel material improved skin entropy, homogeneity and energy (Fig. 4b to 4d) to a larger extent than deionized water and *A. ferox* at 30 and 90 min after application. *Aloe ferox* gel material increased skin energy more than deionized water 30 and 90 min after application.

The p-values revealed statistical significant effects for the treatment and the time-treatment interaction of the Corneometer® measurements (p = 0.0001 and 0.017, respectively) and for the homogeneity parameter (p = 0.066 and 0.084, respectively). Statistical significant effects for treatment was found for the entropy (p = 0.036) and energy (p = 0.00001) parameter. The significant interaction between time and treatment reveals that the effect of the treatment on the Corneometer® measurements and the homogeneity parameter depends on time. However, it is important to note that the significance of the interaction effect of the Corneometer® measurements may be induced by the dominant influence of treatment given its F-value of 10.076, which is 5.6 times larger than that of time (F-value: 1.788).

Pairwise comparisons with a Bonferroni adjustment revealed no significant difference between the levels of time for any of the skin hydration parameters investigated. Pairwise comparisons with a Bonferroni adjustment between the different treatments revealed a statistical significant difference between *A. ferox* and *A. marlothii* gel materials (p = 0.007), between *A. ferox* and *A. vera* gel materials (p = 0.023) and between *A. ferox* and deionized water (p = 0.016) with the Corneometer® measurements. This indicates that *A. ferox* gel material differed significantly from the other aloe materials and the placebo as it showed a dehydrating effect (negative percentage change) over the short-term study. The short-term hydrating effects of *A. vera* and *A. marlothii* was not statistically significantly different from that of deionized water. Skin entropy end homogeneity showed a statistical significant difference between *A. ferox* and *A. marlothii* with p-values of 0.068 and 0.067, respectively. A statistical significant difference existed between *A. ferox* and *A. marlothii* (p = 0.003) and between *A. marlothii* and deionized water (p = 0.003) for the measured energy parameter. This indicates that *A. marlothii* gel material improved the general state of the skin more than deionized water.

Researchers previously investigated the effects of a single application and multiple applications (1- and 2-week daily application) of formulations containing 5% w/w trilaureth-4 phosphate-based blend supplemented with 0.10%, 0.25% or 0.50% w/w *A. vera* extract on the volar forearm of volunteers. From the single application study it was seen that after an hour only the 0.50% w/w formulation significantly increased the stratum corneum (SC) water content (measured with Corneometer® CM 825) when compared to the vehicle. At 2 and 3 h after application, the 0.25% and 0.50% w/w formulations significantly increased the SC water content when compared to the vehicle.[11] It is important to note that these formulations did not contain pure aloe gel materials dissolved in water alone, but contained other excipients that may have had an interactive role in the effects obtained on the skin.

Longer-term hydration study

Results obtained from the present multiple application study as measured by the Corneometer® are in contrast with the results of the single application study where *A. marlothii* and *A. vera* gel materials showed to hydrate the skin more than deionized water. Fig. 5 represents the percentage change in skin hydration relative to the initial conditions (T_0) as measured by the Corneometer® after 1 (T_1), 2 (T_2), 3 (T_3) and 4 (T_4) weeks of treatment. From this figure it can be seen that *A. marlothii* and *A. vera* gel materials had a predominantly dehydrating effect on the skin over the 4 week period of treatment. *Aloe marlothii* gel material dehydrated the skin the most of all the aloe materials investigated from week 1 to week 4. *Aloe ferox* gel material showed a 1.1% increase in skin hydration after 1 week of treatment; but thereafter also exhibited a dehydrating effect on the skin. The dehydration effect caused by *A. ferox* gel material was less than caused by the other two aloe gel materials. The placebo (i.e. deionized water), in contrast to the test materials, increased the level of skin hydration over the 4 week time period.

[Insert Figure 5 here]

Investigation of the skin's topography with the Visioscan® supported the findings obtained with the Corneometer®. The entropy, homogeneity and energy parameters followed a similar pattern as can be seen from Fig. 6a, 6b and 6c, respectively. The three aloe gel materials as well as the placebo increased the skin entropy (Fig. 6a) slightly after the first week of treatment, with *A. ferox* gel material showing the highest percentage increase. A decrease in skin entropy was observed after 2, 3 and 4 weeks of treatment with the *A. marlothii* and *A. ferox* gel materials as well as the placebo (i.e. deionized water). *Aloe vera* gel material showed a small percentage increase in skin entropy after 2 weeks of treatment, thereafter also decreasing after 3 and 4 weeks of treatment. The skin homogeneity almost followed the same pattern, except that initially *A. marlothii* gel material showed to increase this parameter the most after 1 week of treatment.

An increase in skin energy was shown with all the aloe gel materials after the first week of treatment with *A. marlothii* gel material having the highest increase. *Aloe vera* and *A. marlothii* gel materials both showed a decreasing effect on skin energy after 3 weeks of treatment, an increase was observed after 4 weeks of treatment. *Aloe ferox* gel material exhibited an increase in skin energy after 1 and 3 weeks, and a decrease in skin energy after 2 and 4 weeks of treatment. Deionized water increased skin energy after the first week, thereafter decreasing it after 2, 3 and 4 weeks of treatment. However, none of these differences between the treatments were found to be statistically significant.

[Insert Figure 6 here]

R-parameters are highly dependent on moisture content (hydration) of the skin. The R2-parameter which indicates the gross-elasticity of the skin was found to have a negative

percentage change (Fig. 7a) from the baseline value for all the aloe gel materials and the placebo indicating decrease in gross-elasticity. The lowest point in percentage decrease of the gross-elasticity of the skin was seen after 2 weeks of treatment with the various aloe gel materials. *Aloe marlothii* gel material showed the largest negative effect on the skin's gross elasticity (R2) when compared to the other aloes and the placebo. This correlates with the Corneometer® values, which indicated that *A. marlothii* gel material had the most dehydrating effect on the skin.

[Insert Figure 7 here]

The R6-parameter (Fig. 7b) measures the stretch capacity of the skin and negative values reflect improved skin condition.[25] Fig. 7b shows an upward curve (positive percentage change) for all the treatments after the first 2 weeks, thereafter the trend becomes downward. Therefore, the R6-parameter correlates with the other R-parameters in showing that skin conditions did not improve (positive percentage change). The only negative percentage change (indicating improved skin conditions) is after 1 week and 4 weeks of treatment with *A. vera*, 1 week of treatment with *A. marlothii* and after 4 weeks of treatment with *Aloe ferox*. Interesting is that the placebo showed the highest percentage positive change, thus showing the least improvement of the R6-parameter.

The R7-parameter seen in Fig. 7c showed a decrease in the elastic portion of the skin with the highest percentage decrease after 2 weeks of treatment. Negative values in R7 reflect a decrease in biological elasticity.[25] The complete relaxation (R8) of the skin followed almost the same pattern as R7, thus also indicating a decrease in skin elasticity. *Aloe marlothii* proved to have the most negative effect on these parameters after 2, 3 and 4 weeks of treatment. These results

support the findings of the Corneometer® that *A. marlothii* dehydrated the skin the most as it had the most negative effect on the elasticity parameters. However, statistically the Cutometer® results indicate that none of the treatments showed to significantly alter the R2-, R6-, R7- and R8-parameters.

The p-values obtained revealed statistical significant differences between the treatments when investigated with the Corneometer® ($p = 0.001$). The homogeneity parameter and the stretch capacity (R6) of the skin showed a significant difference between the times of treatment with p-values of 0.045 and 0.091, respectively. The gross-elasticity (R2) and complete relaxation (R7) of the skin revealed statistical significant differences for the interaction between time and treatment with p-values of 0.009 and 0.032, respectively. Statistical significant effects for time ($p = 0.067$) and interaction between time and treatment ($p = 0.074$) was observed for R8.

Pairwise comparisons with a Bonferroni adjustment between the levels of time revealed that the time of treatment had no statistical significance, except for the homogeneity parameter where statistical significant difference was seen between 1 week and 4 weeks ($p = 0.037$) of treatment. Pairwise comparisons with a Bonferroni adjustment between the different treatments revealed statistical significant differences in the Corneometer® measurements between the placebo and *A. ferox* gel material ($p = 0.003$), *A. marlothii* gel material ($p = 0.001$) and *A. vera* gel material ($p = 0.007$) gel materials. Thus deionized water was statistically better than the aloes in improving skin hydration.

A previous study found that *A. vera* extract moisturized the skin by significantly increasing the SC water content whilst not changing the TEWL.[11] It was suggested that this could be due to the rich composition of the *A. vera* extract of hygroscope mono- and polysaccharides[11, 33] and

the presence of the amino acids alanine, arginine, glycine, histidine, serine and threonine which contributes to SC hydration.[11, 34] Dal'Belo *et al.*[11] found that relatively low *A. vera* extract containing formulations (i.e. 0.10%, 0.25% and 0.50% w/w) increased skin hydration after 1 and 2 weeks of application compared to the vehicle alone. However, when the formulations were compared with each other after 2 weeks of application the formulation containing 0.50% w/w *A. vera* extract proved to be significantly better than the 0.10% and 0.25% formulations.

The findings of this longer-term, multiple application study are different from other studies described above, which may be ascribed to differences in the composition of the formulations tested (pure aloe gel materials dissolved in water in this study versus formulations containing excipients in other studies), but also due to the difference in composition of the aloe gel from different species.[35] Humectants promote the retention of water within the SC by attracting water from the outside in (from environment to skin) and from the inside out (from dermis to epidermis/SC).[36] Although it was not the aim of this study to determine the mechanism of skin dehydration by the aloe materials, a possible explanation is given here that should be further investigated in future studies. The dehydration of the skin by the aloe gel materials after multiple applications may be attributed to attracting water not only from the dermal/epidermal layers of the skin but also from the SC. The dehydration effect therefore potentially occurred due to absorption of moist from the skin into the aloe gel layer that was applied to the skin which dried by evaporation of the water in which the gel materials were dissolved after application to the skin.

The external use of aloe gel on intact skin is not associated with adverse reactions and is generally regarded as safe.[37] This is in accordance with the findings in other studies, although final conclusions on general safety in one of these studies could not be made due to the rarity of

an allergy after a single application.[12] In contrast to this, case studies on the topical application of aloe-derived products showed some adverse reactions which included contact urticaria, dermatitis and acute eczema.[38] It has been suggested that the adverse effects (such as hypersensitivity) may be due to the presence of apoptosis-inducing antrhaquinones in *A. ferox*.[39]

In the present study, two volunteers were withdrawn from the study due to severe allergic reactions. From weekly questionnaires it was determined that 11 (18.64 %), 14 (23.73 %) and 2 (3.39 %) out of the 59 volunteers reported a mild allergic reaction after topical application of *A. vera*, *A. marlothii* and *A. ferox* gel materials, respectively. The volunteers experienced a red rash, especially when the aloe gel started to dry on the skin, with either a burning or itching sensation.

Erythema study

Various tests have been used to examine the anti-inflammatory efficacy of aloe gel or its various components and generally involve some kind of intentional wounding [5] as the first step in wound healing is anti-inflammation.[8]

In the present study, the SLS exposure test was performed to compare the anti-erythema efficacy of the aloe gel materials to the positive control group (hydrocortisone gel). The percentage change in skin erythema as expressed by hemoglobin content from irritation (T_1) to two time intervals (T_2 and T_3) after treatment with test materials are given in Table 2.

[Insert Table 2 here]

Hydrocortisone gel showed a 13.1% decrease in erythema at T_2; followed by deionized water (7.8%) and *A. ferox* (7.0%), while *A. vera* and *A. marlothii* demonstrated the lowest percentage

decrease in erythema at T_2. However, no statistical significant differences were obtained between hydrocortisone gel, *A. ferox* gel material, deionized water and the untreated irritated skin in terms of anti-erythema effect at T_2. Hydrocortisone gel performed statistically significantly better than *A. vera* and *A. marlothii* gel materials in reducing skin erythema. *Aloe ferox* gel material statistically significantly reduced erythema to a larger extent than *A. marlothii* gel material at T_2.

At T_3 (on the 7^{th} day after 6 days of treatment), hydrocortisone gel decreased erythema by 18.8% followed by *A. vera* (17.0%), *A. ferox* (15.2%), and *A. marlothii* (9.0%). The anti-erythema effect of *A. vera* gel material and hydrocortisone gel was statistically significantly higher than that of *A. marlothii* gel material with p-values of 0.051 and 0.046 respectively. Deionized water and untreated irritated skin showed a similar decrease in skin erythema at T_3 with a percentage decrease of 13.0% and 13.1%, respectively.

Aloe marlothii gel material decreased erythema less than deionized water and untreated irritated skin, although this was not statistically significant. A statistically significant difference ($p = 0.0196$) was observed between T_2 and T_3 when treated with *A. vera* gel material, indicating its anti-erythema effect is time dependent. This correlates with a previous study where a lag phase was observed and the onset of *A. vera* gel's anti-inflammatory activity was found to be delayed.[12]

The differences obtained in the anti-erythema results for the different species of aloe could be explained by differences in their chemical compositions as confirmed with ^{1}H-NMR.

CONCLUSION

The clinical significance of the Corneometer® measurements, which are regarded as the most important indicator of skin hydration, indicates that *A. vera* and *A. marlothii* gel material did improve the hydration of the skin after a single application, although it was not statistically significantly different from the placebo (i.e. deionized water). *Aloe ferox* gel materials showed to dehydrate the skin after a single application when compared to the other aloe gel materials and the placebo. After multiple applications, all the aloe gel materials showed to have a dehydrating effect on the skin as opposed to deionized water, which significantly improved skin hydration. Mexameter® readings showed that *A. vera* and *A. ferox* gel materials were similar in their erythema reducing effects after 6 days of treatment to that of hydrocortisone gel. The anti-erythema effect of *A. vera* gel material was found to be dependent on time as there was a statistical significant difference between the second day of treatment and seventh day of treatment. *Aloe marlothii* gel material dehydrated the skin to the largest extend during the longer-term study and showed to be less effective than deionized water and untreated irritated skin in decreasing erythema. It also caused the highest number of mild skin reactions in the volunteers. While this study has elucidated the effects of gel materials from different aloe species on skin hydration and erythema in human subjects, the mechanisms of action should be investigated in future studies.

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Table legends

Table 1: Hydrocortisone gel formulation (positive control group)

Table 2: Percentage change in skin erythema (hemoglobin) from irritation (T_1) to two time intervals (T_2 and T_3) after treatment

Table 1

Components	**Concentration**
Hydrocortisone acetate	1% w/v
Ethanol (96% v/v)	15% v/v
Polyethylene glycol	15% w/v
Carbopol Ultrez 20	1% w/v
Distilled water up to	Up to 100% of preparation
Tri-ethanol amine	Enough to adjust pH to approximately 6.8

Table 2

Treatment	T_2	T_3
Irritated	-4.5 ± 21.3	-13.1 ± 18.3
Aloe vera	-1.8 ± 19.4	-17.0 ± 19.1
Aloe marlothii	-1.8 ± 19.3	-9.0 ± 21.2
Aloe ferox	-7.0 ± 14.3	-15.2 ± 20.4
Deionized water	-7.8 ± 15.7	-13.0 ± 26.6
Hydrocortisone	-13.1 ± 21.6	-18.8 ± 26.0

Figure legends

Figure 1: Flow chart to indicate enrollment, allocation, follow-up of volunteers and data analysis for the different studies (Adapted portions from CONSORT[17]) [reproduction size: page width]

Figure 2: A typical skin deformation curve obtained with the Cutometer®, which is similar to previously reported curves[24, 25] [reproduction size: column width]

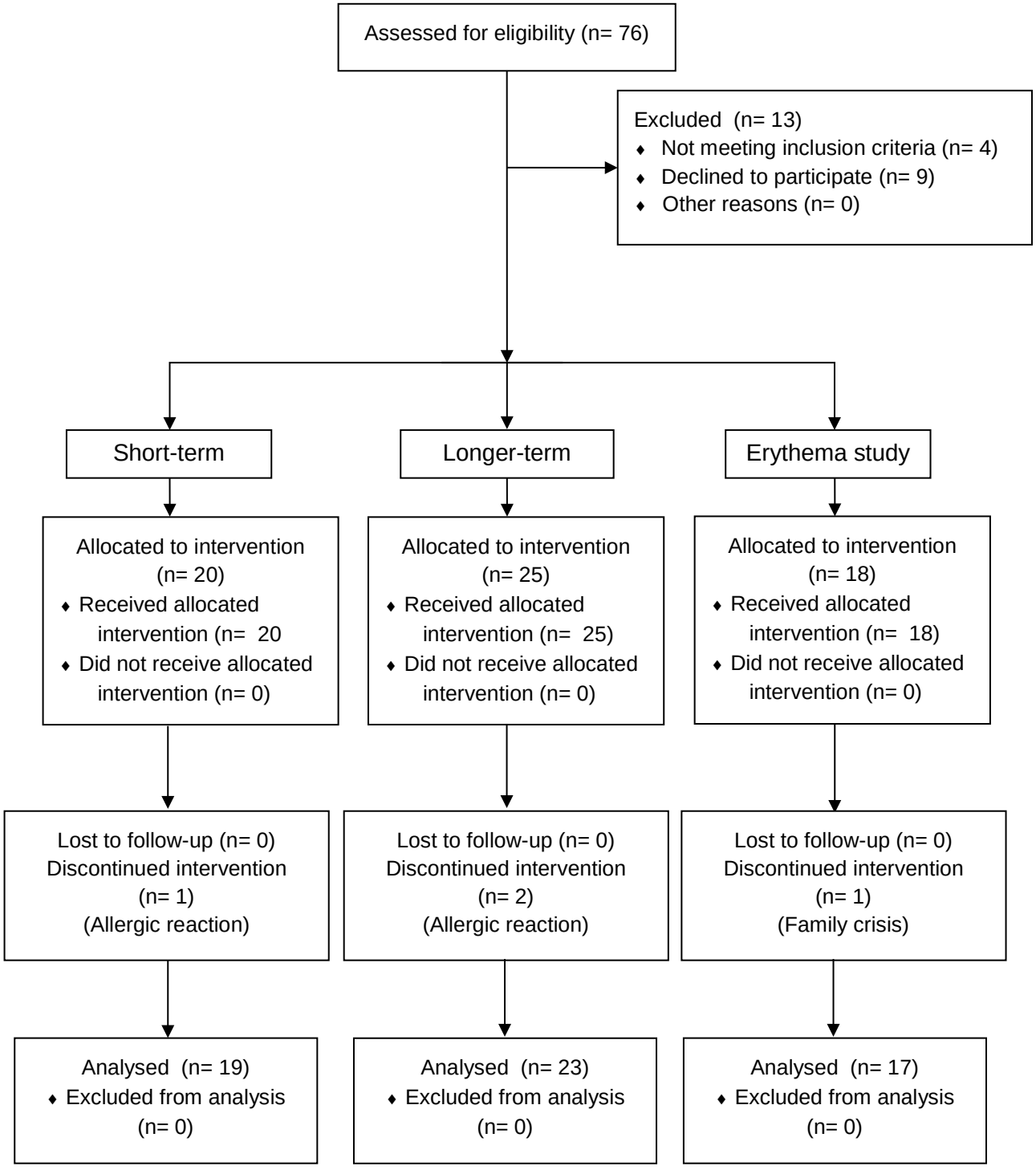
Figure 3: ^{1}H-NMR spectra of *A. vera* (a), *A. marlothii* (b) and *A. ferox* (c) precipitated gel materials [reproduction size: page width]

Figure 4: Percentage change measured by the Corneometer® (a), Visioscan® entropy (b) Visioscan® homogeneity (c) and the Visioscan® energy (d) measurements [reproduction size: page width]

Figure 5: Percentage change in skin hydration relative to initial conditions (T_0) as measured with the Corneometer® [reproduction size: column width]

Figure 6: Percentage change relative to initial conditions (T_0) as determined with the Visioscan® entropy (a) Visioscan® homogeneity (b) and the Visioscan® energy (c) [reproduction size: page width]

Figure 7: Percentage change relative to initial conditions (T_0) for the Cutometer® R2 (a) Cutometer® R6 (b), Cutometer® R7 (c) and the Cutometer® R8 (d) [reproduction size: page width]



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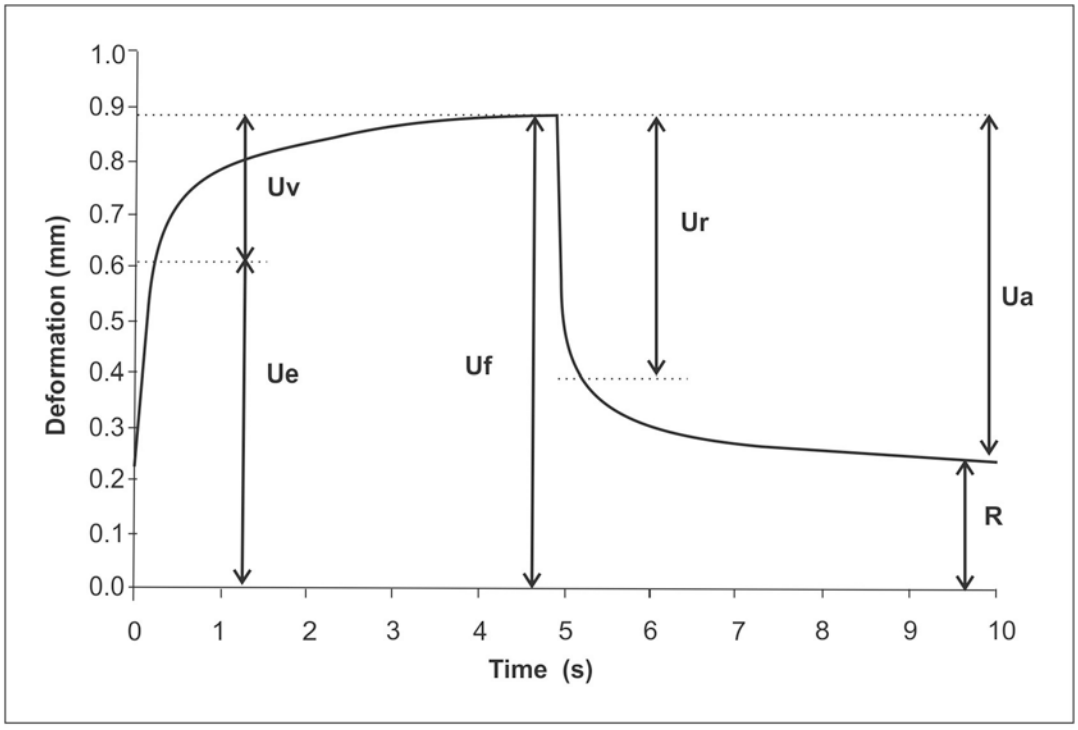

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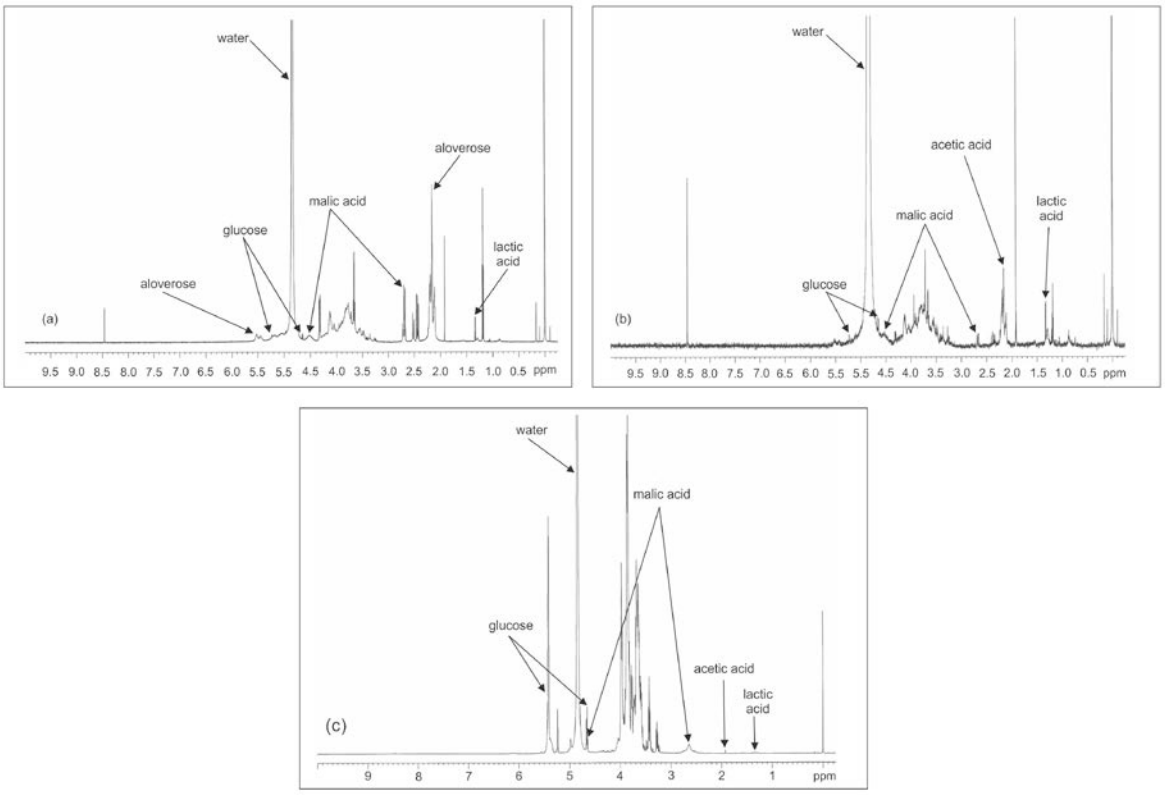

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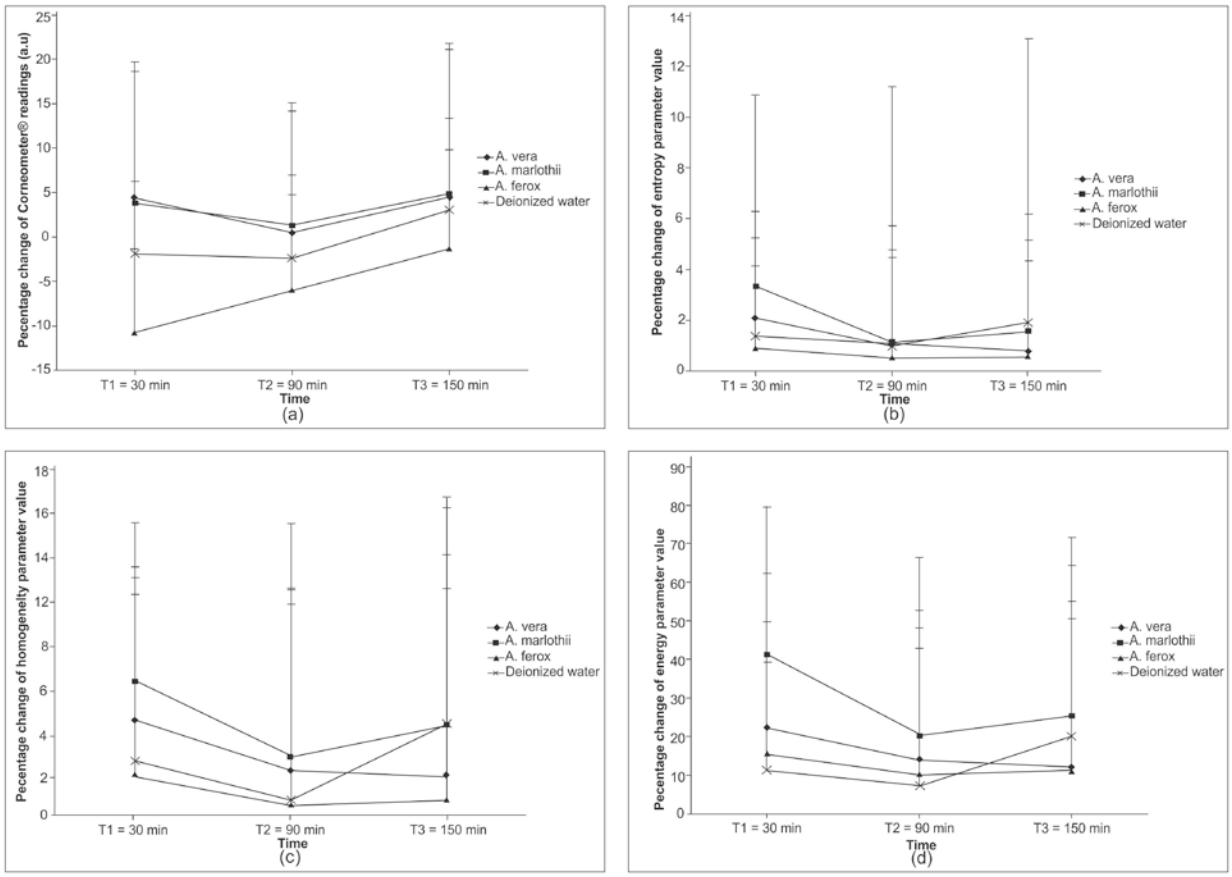


Figure 4: Percentage change measured by the Corneometer® (a), Visioscan® entropy (b) Visioscan® homogeneity (c) and the Visioscan® energy (d) measurements [reproduction size: page width]

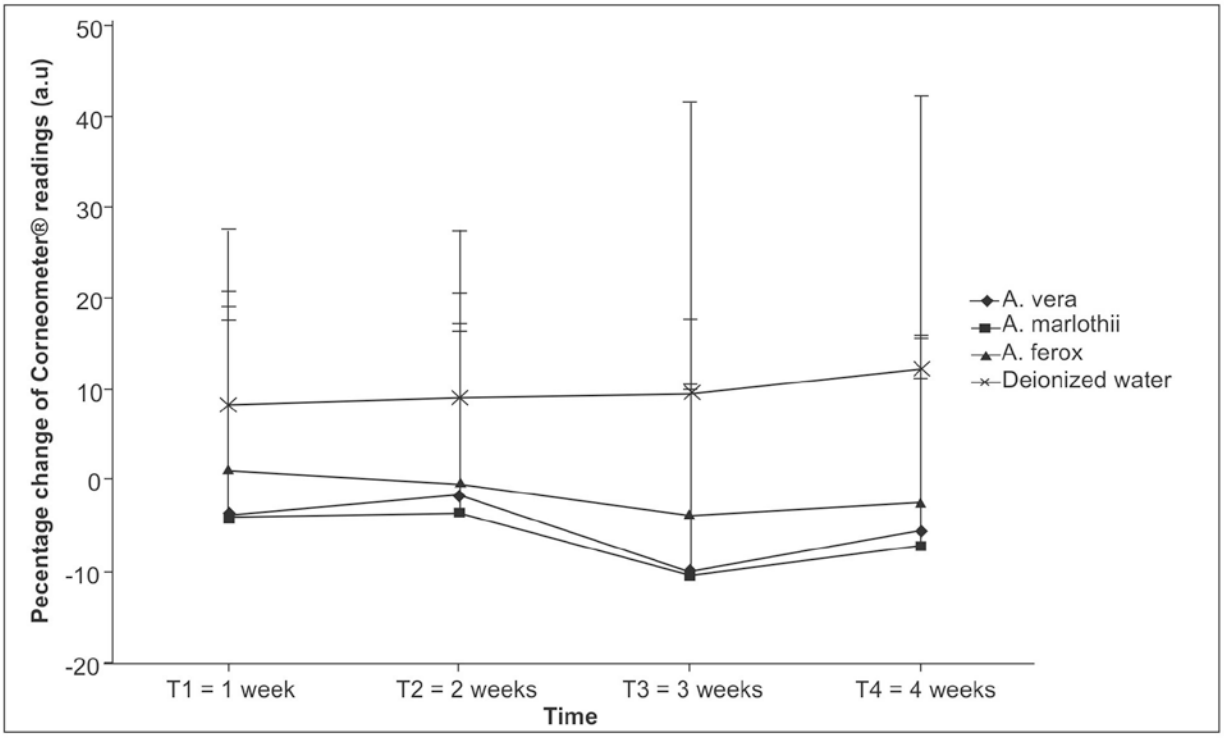


Figure 5: Percentage change in skin hydration relative to initial conditions (T_0) as measured with the Corneometer® [reproduction size: column width]

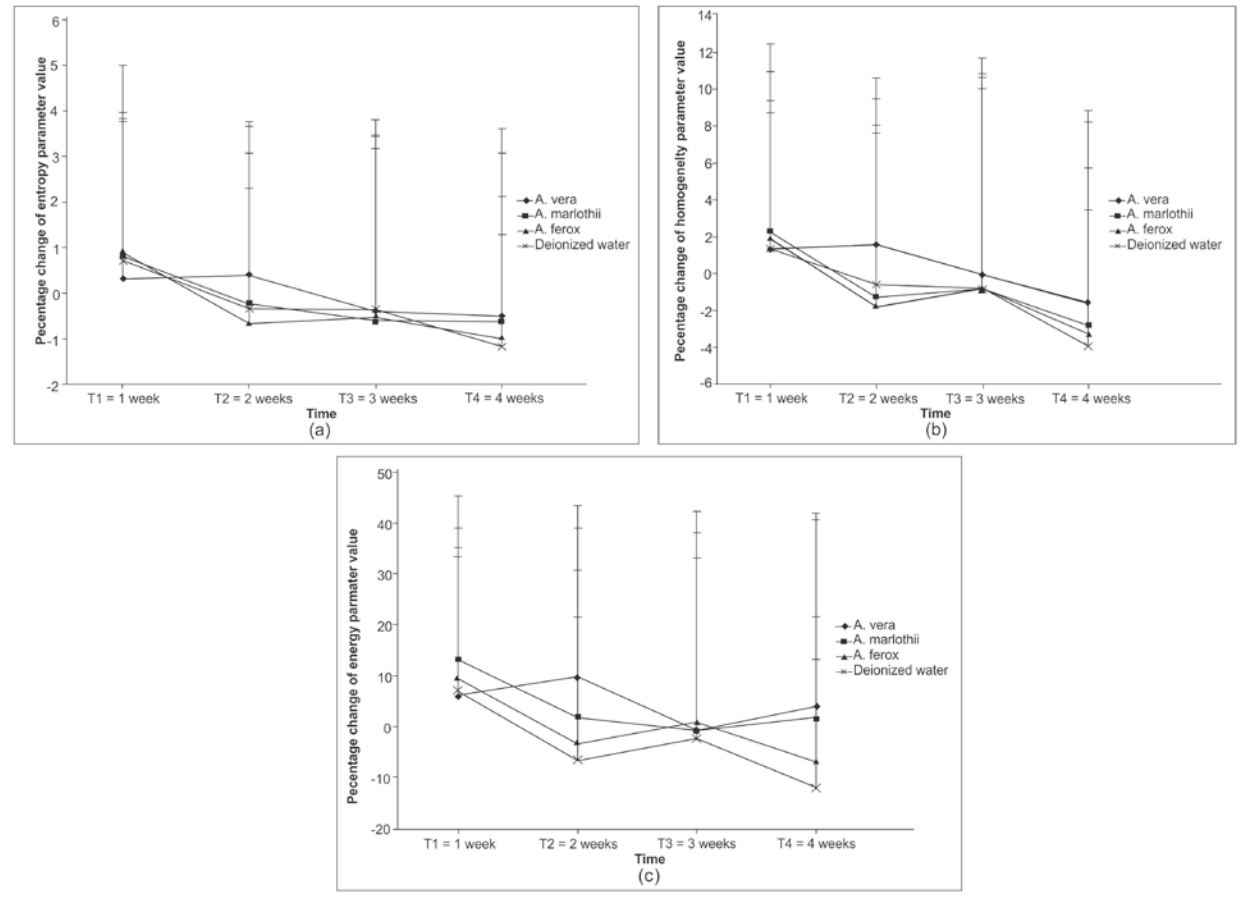
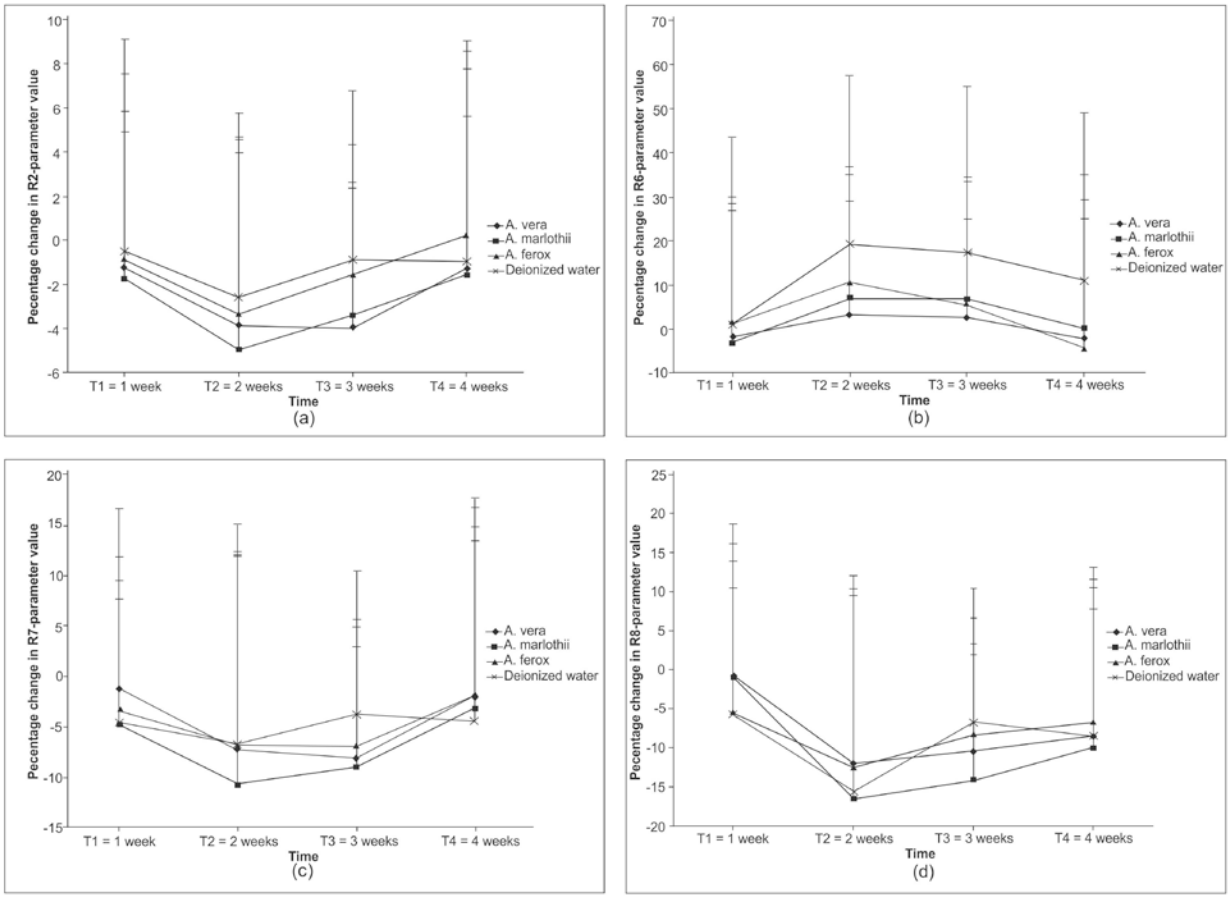


Figure 6: Percentage change relative to initial conditions (T_0) as determined with the Visioscan® entropy (a) Visioscan® homogeneity (b) and the Visioscan® energy (c) [reproduction size: page width]



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