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Skin permeation enhancement effects of the gel and whole-leaf materials of *Aloe vera*, *Aloe marlothii* and *Aloe ferox*

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Keywords
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Abstract

Objectives The aim of this study was to investigate the in-vitro permeation enhancement effects of the gel and whole-leaf materials of *Aloe vera*, *Aloe marlothii* and *Aloe ferox* using ketoprofen as a marker compound.

Methods The permeation studies were conducted across excised female abdominal skin in Franz diffusion cells, and the delivery of ketoprofen into the stratum corneum-epidermis and epidermis-dermis layers of the skin was investigated using a tape-stripping technique.

Key findings *A. vera* gel showed the highest permeation-enhancing effect on ketoprofen (enhancement ratio or ER = 2.551) when compared with the control group, followed by *A. marlothii* gel (ER = 1.590) and *A. ferox* whole-leaf material (ER = 1.520). Non-linear curve fitting calculations indicated that the drug permeation-enhancing effect of *A. vera* gel can be attributed to an increased partitioning of the drug into the skin, while *A. ferox* whole leaf modified the diffusion characteristics of the skin for ketoprofen. The tape stripping results indicated that *A. marlothii* whole leaf delivered the highest concentration of the ketoprofen into the different skin layers.

Conclusions Of the selected aloe species investigated, *A. vera* gel material showed the highest potential as transdermal drug penetration enhancer across human skin.

Introduction

The transdermal route of drug administration offers many advantages, such as avoiding first-pass metabolism, needing less frequent dosing regimens as they produce release for long periods of time, availability of a relatively large surface area for absorption and increased patient acceptability because of its non-invasiveness.[1,2] However, the outermost layer of the skin, the stratum corneum (SC), offers a formidable physical barrier to molecular transport.[1,3] This layer is very specific with regards to the type of molecule that can be transported across the skin and therefore only molecules with certain physicochemical properties can readily cross the skin.[1] This limits the range of potential drugs that can be administered transdermally, which emphasizes the need for formulations to incorporate penetration enhancers to assist in the effective delivery of a larger variety of drugs across the skin.[4]

Penetration enhancers can be used to enhance the penetration rate of drugs across the skin by means of two possible mechanisms of action.[5] Firstly, the penetration enhancer can work by altering the solubility properties of the skin, thereby increasing the solubility of the drug within the SC; secondly, the enhancer disrupts the ordered nature of the skin lipids, which consequently influences diffusion across the SC.[6,7] The use of natural products as effective and safe drug permeation enhancers is receiving considerable attention.[8] One such a natural product, *Aloe vera* (*Aloe barbadensis* Miller) juice, has shown potential to enhance the permeation of certain drug molecules through porcine ear skin membranes.[9]

Aloe is a genus consisting of more than 400 different species belonging to the Xanthorrhoeaceae family. *A. vera* has generally been researched to a larger extent for its

medicinal properties and other applications of all the aloe species. It is therefore important to include more aloe species in further investigations.[10] It was suggested that the mucilaginous gel of the aloe, consisting mainly of polysaccharides, holds the secret to some of the medicinal properties and biological effects of this family of plants,[11] which was confirmed for drug absorption enhancement across intestinal epithelial cells.[12]

The aim of this study was to investigate the in-vitro skin permeation enhancement properties of the gel and whole-leaf materials of *Aloe ferox* (*A. ferox* Mill.) and *Aloe marlothii* (*A. marlothii* A. Berger) and to compare their effects with those of *A. vera*, using ketoprofen as a type of 'marker' compound. Although the permeation enhancement effect of *A. vera* has been shown previously,[9] this is the first study on the permeation enhancement abilities of other aloe species (i.e. *A. ferox* and *A. marlothii*). Membrane release studies were performed before the skin diffusion studies to determine what concentration (3.00%, 1.50% or 0.75% (w/v)) of aloe leaf materials[5] should be used for the ketoprofen skin diffusion studies. Non-linear curve fitting was used to calculate α and β values as well as permeation coefficient (k_p) values to give an indication of the mechanism of ketoprofen permeation enhancement across the skin by the aloe leaf materials.

Materials and Methods

Materials

Ketoprofen was obtained from DB Fine Chemicals (Johannesburg, South Africa). Sodium dihydrogen phosphate anhydrous and HPLC-grade ethanol (99%) was purchased from Fluka (Johannesburg, South Africa) and Associated chemical enterprises (Johannesburg, South Africa), respectively. Sodium hydroxide, potassium dihydrogen orthophosphate, acetonitrile (HPLC grade), glacial acetic acid, deuterium oxide and 3-(trimethylsilyl) propionic acid-D4 sodium were obtained from Merck (Johannesburg, South Africa). Porafil membrane filters (cellulose nitrate) for the membrane release studies were purchased from Separations (Johannesburg, South Africa).

Collection and preparation of aloe leaf materials

Aloe vera dehydrated gel powder (Daltonmax 700) and whole-leaf materials were procured from Improve USA, Inc. (DeSoto, TX, USA, authenticated by the International Aloe Science Council). The *A. ferox* leaves were procured from Organic Aloe (Pty) Ltd. (Albertina, South Africa) whose products are endorsed by the South African Aloe Council. *A. marlothii* leaves were harvested from natural populations near Koster in the North West Province of South Africa, and the plant was identified by Mr Chris van Niekerk from the North-West University Botanical Gardens, South Africa. A specimen voucher (collector: Hamman 2011; collection number: PUC 11151) was deposited at the Herbarium of the North-West University. A hand-filleting method was used to obtain the gel and whole-leaf materials from the *A. marlothii* and *A. ferox* leaves, which involved cutting the leaf base, tapering point, the margins and the rind from the leaves.[13] The pulp was liquidized in a kitchen blender either alone or together with parts of the green rind to obtain the gel and whole-leaf materials, respectively.[14] These materials were lyophilized with a freeze dryer (VirTis, Gardiner, NY, USA) under vacuum (under 100 millitorr) at approximately –60°C.

Nuclear magnetic resonance fingerprinting of aloe gel materials

Approximately 30 mg of the *A. vera*, *A. marlothii* and *A. ferox* gel and whole-leaf materials were weighed off and dissolved in 1.5 ml D_2O. These solutions were filtered through cotton wool, and a small quantity of 3-(trimethylsilyl) propionic acid-D4 sodium salt was added. The nuclear magnetic resonance (^{1}H-NMR) spectra were recorded with an Avance III 600 Hz NMR spectrometer (Bruker, Rheinstetten, Germany) to identify certain marker molecules known to be present in fresh aloe leaf materials such as aloverose, glucose, malic acid and iso-citric acid.[15]

Preparation of receptor and donor phase solutions

Phosphate buffer solution (PBS, pH 7.4), prepared according to the British Pharmacopoeia,[16] containing 10% HPLC-grade ethanol was employed as the receptor phase for the membrane release as well as the skin diffusion studies.

The compositions of the donor test solutions are shown in Table 1. Ketoprofen was dissolved in HPLC-grade ethanol before adding the PBS (pH 6.5, prepared according to the British Pharmacopoeia[17]) and the aloe leaf materials in three different concentrations: 3.00%, 1.50% and 0.75% (w/v). The pH of the solution was adjusted to 6.5 with 2 M

Table 1 Composition of the aloe leaf material solutions

Ingredients	Concentration
Ketoprofen	2.50% (w/v)
Ethanol (99%)	10.00% (v/v)
Aloe leaf material[a]	3.00% or 1.50% or 0.75% (w/v)
PBS (pH 6.5)	Up to 20 ml
2 M NaOH	Enough to adjust pH to approximately 6.5

[a]The gel or whole-leaf materials of *A. vera*, *A. marlothii* or *A. ferox*. PBS, phosphate buffer solution.

NaOH. For the control group, ketoprofen (2.5% (w/v)) was dissolved in ethanol (10% (v/v)), and the solution was made up to volume (20 ml) with PBS (pH 6.5) and the pH was adjusted to 6.5 with 2 M NaOH. Before the membrane and skin diffusion studies, the donor solutions were pre-heated in a water bath at 32°C.

HPLC analysis of ketoprofen

An existing HPLC method was optimized and validated for ketoprofen in terms of linearity, accuracy and precision, limit of detection (LOD), limit of quantification (LOQ), ruggedness, repeatability and specificity. An Agilent 1100 series HPLC device with Quaternary gradient pump, autosampler, diode array UV detector and Chemstation Rev. A.10.01 software for data acquisition and analysis (Agilent Technologies, Palo Alto, CA, USA) were utilized. The column used was an Agela Venusil XBP C_{18} (2) (4.6 × 150 mm) with a 5 μm particle size (Agela Technologies, Newark, DE, USA). The mobile phase consisted of 60 volumes of acetonitrile (CH_3CN) and 40 volumes of distilled water containing 1% acetic acid degassed using an ultrasonic bath before use. The flow rate was set to 1 ml/min, and the injection volume was 1 μl (or 2 μl) and 50 μl for the membrane release and skin diffusion studies, respectively. The UV detector was set at 255 nm, with a total running time of 6.5 min. The retention time of ketoprofen was approximately 4.2 min. Standard solutions of ketoprofen were prepared freshly before each study and injected in the HPLC to construct calibration curves ranging in concentrations of 250–4000 μg/ml for the membrane release studies and 0.125–50 μg/ml for the skin diffusion studies.

Preparation of human skin membranes

Human abdominal skin from Caucasian female patients was obtained after cosmetic surgery and frozen at −20°C within 24 h after removal.[18] Ethical approval for the procurement and exploitation of the skin was obtained from the Research Ethics Committee of the North-West University under reference number NWU-00114-11-A5 (2011-08-25). The donors gave informed consent before the collection of the skin, and their identities were masked to ensure anonymity. The skin was thawed at room temperature before processing with a Zimmer electric dermatome model 8821 (Zimmer, Dover, OH, USA) to a thickness of 400 μm and a width of 2.5 cm as reported previously by Otto *et al.*[19]

Membrane release and skin diffusion studies

Before the skin diffusion studies, membrane release studies were performed to determine at which concentration (i.e. 3.00%, 1.50% of 0.75% (w/v)) aloe leaf material in solution released the marker, ketoprofen, at a suitable rate for use in the diffusion studies. Vertical Franz diffusion cells consisting of donor (top, ±1 ml) and receptor (bottom, ±2 ml) compartments with a diffusional area of 1.13 cm^2 were prepared as previously described.[20] Cellulose nitrate membranes and dermatomed skin (SC facing upward) were used for the membrane release and skin permeation studies, respectively. The entire receptor phase was withdrawn at specific time intervals and subsequently replenished with fresh receptor phase.[9] During the membrane release studies, the time intervals were hourly up to 6 h, whereas the skin diffusion studies samples were taken after 20, 40, 60, 80 and 100 min as well as 2, 4, 6, 8, 10 and 12 h. The samples withdrawn were placed in glass vials and immediately analysed on the HPLC.

Tape stripping

The tape-stripping method was performed as previously described by Pellet *et al.*[21] and Fox *et al.*[20] During this study the tape strips (SC-epidermis) and the remaining skin (epidermis-dermis) were placed in a glass containers filled with 5 ml of a 40% ethanol in PBS (pH 7.4) and kept overnight at 4°C. Subsequently, samples were withdrawn from the tape strip and skin containing dispersions and were filtered by utilizing 0.45-μm syringe filters, then placed in glass vials and analysed on HPLC.

Data analysis

Transdermal data analysis

The cumulative concentration (μg/cm^2) of ketoprofen, which permeated the membranes or skin samples, were plotted against time (hours) for both the membrane release and skin diffusion studies, respectively. The slope of the linear portion of the curve was used to determine the average flux. The enhancement ratio (ER) was obtained by dividing the average flux of ketoprofen from the aloe-containing solution by the average flux of ketoprofen from the control group.[9] The lag time was determined from the data of the skin diffusion studies by extrapolating the steady-state portion of the line to the time axis.[22]

The permeation profiles were further analysed by applying the data to a non-linear curve fitting procedure described by Díez-Sales *et al.*[23] and Otto *et al.*[24] The following Eq. (1)[25] was used to fit the data:

$$Q(t) = AKhC_v\left[D\frac{t}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n-1}^{\infty}\frac{(-1)^n}{n^2}exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right)\right] \quad (1)$$

Where $Q(t)$ is the quantity of the active substance that permeates the skin within time t, A is the diffusion area

(1.13 cm^2), K is the partition coefficient of the active between the skin and vehicle, h is the diffusional path length, D is the diffusion coefficient of the active across the skin and C_v is the actual concentration of the active in the donor vehicle. As t approaches infinity, the exponential term becomes insignificant, and Eq. (1) can be simplified to Eq. (2):

$$Q(t) = AKhC_v\left[D\frac{t}{h^2} - \frac{1}{6}\right] \quad (2)$$

As K and D are unknown, the product $K \times h$ and term D/h^2 were each replaced by α and β respectively, which were determined by fitting Eq. (2) to the obtained permeation plots using a computerized non-linear least square method (EasyPlot, Spiral Software, Norwich, VT, USA). The permeability coefficient (k_p) was calculated using Eq. (3).

$$k_p = \frac{KD}{h}(=\alpha\beta) \quad (3)$$

Statistical data analysis

Statistical analyses for the data obtained during the membrane release, skin diffusion and tape-stripping studies were carried out using Statistica (data analysis software system), version 11.[26] Data were analysed using descriptive as well as inferential statistics. Descriptive statistics involved the calculation of the average (with standard deviation) and median (middle score in a distribution) flux values.[27,28] Box plots were used to depict the tape stripping data graphically. The box plots were constructed by using the median and the first and third quartiles of the distribution.[29] The inferential statistics involved analysis of variance (ANOVA) as well as non-parametric hypothesis testing to compare the different solutions with each other.

To investigate the significant differences among the flux values, α, β and k_p values obtained with the skin diffusion studies, a one-way ANOVA was used. This was followed by a post-hoc Tukey's honestly significant difference (HSD) and Dunnett's *t*-test. However, the non-parametric Kruskal–Wallis *H*-test with multiple comparisons was preferred because of skewness of the data. Data from the membrane release studies were subject to three-way ANOVA analysis to investigate the effects of the different factors (concentration, species and type of material, i.e. whole leaf or gel) and their interactions on the flux. This was followed by a post-hoc Tukey's HSD test to identify exactly between which average values the difference lay. A P-value < 0.05 indicates statistically significant differences between the values that were compared.

Results and Discussion

Nuclear magnetic resonance fingerprinting

The ^{1}H-NMR spectra of the selected aloe leaf materials can be seen in Figure 1. The major marker molecules used for identifying *A. vera* gel material namely aloverose (or partly acetylated polymannose or acemannan), glucose and malic acid[15] were all confirmed to be present in the Daltonmax 700 materials (Figure 1a) by ^{1}H-NMR spectroscopy. These marker molecule quantities in the Daltonmax 700 materials (also used in this study) were previously determined by quantitative NMR and reported by Beneke *et al.*[12] Aloverose was not detected in the *A. marlothii* and *A. ferox* gel materials (Figure 1b and 1c, respectively), although glucose and malic acid were present as reported previously.[12] The whole-leaf materials of *A. vera*, *A. marlothii* and *A. ferox* (Figure 1d, 1e and 1f, respectively) contained the marker compounds glucose and malic acid, plus an additional whole-leaf marker (i.e. iso-citric acid), which is characteristic of fresh aloe whole-leaf material.[15,30] Aloverose was also detected in the whole-leaf material of *A. vera* but not in the whole-leaf materials of *A. marlothii* and *A. ferox* as previously found.[12]

Validation of the HPLC method for ketoprofen

The optimized HPLC method for ketoprofen was found to be reliable and sensitive enough for the determination of the concentration ketoprofen present in the membrane release and skin diffusion samples. The regression value ($r^2 = 0.999$) obtained from the linear regression curve indicated a high degree of linearity and therefore demonstrated a direct correlation between response and analyte concentration. The accuracy of the method (as reflected by the mean percentage recovery of the analyte) showed that the method yielded an acceptable mean recovery of 98.88%.[31] Interday precision was acceptable with a percentage relative standard deviation (RSD) of less than 5%.[31] The LOD was 0.001 μg/ml and the LOQ was 0.005 μg/ml. A ketoprofen sample (50 μg/ml) was injected hourly up to 24 h (ruggedness) and showed that ketoprofen was stable over this period. Repeatability was within the accepted range with a %RSD of less than 2% (even at an injection volume of 1 μl).[32] Chromatograms of *A. vera* gel alone, as well as *A. vera* gel in combination with ketoprofen are given in Figure 2 to demonstrate the specificity of the method. Only *A. vera* gel's chromatograms were included as the rest of the aloe leaf materials showed similar results.

Membrane release studies

The percentage of ketoprofen released from the solution, the average flux as well as the median flux values of the

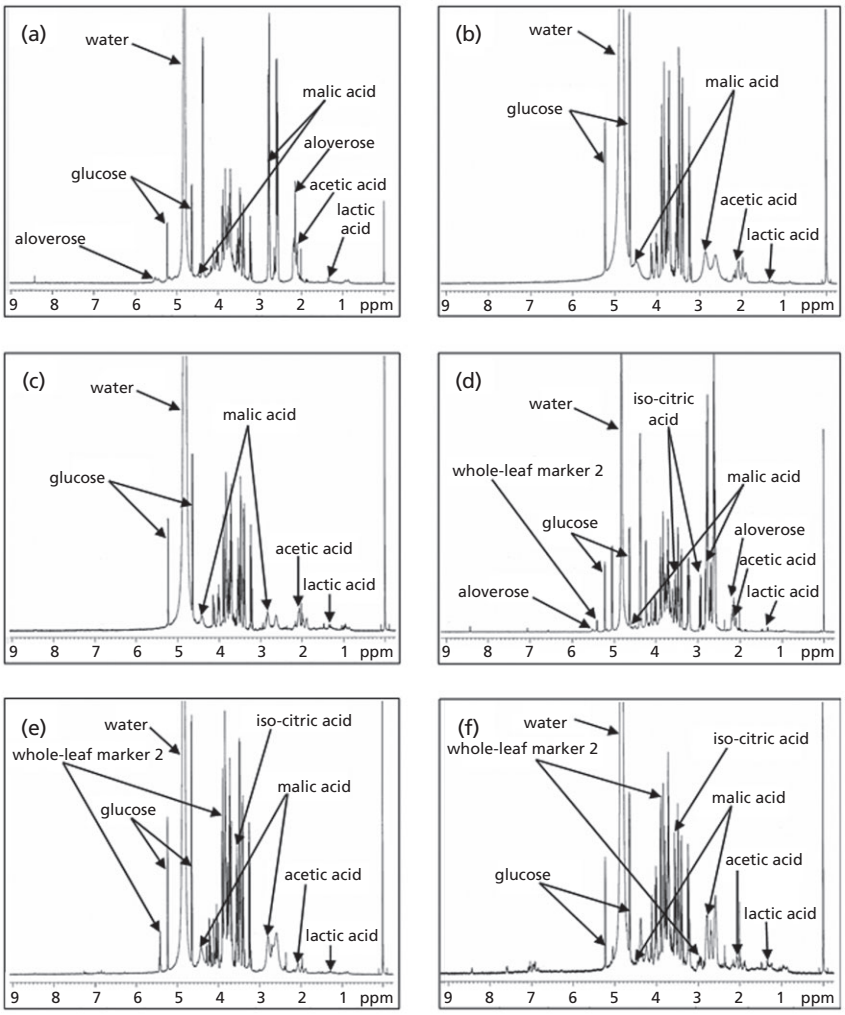

Figure 1 Nuclear magnetic resonance (^{1}H-NMR) spectra of (a) *A. vera* gel, (b) *A. marlothii* gel, (c) *A. ferox* gel, (d) *A. vera* whole leaf, (e) *A. marlothii* whole leaf and (f) *A. ferox* whole leaf.

membrane release studies are listed in Table 2. These results show that the marker compound, ketoprofen, permeated through the cellulose nitrate membranes from all the aloe-containing solutions investigated in this study, although to different extents.

When comparing the different aloe leaf material concentrations with each other (i.e. 75, 1.50 and 3.00% (w/v)), it is clear that the 0.75% (w/v) concentration had the highest average percentage of ketoprofen released of all concentrations tested, except for *A. marlothii* whole leaf and *A. ferox* gel, which showed lower percentage ketoprofen release at 0.75% than at 3.00%. Comparison of the different aloe leaf material solutions at 0.75% (w/v) concentration, indicated that the *A. vera* whole leaf-containing solution released the highest percentage ketoprofen from its gel-like structure followed by *A. vera* gel, *A. marlothii* gel, *A. ferox* gel, *A. marlothii* whole leaf and *A. ferox* whole leaf.

Statistical comparison of the average flux values with three-way ANOVA showed statistical significant differences between the concentrations ($P < 0.0001$), between the types of leaf material (i.e. gel or whole leaf) ($P = 0.001$) and between the species ($P < 0.0001$). Statistical significant effects were also found for the interactions between concentration and type ($P = 0.0063$), concentration and species ($P < 0.0001$), type and species ($P < 0.0001$), concentration and type and species ($P < 0.0001$). Post-hoc comparisons using Tukey's HSD (honestly significant difference) test revealed statistical significant differences ($P < 0.0001$) between the different concentrations (0.75%, 1.50% and 3.00%) and the different types of leaf material (i.e. gel and whole leaf). The data for the release of ketoprofen from *A. vera* solutions was statistically significantly different ($P < 0.0001$) from *A. marlothii* and *A. ferox*.

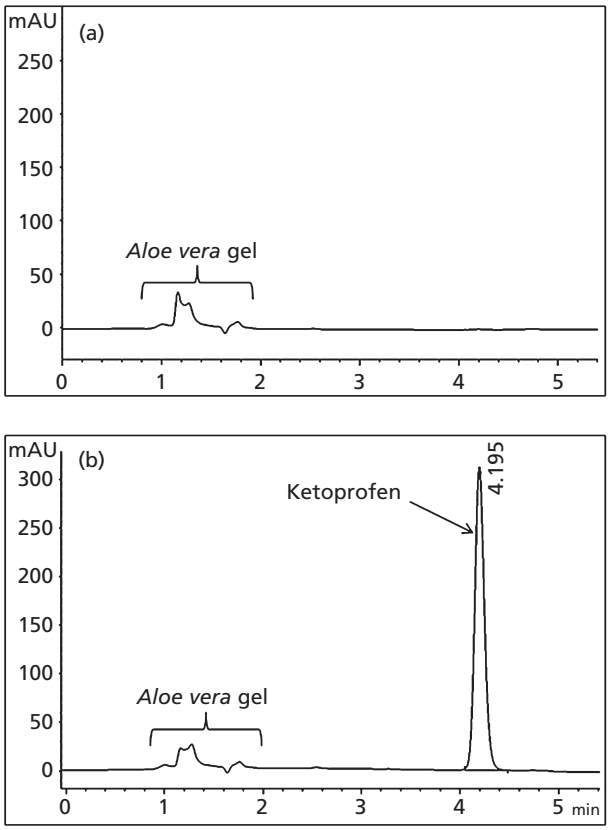

Figure 2 HPLC chromatogram illustrating the retention time of (a) *A. vera* gel and (b) ketoprofen in the presence of *A. vera* gel.

Transdermal skin diffusion studies

Flux, percentage ketoprofen diffused and enhancement ratio by aloe leaf materials

Based on the results of the membrane release studies, it was decided to test the aloe leaf materials for their penetration enhancing effects at a concentration of 0.75% (w/v). Table 3 presents the average, as well as the median flux values, percentage ketoprofen diffused and the ER values for the in-vitro skin permeation studies.

Results in Table 3 clearly show that the test solutions containing *A. vera* gel, *A. marlothii* gel and *A. ferox* whole leaf exhibited higher average ketoprofen flux values, median flux values as well as a higher percentage ketoprofen diffused across the skin than the control group (ketoprofen alone) after 12 h. *A. vera* whole leaf, *A. marlothii* whole leaf and *A. ferox* gel had only slightly higher, comparable or even slightly lower average flux, median flux and percentage diffused values than the control group. With an ER of 2.551, *A. vera* gel was superior to all the other aloe test solutions including *A. marlothii* gel (ER = 1.590) and *A. ferox* whole leaf (ER = 1.520) in enhancing the permeation of ketoprofen across the skin.

The Kruskal–Wallis multiple comparisons test revealed that *A. vera* gel statistically significantly ($P = 0.0003$) enhanced the permeation of ketoprofen compared with the control group, *A. vera* whole leaf ($P = 0.0004$), *A. marlothii* whole leaf ($P = 0.0002$) and *A. ferox* gel ($P = 0.000$). Statistically significant differences were also found between *A. marlothii* gel and *A. ferox* gel ($P = 0.008$) as well as between *A. ferox* whole leaf and *A. ferox* gel ($P = 0.011$). However, it is possible that the presence of more data points

Table 2 Membrane release data for ketoprofen from the different aloe material solutions after 6 h

Aloe leaf material	Concentration (w/v)	Average flux (μg/cm²·h)	Median flux (μg/cm²·h)	Average % released
A. vera gel	3.00% ($n = 10$)	1694.8 ± 102.954	1775.17	26.733
	1.50% ($n = 10$)	2508.6 ± 209.908	2524.73	39.171
	0.75% ($n = 10$)	2572.4 ± 208.945	2699.76	43.961
A. vera whole leaf	3.00% ($n = 10$)	3173.4 ± 375.626	2724.13	38.833
	1.50% ($n = 9$)	1753.8 ± 119.413	2454.30	34.014
	0.75% ($n = 10$)	2941.0 ± 297.825	2928.72	47.032
A. marlothii gel	3.00% ($n = 10$)	1747.5 ± 178.909	1930.78	29.290
	1.50% ($n = 10$)	1729.9 ± 91.526	1866.01	29.077
	0.75% ($n = 10$)	2282.2 ± 90.555	2367.88	35.710
A. marlothii whole leaf	3.00% ($n = 10$)	2180.2 ± 127.121	2206.38	33.887
	1.50% ($n = 10$)	2148.5 ± 204.894	2206.42	33.557
	0.75% ($n = 10$)	2216.1 ± 139.911	2224.37	32.962
A. ferox gel	3.00% ($n = 10$)	2275.4 ± 149.469	2356.74	35.320
	1.50% ($n = 10$)	2178.4 ± 86.286	2172.69	32.623
	0.75% ($n = 10$)	2219.5 ± 108.341	2249.41	33.419
A. ferox whole leaf	3.00% ($n = 10$)	1663.2 ± 201.892	1795.52	27.097
	1.50% ($n = 7$)	2490.2 ± 275.723	1956.75	24.042
	0.75% ($n = 10$)	2066.7 ± 177.705	2138.62	32.115

Table 3 Average flux (μg/cm²·h), median flux (μg/cm²·h), average percentage ketoprofen diffused and enhancement ratio (ER) values obtained from the different aloe leaf material solutions across skin over a 12-h period

Solution	Average flux (μg/cm²·h)	Median flux (μg/cm²·h)	Average % diffused	Enhancement ratio
Control ($n = 9$)	8.020 ± 1.497	6.859	0.169	
A. vera gel ($n = 10$)	20.464 ± 3.941*	16.776	0.446	2.551
A. vera whole leaf ($n = 10$)	9.006 ± 2.997	6.040	0.185	1.123
A. marlothii gel ($n = 9$)	12.756 ± 1.701	11.402	0.279	1.590
A. marlothii whole leaf ($n = 10$)	7.821 ± 1.471	6.383	0.169	0.975
A. ferox gel ($n = 10$)	6.626 ± 2.332	5.261	0.148	0.826
A. ferox whole leaf ($n = 9$)	12.187 ± 3.229	10.237	0.277	1.520

*$P < 0.05$ (comparison with control).

could have revealed more statistical significant differences between the different comparisons made.

Previously, the in-vitro skin permeation enhancement potential of *A. vera* juice was investigated by employing porcine ear skin membranes and saturated solutions of various model drugs (i.e. 'within-vehicle') with different molecular weights and lipophilicities (i.e. caffeine, colchicine, mefenamic acid, oxybutynin and quinine). No link was found between the lipophilicity of the drug and the permeation enhancement effect of the *A. vera* juice; however, it had a higher skin permeation enhancement effect on drugs with a higher molecular weight.[9]

Subsequently, a mechanism was proposed whereby the smaller molecules were less efficient at blocking *A. vera* constituents from the permeation pathways, leading to a reduced opportunity for the drug to interact with the enhancing factor, which was 'lost' from the solution because of its permeation. In contrast, a drug with a larger molecular weight effectively blocked the permeation routes, allowing increased possibility for the drug to interact with the enhancing factor and complex with it before being transported across the skin, that is, permeation enhancement occurs by a 'pull effect'.[9] Numerous studies[33,34] have explained the skin penetration enhancement of drugs by the 'pull' effect, whereby the permeation of the enhancer facilitates the permeation of the solute via a solvation or complexation interaction.[35]

Another study[36] found that pretreatment with *A. vera* juice did not enhance the in-vitro permeation of ketoprofen across the skin. It was suggested that the ketoprofen did not complex with the, as yet unidentified, enhancing factor the same way as described by Cole and Heard[9] when utilized 'within-vehicle'. The results of this study clearly showed a significant permeation-enhancing effect by *A. vera* gel when ketoprofen was incorporated into the solution (i.e. 'within-vehicle'). Therefore, it can be hypothesized that ketoprofen had the opportunity to interact with the aloe phytochemicals (i.e. enhancing factor) in the aloe-containing solutions to facilitate its transport across the skin.

A. vera gel and *A. marlothii* gel showed higher permeation-enhancing effects than *A. vera* whole leaf and *A. marlothii* whole leaf, but this was not the case for *A. ferox*. The *A. ferox* whole leaf had a much higher ER (1.52) than the *A. ferox* gel (ER = 0.826). It is not clear from the current chemical characterization why this effect was obtained; therefore, further investigation is needed to elucidate the causative factor.

It is known that the composition of the plants including aloe leaf materials may be influenced by factors such as soil composition, location, climate, species, growth conditions and harvesting processes.[35,37]

Non-linear curve fitting and lag times

There are two main mechanisms of penetration enhancement, namely changing the partitioning of the drug into the skin layers or changing the diffusion of the drug molecule across the skin layers.[6,7] To investigate by which of these two mechanisms the aloe leaf materials possibly enhanced the permeation of ketoprofen, the α-values and β-values were obtained by applying the various permeation profiles to a non-linear curve-fitting procedure.[23] A change in α indicates an effect on the partition coefficient (K) and a change in β indicates an effect on the diffusivity (D) (with the assumption that h, the diffusional path length, is constant).[19] The obtained α, β and k_p values as well as the calculated lag times are given in Table 4.

The relatively high value of α (Table 4) for the *A. vera* gel and *A. marlothii* gel groups indicates tha these two aloe leaf materials increased the skin partitioning of the ketoprofen when compared with the control group. However, a statistical significant difference ($P = 0.005$, obtained with the Kruskal–Wallis multiple comparisons test) existed only between the control group and *A. vera* gel. Furthermore, the relatively higher k_p values obtained for *A. vera* gel and *A. marlothii* gel leaf groups may be attributed to the higher α-value (higher partitioning of ketoprofen into the outer layers of the SC). The permeability coefficient of *A. vera* gel was statistically significantly different from the permeability coefficient of the control group ($P = 0.0002$).

The obtained β-value for *A. ferox* whole leaf was statistically significantly higher ($P = 0.0002$, Kruskal–Wallis

Table 4 Calculated α, β and the permeability coefficient (k_p) values after analysing the permeation profiles using a non-linear curve-fitting procedure as well the lag times of the different test materials (with standard deviation)

Solution	α	β	k_p (cm/h)	Lag time (h)
Control	0.0015 ± 0.0002	0.155 ± 0.016	0.00024 ± 0.00005	2.387 ± 0.214
A. vera gel	0.0034 ± 0.0005*	0.185 ± 0.028	0.00062 ± 0.00010*	2.061 ± 0.290
A. vera whole leaf	0.0018 ± 0.0006	0.139 ± 0.010	0.00026 ± 0.00009	2.655 ± 0.229
A. marlothii gel	0.0021 ± 0.0003	0.183 ± 0.011	0.00039 ± 0.00005	1.965 ± 0.148
A. marlothii whole leaf	0.0014 ± 0.0003	0.174 ± 0.014	0.00024 ± 0.00005	2.045 ± 0.185
A. ferox gel	0.0011 ± 0.0002	0.190 ± 0.061	0.00021 ± 0.00008	1.773 ± 0.411*
A. ferox whole leaf	0.0016 ± 0.0005	0.244 ± 0.024*	0.00039 ± 0.00010	1.474 ± 0.139*

*$P < 0.05$ (compared with the control).

multiple comparisons test) compared with the control group. This was followed by *A. ferox* gel and *A. vera* gel, although their obtained β-values did not statistically significantly differ from the control group. These higher β-values indicate these aloe leaf materials most probably modified the diffusion characteristics of the skin for ketoprofen.[38] The relatively higher β-values also explain the higher k_p obtained for *A. ferox* whole leaf and *A. vera* gel. The k_p values obtained for *A. vera* whole leaf and *A. marlothii* whole leaf were similar to those of the control group.

Aloe vera gel had an effect on both the partitioning coefficient as well as the diffusion coefficient of ketoprofen, as reflected by the change in the values of both α and β, respectively. However, *A. vera* gel's effect on the partitioning of the drug (α-value) was much more prominent than its effect on the diffusivity (β-value). Even though *A. ferox* gel had an increased β-value, it was counteracted by a reduced α-value (which was lower compared with the control); this could possibly explain why *A. ferox* gel did not enhance the flux of ketoprofen.

The lag times (Table 4) of the different aloes and control group can be arranged in the following decreasing order: *A. vera* whole leaf (2.655 h) > control group (2.387 h) > *A. vera* gel (2.061 h) > *A. marlothii* whole leaf (2.046 h) > *A. marlothii* gel (1.965 h) > *A. ferox* gel (1.773 h) > *A. ferox* whole leaf (1.474 h). Compared with the control group (ketoprofen alone), all the aloe leaf materials, except *A. vera* whole leaf, decreased the lag time. *A. ferox* gel and *A. ferox* whole leaf groups showed to decrease lag time statistically significantly from the control group with *P*-values of 0.01900 and 0.00005, respectively. However, the data indicated no correlation between the lag time and the permeation enhancing effects of the aloe leaf materials.

Tape stripping

Concentration ketoprofen in the stratum corneum-epidermis

Comparing the average SC-epidermis concentrations (Figure 3) revealed the following ranking order: *A. marlothii* whole leaf (3.285 μg/ml) > *A. vera* gel (2.817 μg/ml) > *A. vera* whole leaf (2.292 μg/ml) > *A. marlothii* gel (2.107 μg/ml) > control group (1.812 μg/ml) > *A. ferox* whole leaf (1.709 μg/ml) > *A. ferox* gel (1.709 μg/ml).

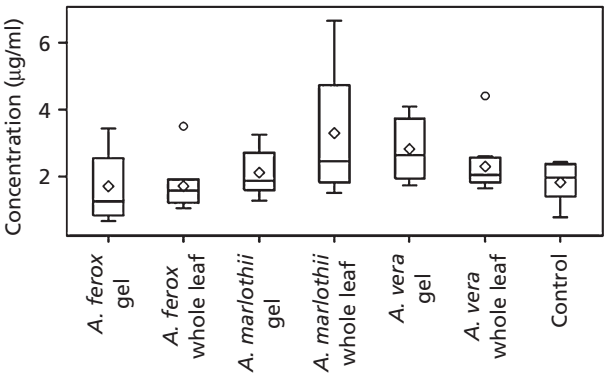

Figure 3 Box plots depicting the concentration (μg/ml) ketoprofen present in the stratum corneum (SC)-epidermis for the different aloe leaf material solutions after tape stripping ($n = 9$ for control, *A. ferox* whole leaf and *A. marlothii gel*; $n = 10$ for *A. ferox* gel, *A. marlothii* whole leaf, *A. vera gel* and *A. vera* whole leaf). The average and median concentration values are indicated by the diamond shapes and lines, respectively.

Tape stripping is a technique employed to remove the outermost layer of the skin, the SC, in a stepwise manner by use of adhesive films.[39] By doing this the penetration of topically applied active ingredients into the uppermost layers of the skin can be examined.[40]

Comparing the average SC-epidermis concentrations (Figure 3) revealed that although *A. marlothii* whole leaf did not enhance the permeation of ketoprofen through the skin (as reflected by its low flux value), it did however deliver a high concentration of ketoprofen into the SC-epidermis. Conversely, *A. vera* gel, which had the highest permeation-enhancing effect (high flux value), also delivered a high concentration ketoprofen into the SC-epidermis. The high average concentration of ketoprofen in this skin layer when applied in the presence of *A. vera* gel correlates with its high

α-value in that it enhanced the partition of the ketoprofen into the skin. Important to note is that *A. ferox* gel did not only have a lower flux value than the control group, but also delivered ketoprofen into the SC-epidermis at the lowest concentration.

Only small differences were noted when comparing the average and median concentrations (Figure 3) except for *A. marlothii* whole leaf, which had a median concentration value of 2.461 μg/ml. This indicates that both the average and median concentration values can be used, but it is proposed that only the median concentrations be used as they are unaffected by a distortion in the spread of the data.[29]

However, statistical comparison among the different solutions with the Kruskal–Wallis multiple comparisons test revealed no statistical significant differences.

Concentration ketoprofen in the epidermis-dermis

The epidermis-dermis ketoprofen concentration values of the different test solutions are depicted in box plots in Figure 4. The following rank order was observed when comparing the average concentrations of the marker ketoprofen in this skin layer for the different test solutions: *A. marlothii* whole leaf (2.646 μg/ml) > *A. marlothii* gel (1.505 μg/ml) > *A. vera* whole leaf (1.382 μg/ml) > *A. vera* gel (1.295 μg/ml) > *A. ferox* whole leaf (1.125 μg/ml) > *A. ferox* gel (1.110 μg/ml) > control group (0.467 μg/ml). Therefore, the results indicate that all the aloe leaf material-containing solutions were better than the control group in delivering ketoprofen into the epidermis-dermis layer of the skin.

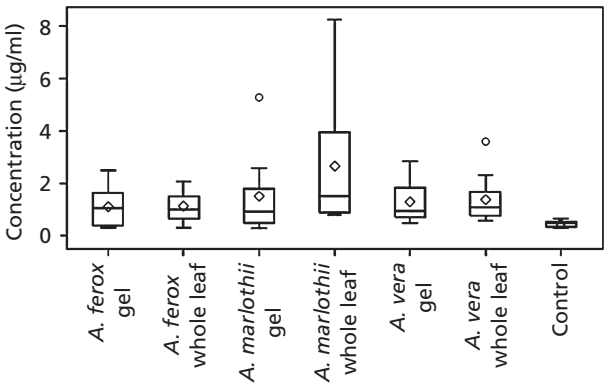

Figure 4 Box plots depicting the concentration (μg/ml) ketoprofen present in the epidermis-dermis for the different aloe leaf material solutions after tape stripping ($n = 9$ for control, *A. ferox* whole leaf and *A. marlothii* gel; $n = 10$ for *A. ferox* gel, *A. marlothii* whole leaf, *A. vera* gel and *A. vera* whole leaf). The average and median concentration values are indicated by the diamond shapes and lines, respectively.

No major differences were noted between the average and median concentrations, except for the *A. marlothii* whole-leaf solution, which had a median concentration value of 1.508 μg/ml and an average concentration value of 2.646 μg/ml. Because the median concentration considered all the data and was not affected by outliers in the data (as in the case with average concentration values), the median epidermis-dermis values provided a more accurate representation of the true concentration.[29]

Of the possible 21 comparisons among the different solutions, the Kruskal–Wallis multiple comparisons test revealed only two statistical significant differences, which were found between the control group and *A. vera* whole leaf ($P = 0.036$), as well as between the control group and *A. marlothii* whole leaf ($P = 0.0007$).

Overall, the average and median concentrations of ketoprofen in the epidermis-dermis were lower than for the SC-epidermis ketoprofen concentrations. This indicates that ketoprofen had a high propensity to leave the aqueous vehicle and migrate into the SC but had some difficulty in penetrating the hydrophilic viable epidermis.[41]

Conclusion

Aloe vera gel test solution statistically significantly enhanced the permeation of ketoprofen across dermatomed skin compared with the control group, followed by *A. marlothii* gel and *A. ferox* whole-leaf groups, although their effects were not statistically significantly different from the control group. The high α-values of *A. vera* gel and *A. marlothii* gel groups gives an indication that these two aloe leaf materials increased the skin partitioning of the ketoprofen when compared with the control group. In contrast, *A. ferox* whole leaf modified the diffusion characteristics of the skin towards ketoprofen as reflected by its relatively high β-value.[38]

When comparing all the tested solutions, in terms of the ketoprofen concentration present in the SC-epidermis and epidermis-dermis skin layers, *A. marlothii* whole leaf displayed the highest values.

In general, the *A. vera* leaf materials proved to be more effective in ketoprofen penetration enhancement across skin compared with *A. marlothii* and *A. ferox*. The differences in the penetration-enhancing abilities of the different aloe leaf materials can possibly be ascribed to differences in their chemical compositions. Although additional studies need to be done to determine the permeation enhancement effects of these aloe species on other actives (i.e. with different physico-chemical characteristics than ketoprofen), the results in this study shows the potential use of aloe leaf materials in improving transdermal drug delivery. Further research is also needed to determine the specific enhancing factor present in these aloe leaf materials as well as to

confirm the mechanism by which aloe leaf materials enhance the skin permeation of drug compounds.

Declarations

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