Chapter 4

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Chapter 4 is written in an article format for the purpose of publication in *European Journal of Pharmaceutics and Biopharmaceutics.* The complete guide for this journal is given in Appendix H. For publishing purposes Chapter 4 contains no formatting, other than advised by the guide for authors. However, the text of the paragraphs was justified to ease reading and improve neatness.

In vitro skin permeation enhancement potential of the gel and whole leaf materials of *A. vera, A. marlothii* and *A. ferox*

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Abstract

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. 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. *Aloe vera* gel showed the highest permeation enhancing effect on ketoprofen (enhancement ratio = 2.551) when compared to the control group, followed by *Aloe marlothii* (enhancement ratio = 1.590) gel and *Aloe ferox* whole leaf material (enhancement ratio = 1.520). Based on the results obtained from non-linear curve fitting calculations, the drug permeation enhancing effect of *Aloe vera* gel can be attributed to an increased partitioning of the drug into the skin, while *Aloe ferox* whole leaf modified the diffusion characteristics of the skin for ketoprofen. From the tape stripping results it is clear that *Aloe marlothii* whole leaf delivered the highest concentration of the ketoprofen into the different skin layers. *Aloe vera* gel material is the most effective transdermal drug penetration enhancer across human skin of the selected aloe species investigated.

Keywords: *Aloe vera*, *Aloe marlothii*, *Aloe ferox,* gel, whole leaf, penetration enhancer, ketoprofen, tape stripping

Abbreviations: stratum corneum (SC), phosphate buffer solution (PBS), enhancement ratio (ER)

1 Introduction

The transdermal route of drug administration offers many advantages, such as avoiding first-pass metabolism, needing less frequent dosing regimens, availability of a relatively large surface area for absorption and increased patient acceptability due to its non-invasiveness [1, 2]. However, the outermost layer of the skin, the stratum corneum (SC), offers a formidable physical barrier to molecular transport [2, 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 [2]. This limits the range of potential drugs that can be administered transdermally, which emphasises 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* juice, has shown potential to enhance the permeation of certain drug molecules through porcine ear skin membranes [9].

Considering that *A. vera* has generally been researched to a large extent for its medicinal properties and other applications, it is important for researchers to investigate other aloe species [10]. Aloe is a genus consisting of more than 400 different species belonging to the Xanthorrhoeaceae family. Aloe plants are characterised by thick, stemless, fleshy leaves which are enlarged to accommodate aqueous tissue to survive in areas of low or erratic rainfall [11,12]. Aloe leaves have a thick epidermis (skin/rind) that covers the mesophyll, which can be differentiated into chlorenchyma cells and thinner walled cells, also known as parenchyma cells (i.e. the fillet or pulp) and these cells contain the gel material, which is a clear mucilaginous jelly [13, 14].

Polysaccharides, as well as miscellaneous bioactive constituents, have been identified from the leaves of the aloe plant [15] and can exhibit physiological as well as pharmacological activities [13]. 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 [13], which was confirmed for drug absorption enhancement across intestinal epithelial cells [16].

The aim of this study was to investigate the *in vitro* skin permeation enhancement properties of the gel and whole leaf materials of *A. ferox* and *A. marlothii* and to compare their effects with those of *A. vera,* using ketoprofen as a type of 'marker' compound. Membrane release studies were performed prior to 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

2 Materials and methods

2.1 Materials

Ketoprofen was obtained from DB Fine Chemicals (Johannesburg, South Africa). Sodium dihydrogen phosphate anhydrous (H_2NaO_4P) and HPLC-grade ethanol (99%) was purchased from Fluka (Johannesburg, South Africa) and Associated chemical enterprises (Johannesburg, South Africa), respectively. Sodium hydroxide (NaOH), potassium dihydrogen orthophosphate

(KH_2PO_4), acetonitrile (HPLC grade), glacial acetic acid ($(CH_3)OOH$, 100%), deuterium oxide (D_2O), 3-(trimethylsilyl) propionic acid-D4 sodium and Dow Corning® high vacuum grease were obtained from Merck (Johannesburg, South Africa). Porafil® membrane filters (cellulose nitrate) for the membrane release studies were purchased from Separations (Johannesburg, South Africa). Water used during this study was purified by a Milli-Q Academic purification system (Millipore, Milford, Mass., USA).

2.2 Collection and preparation of aloe leaf materials

The *A. vera* dehydrated gel powder (Daltonmax 700®) and whole leaf material was donated by Improve USA, Inc. (Texas, United States of America). *Aloe marlothii* leaves were harvested sustainably from natural populations near Koster in the North West Province of South Africa, while *A. ferox* leaves were harvested from natural populations near Albertina in the Western Cape Province of South Africa. The traditional hand-filleting method to obtain the gel material of the *A. marlothii* and *A. ferox* leaves was used as it was developed to prevent contamination of the gel with the yellow sap (latex/aloin). Briefly, it involved cutting off the leaf base, tapering point, the margins and the rind from both the top and bottom flat sides of the leaves [14]. The gel was then rinsed with water to wash off the exudate (yellow sap) and was subsequently liquidised in a kitchen blender either alone or together with parts of the green rind to obtain the gel and whole leaf materials, respectively [17]. It was lyophylised with a freeze dryer (VirTis, United Kingdom) and the dried powder was passed through a sieve (150 - 180 µm) and stored in airtight containers.

2.3 Nuclear magnetic resonance (^{1}H-NMR) 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 ^{1}H-NMR spectra was recorded with an Avance III 600 Hz NMR spectrometer (Bruker, Germany). The obtained ^{1}H-NMR spectra was used to identify certain marker molecules known to be present in fresh aloe leaf materials such as aloverose, glucose, malic acid and iso-citric acid [18].

2.4 Preparation of phosphate buffer solutions

Phosphate buffer solution (PBS, pH 7.4) utilised during this study was prepared by combining 250 ml of 0.2 M KH_2PO_4 with 393.4 ml of 0.1 M NaOH and adjusting the pH with 10% H_3PO_4 [19]. The PBS (pH 6.5) used in the donor solutions was prepared by dissolving 11.99 g H_2NaO_4P in 900 ml distilled water, adjusting the pH with 2 M NaOH and making it up to a volume of 1000 ml with distilled water [20].

2.5 Preparation of receptor and donor phase solutions

PBS (pH 7.4) containing 10% HPLC-grade ethanol was employed as the receptor phase for the membrane release as well as the skin diffusion studies. For relatively lipophilic compounds such as ketoprofen (log P value of 1.0, pH 7.4) [21], it is recommended a receptor fluid be selected into which the compound will freely partition [22]. Therefore 10% ethanol (v/v) was incorporated into the receptor fluid (PBS, pH 7.4) as it enhanced the solubility of ketoprofen and provided sink conditions [23]. The receptor solution was kept at a temperature of 37 °C, prior to and during the permeation studies, in a water bath.

The basic composition of the aloe-containing donor test solutions can be seen in Table 1. Ketoprofen was dissolved in HPLC-grade ethanol before adding the PBS (pH 6.5) and the aloe leaf materials in three different concentrations: 3.00%, 1.50%, 0.75% (w/v). It was observed the ketoprofen tended to lower the pH of the solution therefore the final pH was adjusted to 6.5 with 2 M NaOH. During the membrane release studies all the solutions containing the various concentrations of the aloe leaf materials (i.e. 3.00%, 1.50% or 0.75% (w/v)) were tested for their release characteristics. 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. Prior to the membrane and skin diffusion studies, the donor solutions were preheated in a water bath at 32 °C.

2.6 High performance liquid chromatography analysis of ketoprofen

A high performance liquid chromatography (HPLC) method was developed and validated for ketoprofen at the Analytical Technology Laboratory (ATL) of the North-West University, Potchefstroom Campus, South Africa. This was done to demonstrate that the method was reliable and sensitive in the determination of the amount of ketoprofen in the samples analysed for both the receptor phase as well as in the skin tissue. The limit of detection was 0.001 µg/ml and the limit of quantification was 0.005 µg/ml. Calibration curves were constructed ranging in concentrations of 250 – 4000 µg/ml for the membrane release studies and 0.125 – 50 µg/ml for the skin diffusion studies.

An Agilent® 1100 series HPLC device with isocratic pump, autosampler and diode array detector and Chemstation Rev. A.10.01 software for data acquisition and analysis (Agilent Technologies, Palo Alto, CA) were utilised. The column used was an Agela® Venusil XBP C_{18} (2) (4.6 x 150mm) with a 5 µm particle size (Agela® Technologies, Newark, DE). The mobile phase consisted of 60 volumes of acetonitrile (CH_3CN) and 40 volumes of distilled water containing 1% acetic acid (CH_3COOH) degassed using an ultrasonic bath prior to use. The flow rate was set to 1.0 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.

2.7 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 [23]. 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. The donors gave informed consent prior to the collection of the skin and their identities were masked to ensure anonymity.

The skin was thawed at room temperature prior to processing. A Zimmer™ electric dermatome model 8821 was utilised to dermatome the skin to a thickness of 400 µm and a width of 2.5 cm. The harvested skin consisted of the SC, viable epidermis and upper dermis [24]. The harvested skin samples were examined for any defects (scars, stretch marks, etc.) and positioned onto Whatman® filter paper with the SC facing upwards. Thereafter, it was placed in aluminium foil sheets and kept frozen at -20 °C until use. The frozen skin was thawed at room temperature and cut into circles with a diameter of approximately 15 mm before the onset of the diffusion study [24].

2.8 Membrane release and skin diffusion studies

Prior to 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 (with a gel-like structure) released the marker, ketoprofen, at the highest 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 used for the membrane release and skin permeation studies.

Magnetic stirrer bars were added to the receptor chambers and either the cellulose nitrate membranes or dermatomed skin (SC facing upward) was mounted between the donor and receptor compartments for the release and skin diffusion studies, respectively. To prevent any leakage, Dow Corning® high vacuum grease was applied to each Franz cell. The donor and receptor compartments, where the drug solution and the receptor fluid were placed respectively, were then clamped together by a horseshoe clamp. The receptor compartments were filled in such a way as to avoid the entrapment of air bubbles under the artificial membrane or dermatomed skin. The donor compartments were filled with 1 ml of the donor solution and subsequently covered with Parafilm® and a plastic cap to prevent evaporation during the experiment. To maintain a constant temperature, the Franz cells were then placed on a submersible magnetic stirrer plate (750 rpm) in a 37 °C water bath to attain a skin temperature

of 32 °C [25]. The entire receptor phase was withdrawn at specific time intervals and subsequently replenished with fresh receptor phase, kept at a constant temperature of 37 °C [9]. During the membrane release studies the time interval was 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 withdrawn samples were placed in glass vials and immediately analysed on the HPLC.

2.9 Tape stripping method

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 [26]. By doing this the penetration of topically applied active ingredients into the uppermost layers of the skin can be examined [27]. The tape stripping method was performed as previously described by Pellet *et al.* [28]. Following the 12 h diffusion study, the receptor and donor phases were removed and the diffusion cells gently taken apart, then the skin was pinned on a piece of Parafilm® and stapled to a solid surface. Indentation from the diffusion cells clearly marked the exposed diffusional area. The skin was dabbed dry with tissue paper to remove any remaining drug solution present on the surface of the skin.

Pieces of 3M Scotch® Magic™ tape were cut to cover the diffusional area, ensuring that it did not overlap the areas outside the diffusion cell imprints. The first tape strip, which may have been contaminated by the drug solution that adhered on the surface of the skin, was discarded as part of the cleaning process. The following 15 tape strips (SC-epidermis) were placed in a glass container filled with 5 ml of a 40% ethanol in PBS (pH 7.4) and kept overnight at 4 °C

Previous studies [29] found that hydration of the SC assists the removal of a larger quantity of SC than compared to dry skin. Therefore, hydrated skin requires less stripping (10 strips) to remove the SC than dry skin (29 strips) [29]. Hence, in the present study, it was assumed that the increased application time (12 h) of the ketoprofen solutions hydrated the skin and therefore only 15 tape strips were used to facilitate the removal of the SC. An indication of the complete removal of the SC is when the viable epidermal layer glistens. Excess skin was cut away from the flange imprints left by the diffusion cells and the remaining skin (epidermis-dermis) was cut into pieces to enlarge surface area. It was then placed in a glass container containing 5 ml of 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 utilising 0.45 µm syringe filters, then placed in glass vials and analysed on HPLC.

2.10 Data analysis

2.10.1 Transdermal data analysis

The cumulative concentration (µg/cm^2) of ketoprofen, which permeated the membranes and/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 average percentage released or diffused from the total amount applied to the donor compartment of the Franz cells also determined for both the membrane release or skin diffusion studies, respectively. 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 [30].

The permeation profiles were further analysed by applying the data to a non-linear curve fitting procedure described by Díez-Sales *et al.* [31] and Otto *et al.* [24]. The following Eq. (1) [32] 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 x 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 computerised non-linear least square method (EasyPlot, Spiral Software, Norwich, Vt., USA). The permeability coefficient (k_p) was calculated using Eq. (3).

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

2.10.2 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 [33]. Data was 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 [34, 35]. The average can be used for normally distributed (symmetric) data; whereas the median can be used for skewed data as it is unaffected by outliers [36]. 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 [36]. The box-plots were drawn with the top at the third quartile and the bottom at the first quartile; the length visually represents the middle 50% of the data. The whiskers (straight lines) extend 1.5 times the interquartile range above and below the 75^{th} and 25^{th} percentiles and the outliers (values above or below the whiskers) are represented by circles [36]. The inferential statistics involved analysis of variance (ANOVA) as well as non-parametric hypothesis testing in order to compare the different solutions with each other.

Data from the membrane release studies were subject to three-way ANOVA analysis in order 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 (honestly significant difference) test to identify exactly between which average values the difference lay. The Tukey's HSD test is generally performed for unplanned comparisons when all possible pairwise comparisons in a set of data are made [34].

In order to investigate the significant differences between 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 HSD and Dunnett's t-test. In order to make use of these parametric statistical tests, certain assumptions need to be met such as needing to have a normal distribution [37]. The construction of normal probability plots was to evaluate the normality of the data and it was found the data was skew and not close to normal distribution. Therefore the non-parametric Kruskal-Wallis *H*-test with multiple comparisons was preferred, although it revealed less significant differences than the Tukey post-hoc test. A p-value <0.05 indicates statistically significant differences between the values that were compared.

3 Results and discussion

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

Aloverose (or partly acetylated polymannose or acemannan), glucose and malic acid, which are the major marker molecules used for identifying *A. vera* gel material [18], were all detected by ^{1}H-NMR spectroscopy. Aloverose was not detected in the *A. marlothii* and *A. ferox* gel materials, although glucose and malic acid were present as found previously [16]. All the whole leaf materials (*A. vera*, *A. marlothii* and *A. ferox*) contained the marker compounds glucose and malic acid, plus an additional whole leaf marker (i.e. iso-citric acid) was detected which is

characteristic of fresh aloe whole leaf extract material [18, 38]. 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 [16].

3.2 Membrane release studies

The percentage of ketoprofen released from the solution, the average flux as well as the median flux values of the 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. This indicates that a portion of ketoprofen was released from the gel-like structures of all the aloe leaf material-containing solutions, although to different extents.

The average flux values were however, influenced by skewed distributions around the central location and could give an inaccurate estimation of the true flux values. Thus, the median flux values were calculated, as outliers in the data (Dawson & Trapp, 2004) did not affect them and were more representative of the true flux value of ketoprofen from the different aloe leaf material solutions. Comparison of the median flux values of the different aloe leaf material concentrations (0.75, 1.50 and 3.00% (w/v)) indicated the highest median flux values were obtained at the lowest concentration (i.e. 0.75% (w/v)) for most of the solutions tested, except for *A. vera* gel.

When comparing the different aloe leaf material concentrations with each other (i.e. 0.75, 1.50 and 3.00% (w/v)), it is clear 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 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.*

3.3 Transdermal skin diffusion studies

3.3.1 Flux, percentage ketoprofen diffused and enhancement ratio (ER)

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 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. *Aloe 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 = 1.590) 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 to 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 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, oxybitynin 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 due to 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 prior to being transported across the skin, i.e. permeation enhancement occurs by a 'pull effect' [9]. Numerous studies [39, 40] 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 [41].

Another study [42] found that pre-treatment with *A. vera* juice did not enhance the *in vitro* permeation of ketoprofen across the skin. The *A. vera* juice was applied and subsequently removed after 1 h before dosing the skin with a saturated solution of ketoprofen in polyethylene glycol 400 [42]. It was suggested that the ketoprofen did not complex with the, as yet unidentified, enhancing factor the same way as described by Cole & Heard [9] when utilised '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 hypothesised that ketoprofen had the opportunity to interact with the aloe phytochemicals (i.e. enhancing factor) in the aloe-containing solutions in order to facilitate its transport across the skin.

Certain polysaccharides are known penetration enhancers [43] and the mucilaginous gel of the aloe consists mainly of polysaccharides [13]. *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), which should be further investigated in order to elucidate the causative factor.

These differences in the penetration enhancing effects for the different aloe leaf materials can be explained by differences in their chemical compositions. 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 [41, 44].

3.3.2 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]. In order to investigate by which of these two mechanisms the aloe leaf materials possibly enhanced the permeation of ketoprofen, the α- and β-values were obtained by applying the various permeation profiles to a non-linear curve-fitting procedure [31]. 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) [45]. 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 these two aloe leaf materials increased the skin partitioning of the ketoprofen when compared to

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 multiple comparisons test) compared to 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 [46]. 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 to 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 to the control group (ketoprofen alone) all the aloe leaf materials, except *A. vera* whole leaf, decreased the lag time. *Aloe ferox* gel and *A. ferox* whole leaf groups decreased 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.

3.4 Tape stripping

3.4.1 Ketoprofen concentration in the SC-epidermis

Comparing the average SC-epidermis concentrations (Figure 1) 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).

The results showed *A. marlothii* whole leaf did not enhance the permeation of ketoprofen through the skin (as reflected by its low flux value), but did 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. It is important to note 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 1) except, for *A. marlothii* whole leaf, which had a median concentration value of 2.461 µg/ml. This indicates both the average and median concentration values can be used, but it is proposed only the median concentrations be used, as they are unaffected by a distortion in the spread of the data [36].

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

3.4.2 Ketoprofen concentration in the epidermis-dermis

The epidermis-dermis ketoprofen concentration values of the different test solutions are depicted in box-plots in Figure 2. 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.

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. Since 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 [36].

Of the possible twenty-one comparisons amongst 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 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 [47].

4 Conclusion

Results from the membrane release studies indicated that ketoprofen was released from all the aloe-containing solutions investigated in this study. However, the 0.75% (w/v) aloe solution concentration had the highest ketoprofen flux values as well as the highest average percentage ketoprofen released for the majority of tested aloe leaf material-containing solutions. Subsequently, the permeation enhancing abilities of the various aloe leaf materials were tested at this concentration (i.e. 0.75% (w/v)) during the skin diffusion studies.

Aloe vera gel test solution statistically significantly enhanced the permeation of ketoprofen across dermatomed skin compared to 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 give an indication that these two aloe leaf materials increased the skin partitioning of the ketoprofen when compared to the control group. In contrast, *A. ferox* whole leaf modified the diffusion characteristics of the skin toward ketoprofen as reflected by its high relatively high β-value [46].

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 with average ketoprofen concentrations of 3.285 µg/ml and 2.646 µg/ml, respectively. In general, the epidermis-dermis concentrations of ketoprofen was found to be lower than for the SC-epidermis concentrations, indicating the ketoprofen had a high tendency to leave the aqueous vehicle and migrate into the SC, but had some difficulty in penetrating the hydrophilic viable epidermis [47].

In general, the *A. vera* leaf materials proved to be more effective in ketoprofen penetration enhancement across skin compared to *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. Further research is needed to determine the specific enhancing factor present in these aloe leaf materials.

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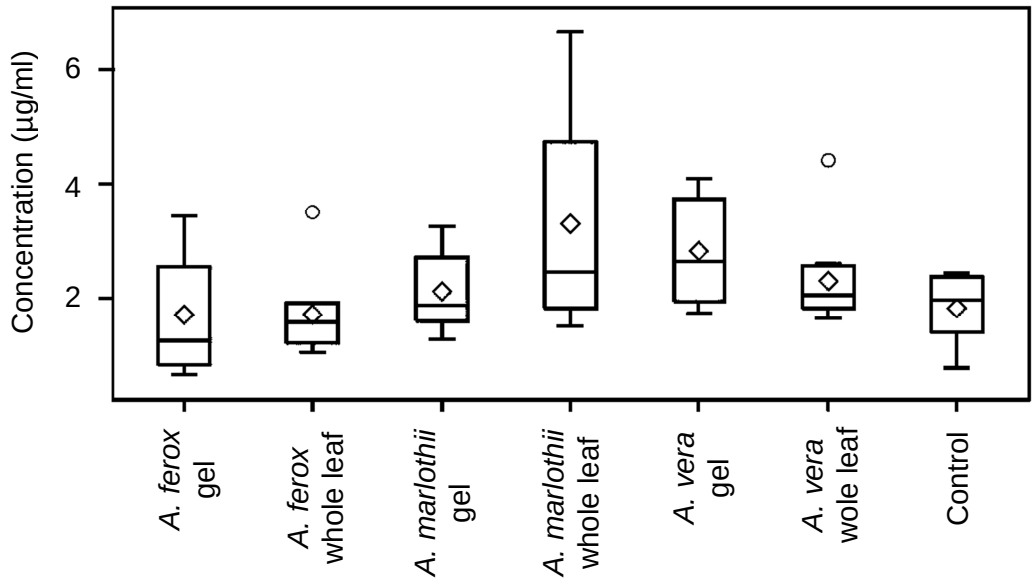
Figure legends

Figure 1: Box-plots depicting the concentration (µg/ml) ketoprofen present in the SC-epidermis for the different aloe leaf material solutions after tape stripping. The average and median concentration values are indicated by the diamond shapes and lines, respectively.

Figure 2: Box-plots depicting the concentration (µg/ml) ketoprofen present in the epidermis-dermis for the different aloe leaf material solutions after tape stripping. The average and median concentration values are indicated by the diamond shapes and lines, respectively.



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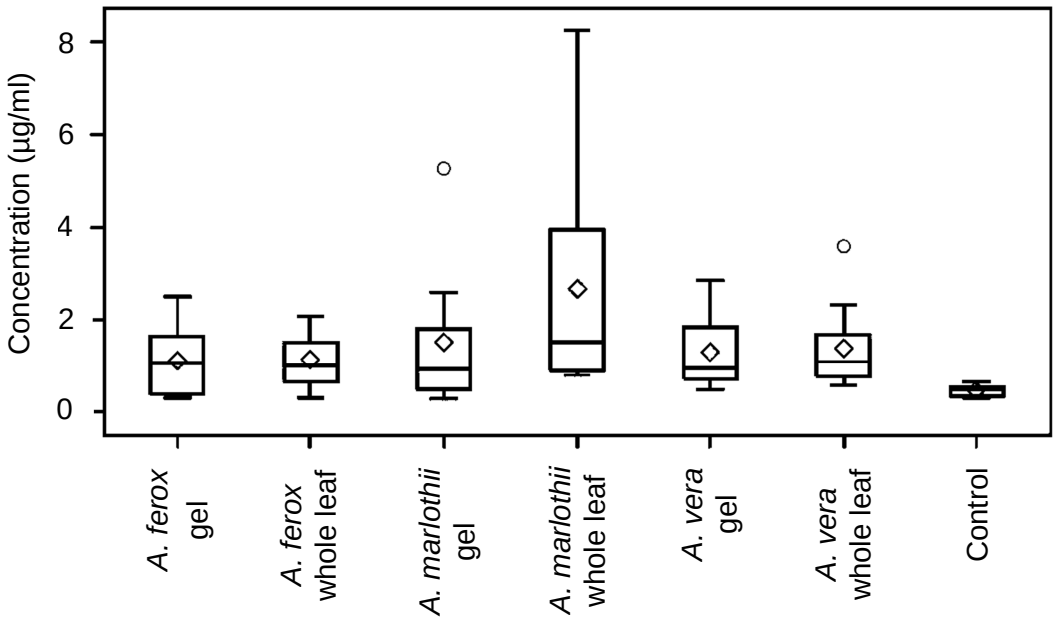

Figure 2: Box-plots depicting the concentration (µg/ml) ketoprofen present in the epidermis-dermis for the different aloe leaf material solutions after tape stripping. The average and median concentration values are indicated by the diamond shapes and lines, respectively.

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*

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/cm^2.h)	Median flux (µg/cm^2.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/cm^2.h), median flux (µg/cm^2.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/cm^2.h)	Median Flux (µg/cm^2.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

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