Transdermal penetration enhancement and clinical efficacy of *Aloe marlothii* and *Aloe ferox* compared to *Aloe vera*

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Abbreviations

AF	*Aloe ferox*
AFG	*Aloe ferox* gel
AFWL	*Aloe ferox* whole leaf
AM	*Aloe marlothii*
AMG	*Aloe marlothii* gel
AMWL	*Aloe marlothii* whole leaf
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
ATL	Analytical Technology Laboratory
AV	*Aloe vera*
AVG	*Aloe vera* gel
AVWL	*Aloe vera* whole leaf
CEL	Cosmetic Efficacy Laboratory
CH_3CN	Acetonitrile
CH_3COOH	Acetic acid
D	Diffusion coefficient
D_2O	Deuterium oxide
ED	Epidermis-dermis
ENT	Entropy
ER	Enhancement ratio
G	Gel

H_2NaO_4P	Sodium dihydrogen phosphate anhydrous
^{1}H-NMR	Proton nuclear magnetic resonance
HOM	Homogeneity
HPLC	High performance liquid chromatography
K	Partition coefficient
KH_2PO_4	Potassium dihydrogen orthophosphate
k_p	Permeation coefficient
LOD	Limit of detection
log P	Octanol-water partition coefficient
LOQ	Limit of quantification
mAU	Mean peak area
NaOH	Sodium hydroxide
NMF	Natural moisturising factor
NRJ	Energy
PBS	Phosphate buffer solution
%RSD	Percentage relative standard deviation
SC	Stratum Corneum
SCE	Stratum corneum-epidermis
SD	Standard deviation
SLS	Sodium lauryl sulphate
TEWL	Transepidermal water loss
UV	Ultraviolet
WL	Whole leaf

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Abstract

Extensive research has already been performed on *Aloe vera* therefore it is important that researchers include other aloe species, such as *Aloe marlothii* and *Aloe ferox*, in studies involving aloe plant materials (Loots *et al.*, 2007:6891). The use of natural products has regained popularity and in recent years the demand for alternative medication has risen considerably (Walji & Wiktorowicz, 2013:86).

The hydration state of the human skin is fundamental for its normal functioning (Verdier-Sévrain & Bonté, 2007:75), with healthy skin possessing a water content higher than 10% (w/v) (Blank, 1952:439). This demonstrates the importance of the topical application of skin moisturisers as part of basic skin care regime (Verdier-Sévrain & Bonté, 2007:75).

The first part of this project focused on the *in vivo* skin hydration effects of the precipitated polysaccharide components of *A. vera*, *A. ferox* and *A. marlothii* leaf gel materials (3% (w/v)) after single (30, 90 and 150 min after application) and multiple applications (twice daily application over a period of four weeks) on healthy volunteers, respectively. The anti-erythema effects of these aloe materials on sodium lauryl sulphate irritated skin were also examined.

The skin hydration effects of the aloe materials were determined with the Corneometer® CM 825 and Visioscan® VC 98 during the short term study (single application) and longer term study (multiple applications). In addition, as an indirect measurement of skin hydration, the Cutometer® dual MPA 580 was used to measure skin elasticity during the longer term study. To determine the anti-erythema effects of the aloe materials when applied to irritated skin areas, the haemoglobin content of the skin was measured with a Mexameter® MX 18.

The results from the *in vivo* study indicated that *A. ferox* gel material dehydrated the skin, whereas *A. vera* and *A. marlothii* gel materials hydrated the skin during the short term study. Results from the longer term study showed that all the aloe leaf materials have skin dehydration effects, probably due to the aloe absorbing moisture from the skin into the applied gel layer upon drying. From the anti-erythema study, it was seen that *A. vera* and *A. ferox* materials had the potential to reduce erythema on the skin similar to that of the positive control group (i.e. hydrocortisone gel) after six days of treatment.

The skin possesses exceptional barrier properties which can mostly be ascribed to the outermost layer of the skin, the stratum corneum (SC). Due to the physical barrier the skin has against drug permeation, the delivery of drug molecules into and across the skin continues to be

challenging (Lane, 2013:13) and to overcome this barrier, penetration enhancers can be used to efficiently deliver drugs across the skin (Barry, 2002:522).

The aim of the second part of this project was to determine the skin penetration enhancing effects of the gel and whole leaf materials of *A. vera*, *A. marlothii* and *A. ferox*. Ketoprofen was used as the marker compound and a high performance liquid chromatography (HPLC) method was developed and validated to determine the amount of ketoprofen present in the samples.

Prior to the skin diffusion studies, membrane release studies were performed to test whether the solutions containing different concentrations of the aloe leaf materials (i.e. 3.00%, 1.50% and 0.75% (w/v)) released ketoprofen from their gel-like structures. From these studies, it was evident the 0.75% (w/v) concentration had the highest average percentage ketoprofen release, which was subsequently chosen as the concentration for the aloe leaf materials tested in the transdermal skin diffusion studies.

The *in vitro* permeation study was conducted across dermatomed (400 µm thick) skin in Franz diffusion cells. Tape stripping was performed after completion of the diffusion studies to determine the concentration ketoprofen present in the SC-epidermis and epidermis-dermis layers of the skin.

Results from the *in vitro* permeation study showed that *A. vera* gel enhanced the flux of ketoprofen to the highest extent (20.464 µg/cm^2.h) when compared to the control group (8.020 µg/cm^2.h). *Aloe marlothii* gel (12.756 µg/cm^2.h) and *A. ferox* whole leaf material (12.187 µg/cm^2.h) also enhanced the permeation of ketoprofen across the skin compared to the control group. *A. vera* gel material was the most efficient transdermal drug penetration enhancer of the selected aloe species investigated.

In order to determine by which mechanism the aloe leaf materials enhanced the skin permeation of ketoprofen (Hadgraft *et al.*, 2003:141), the permeation profiles were analysed using a non-linear curve-fitting procedure (Díez-Sales *et al.,* 1991:3) to obtain α, β and k_p values. A change in the α-value indicated the aloe leaf material influenced the partition coefficient (K), whereas a change in β indicated the aloe leaf material influenced the diffusivity (D) (with the assumption that *h*, the diffusional path length is constant) (Otto *et al*., 2010:278).

The calculated α-values indicated the drug permeation enhancing effect of *A. vera* gel can be ascribed to an increased partitioning of the drug into the skin. The calculated β-values showed *A. ferox* whole leaf altered the diffusion characteristics of the skin for ketoprofen. The tape stripping results showed *A. marlothii* whole leaf delivered the highest concentration of the ketoprofen into the SC-epidermis and epidermis-dermis layers of the skin.

Keywords: *Aloe vera*, *Aloe marlothii*, *Aloe ferox*, skin hydration, anti-erythema, gel, whole leaf, penetration enhancer, tape stripping

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Uittreksel

Ekstensiewe navorsing is reeds op *Aloe vera* gedoen, daarom is dit belangrik dat navorsers ander aalwynspesies, soos byvoorbeeld *Aloe marlothii* en *Aloe ferox*, insluit in ondersoeke wat op aalwyn-plantmateriale uitgevoer word (Loots *et al.*, 2007:6891). Die gebruik van natuurlike produkte het gewildheid herwin en in die afgelope jare het die navraag na alternatiewe medisynes aansienlik toegeneem (Walji & Wiktorowicz, 2013:86).

Die hidrasietoestand van die vel is fundamenteel vir sy normale funksionering (Verdier-Sévrain & Bonté, 2007:75); waar gesonde vel 'n waterinhoud van hoër as 10% (w/v) besit (Blank, 1952:439). Dit demonstreer die belangrikheid van die topikale aanwending van vel bevogtigingsmiddels as deel van 'n basiese velsorgregime (Verdier-Sévrain & Bonté, 2007:75).

Die eerste deel van die projek het op die *in vivo* velbevogtigingseienskappe van die polisakkaried bevattende komponente van die *A. vera*, *A. ferox* en *A. marlothii* jel materiale (3% (w/v)) gefokus na 'n enkele aanwending (30, 90 en 150 min na aanwending) sowel as na veelvuldige aanwendings (twee keer daaglikse aanwending oor 'n tydperk van vier weke) op vrywillige deelnemers. Die anti-eriteem effekte van die drie aalwynmateriale op natriumlaurielsulfaat (NLS) geïrriteerde vel is ook ondersoek.

Die velbevogtigingseienskappe is met die Corneometer® CM 825 en die Visioscan® VC 98 bepaal tydens die korttermyn (enkele aanwending) en langtermyn (veelvuldige) studies. Die Cutometer® dual MPA 580 is as 'n indirekte bepaling van velhidrasie gebruik om die vel se elastisiteit te meet tydens die langtermynstudie. Om die anti-eriteemeffekte van die aalwynmateriale te bepaal nadat dit op geïrriteerde vel areas aangewend is, was die hemoglobieninhoud van die vel met behulp van die Mexameter® MX 18 gemeet.

Resultate het aangedui dat die *A. ferox* materiaal die vel gedehidreer het tydens die korttermynstudie, terwyl *A. vera* en *A. marlothii* materiale die vel gehidreer het. Resultate tydens die langtermynstudie het getoon dat al die aalwynmateriale dehidrerende eienskappe op die vel aandui, waarskynlik as gevolg van vog wat vanuit die vel geabsorbeer is wanneer die aangewende aalwynjellaag droog geword het.

Vanuit die anti-eriteemstudie, is dit gesien dat *A. vera* en *A. ferox* die potensiaal besit om veleriteem te verminder soortgelyk aan die effek van die positiewe kontrole groep (d.i. hidrokortisoonjel) na ses dae van behandeling.

Die vel besit uitstekende fisiese skansfunksie eienskappe wat meestal toegeskryf kan word aan die buitenste laag van die vel, naamlik die stratum korneum (SK). As gevolg van die skans wat die vel bied teen die deurdringing van geneesmiddels, is die aflewering van geneesmiddelmolekules binne-in en deur die vel 'n voortdurende uitdaging (Lane, 2013:13). Om die fisiese skans te oorkom, kan penetrasiebevorderaars gebruik word om die beweging van geneesmiddels oor die vel te verbeter (Barry, 2002:522).

Die doel van die tweede deel van die projek was om die penetrasiebevorderingsvermoë van die jel en heelblaarmateriale van *A. vera*, *A. marlothii* en *A. ferox* te ondersoek. Ketoprofen is gebruik as die modelverbinding en 'n hoë drukvloeistofchromatografie (HDVK) metode was ontwikkel en gevalideer om sodoende die hoeveelheid ketoprofen in die monsters te bepaal.

Voordat die veldiffusiestudies gedoen is, was die membraanvrystellingsstudies uitgevoer om te bepaal of die oplossings wat die verskillende konsentrasies aalwyn-blaarmateriale bevat (i.e. 3.00%, 1.50% en 0.75% (w/v)) die ketoprofen van hulle jelagtige strukture vrygestel het. Uit hierdie studies was dit duidelik dat die 0.75% (w/v) konsentrasie die hoogste gemiddelde % ketoprofen vrystelling gehad het en daarom is hierdie konsentrasie tydens die transdermale veldiffusiestudies gebruik.

Die *in vitro* ketoprofen permeasie was bepaal met gedermatoomde (400 µm in dikte) vel in Franz diffusie selle. 'n Kleefbandafstropingstudie is uitgevoer na afloop van die diffusiestudies om die konsentrasie ketoprofen teenwoordig in die SK-epidermis en epidermis-dermis vellae te bepaal.

Resultate van die permeasiestudie het gewys dat *A. vera* jel materiaal die aflewering van ketoprofen oor die vel die meeste bevorder het (20.464 µg/cm^2.h) wanneer dit met die kontrole groep (8.020 µg/cm^2.h) vergelyk word. *Aloe marlothii* jel (12.756 µg/cm^2.h) en *A. ferox* heelblaarmateriaal (12.187 µg/cm^2.h) het ook die permeasie van ketoprofen oor die vel verhoog in vergelyking met die kontrole groep. *A. vera* jel materiaal was die effektiefste transdermale geneesmiddel penetrasiebevorderaar van al die geselekteerde aalwynspesies wat ondersoek is.

Ten einde die meganisme te bepaal waardeur die aalwyn-blaarmateriale die penetrasie van ketoprofen oor die vel bevorder het (Hadgraft *et al.*, 2003:141), is die penetrasieprofiele geanaliseer deur middel van 'n nie-liniêre kurwe-passende prosedure (Díez-Sales *et al.,* 1991:3) om die α, β en k_p waardes te verkry. 'n Verandering in α-waarde wys daarop dat die aalwyn-blaarmateriale die partisiekoëffisiënt (K) beïnvloed, terwyl 'n verandering in β-waarde 'n aanduiding is dat die aalwyn-blaarmateriale die diffusie (D) beïnvloed (met die veronderstelling dat *h*, die diffusie padlengte, konstant is) (Otto *et al*., 2010:278).

Die berekende α-waardes het aangedui dat die geneesmiddelpenetrasie-effek van die *A. vera* jel toegeskryf kan word aan die verhoogde partisie van die geneesmiddel in die vel. Die berekende β-waardes wys daarop dat die *A. ferox* heelblaarmateriaal die diffusie karakteristieke van die vel teenoor ketoprofen verander het. Die kleefbandafstropingsresultate het daarop gewys dat die *A. marlothii* heelblaarmateriaal die hooste konsentrasie ketoprofen afgelewer het in die SK-epidermis en epidermis-dermislae van die vel.

Sleutelwoorde: *Aloe vera*, *Aloe marlothii*, *Aloe ferox*, velhidrasie, anti-eriteem, jel, heelblaar, penetrasiebevorderaar, kleefbandafstroping

Verwysings

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Foreword

The aim of this study was to investigate the *in vivo* skin hydration and anti-erythema effects as well as the *in vitro* skin penetration enhancing effects of isolated leaf materials from three aloe species, namely *Aloe vera*, *Aloe marlothii* and *Aloe ferox*.

This thesis is presented in the article format as prescribed by guidelines of the North-West University and contains introductory chapters, which include an already published review article in the peer-reviewed journal "*Molecules"*. Also included are two full length research articles for publication in the journals "*Pharmacognosy"* and the "*European Journal of Pharmaceutics and Biopharmaceutics"* for which the complete guides for authors are included in Appendix G and H, respectively. In addition to these research articles, detailed experimental methods and data are given in different appendices of this thesis.

I truly feel blessed for having the opportunity to fulfil my dream in completing a PhD project. Not only have I grown as a young researcher, but also as a person. I have learnt a great deal and have gained countless experience. I am looking forward to the new chapters in my life!