The activity of *Aloe arborescens* Miller varieties on wound-associated pathogens, wound healing and growth factor production

Samantha Loggenberg[1], Danielle Twilley[1], Marco Nuno De Canha[1], Debra Meyer[2], Namrita Lall[1,3,4,5,*]

[1] *Department of Plant and Soil Sciences, University of Pretoria, Pretoria 0002, South Africa*

[2] *Department of Biochemistry, Genetics & Microbiology, Centre for Bioinformatics & Computational Biology, University of Pretoria, Pretoria 0002, South Africa.*

[3] *School of Natural Resources, University of Missouri, Columbia, MO, United States*

[4] *College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India*

[5] *Senior Research fellow, Bio-Tech R&D Institute, University of the West Indies, Kingston, Jamaica*

[*] Corresponding author: Namrita Lall

Email address: namrita.lall@up.ac.za

Highlights

- Varying bioactivity between *Aloe arborescens* varieties and extract preparation (gel or leaf).
- 'Eloff' and 'Jack Marais' ethanolic leaf extracts (at 50 μg/mL) exhibited percentage closure of 56.06 ± 1.47 and 73.72 ± 0.65 %, respectively ($p < 0.05$).
- 'Jack Marais' gel and ethanolic leaf extract (at 50 μg/mL) increased platelet-derived growth factor-AA secretion to 601.09 ± 97.77 and 559.43 ± 112.52 pg/mL, respectively ($p < 0.05$).

Abstract

Various *Aloe* L. species have been used worldwide to soothe and treat dermal wounds and burns. However, there is a lack of substantiative research on the efficacy of *Aloe* L. species for wound healing. The aim of this study was to investigate the differences in biological activities of the gel and ethanolic (EtOH) leaf extracts of seven *Aloe arborescens* Miller varieties. The extracts were investigated for their antibacterial activity against wound-associated bacteria, *Staphylococcus aureus* (ATCC 6538 and ATCC 25293) and *Pseudomonas aeruginosa* (ATCC 9027), as well as their nitric oxide (NO) scavenging potential. Varieties with antibacterial activity were further evaluated for wound closure and growth factor stimulation in human keratinocytes (HaCaT). The EtOH leaf extract of the 'Eloff' and 'Jack Marais' varieties displayed antibacterial activity against *S. aureus*

ATCC 25293 (MIC of 500 and 250 µg/mL, respectively). The EtOH leaf extract of 'Jack Marais' displayed an MIC of 500 µg/mL against *S. aureus* ATCC 6538. The gel extract of 'Le Roux' exhibited antioxidant activity with a half maximal inhibitory concentration (IC$_{50}$ of 2696 $\pm$ 582.66 µg/mL). The EtOH leaf extracts of 'Eloff' and 'Jack Marais' showed significant ($p < 0.05$) wound closure of 56.06 $\pm$ 1.47 and 73.72 $\pm$ 0.65%, respectively at 50 µg/mL and the 'Jack Marais' gel extract significantly stimulated wound closure ($p < 0.05$) by 77.02 $\pm$ 1.97 and 71.51 $\pm$ 1.11% at 50 and 100 µg/mL, respectively. Both the 'Jack Marais' gel extract (at 50 and 100 µg/mL) and the EtOH leaf extract (50 µg/mL), significantly ($p < 0.05$) increased platelet-derived growth factor (PDGF-AA) secretion to 601.09 $\pm$ 97.77, 1035.00 $\pm$ 913.98 and 559.43 $\pm$ 112.52 pg/mL, respectively. The 'Jack Marais' EtOH leaf extract exhibited the highest antibacterial activity, whereas the gel extract displayed the greatest potential to stimulate wound closure. This not only suggests a difference in biological activity among varieties but also between the type of extract (gel or leaf).

Keywords: Wound healing, *Aloe arborescencs* Miller, antibacterial, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, nitric oxide scavenging, scratch assay, platelet-derived growth factor (PDGF-AA)

1. Introduction

The wound healing process is a dynamic and complex system that involves numerous cellular factors and four overlapping stages, namely, haemostasis, inflammation, cellular proliferation and tissue maturation (Halloran and Slavin, 2002). Upon injury to the dermal layer, blood rushes to the wound site to allow inactive blood platelets to interact with exposed cellular collagen and become activated. This interaction facilitates blood clotting, through the recruitment and coagulation of red blood cells (RBCs) and actin filaments, to prevent further bleeding from the wound (Barrientos et al., 2008). White blood cells (WBCs) and neutrophils, also known as natural killer cells, near the wound site detect and destroy pathogens that may have entered the exposed tissue, whilst simultaneously clearing away cell debris (Fang et al., 2010). This detection of pathogenic proteins leads to the production and secretion of pro-inflammatory signaling molecules, such as nitric oxide (NO), by macrophages and neutrophils (Haroon et al., 2003). The secreted signaling molecules initiate the inflammatory response recruiting additional immune cells to the wound site to fight pathogenic entry. Excessive production of nitric oxide at the wound site has been implicated in prolonged recovery times, particularly in chronic wounds, therefore a balance between excessive and baseline nitric oxide production is necessary (Schulz and Stechmiller, 2006). This leads to inflammation around the wound as blood rushes to the injury site facilitating clotting and pathogenic destruction (Halloran and Slavin, 2002). Activated macrophages continue to recruit immune cells and secrete cytokines that stimulate the formation of granulation tissue, which consists of collagen and actin filaments. The granulation tissue acts as an extra-cellular matrix in which normal cellular functions may occur to facilitate tissue repair (Almine et al., 2012). The actin filaments within the granulation tissue contract while nascent dermal cells, like human keratinocytes, concurrently proliferate and migrate, to aid wound closure. Once the wound has completely closed, the final remodeling stage of the wound healing process ensues where the tensile strength of the nascent tissue increases (Li et al., 2013). The proliferation of human keratinocytes, is stimulated by various growth factors that are secreted by numerous somatic cells, including keratinocytes, during the stages of wound healing.

Growth factors secreted by keratinocytes which are involved in the regulation of wound healing include; angiopoietin-2 (ANGPT2), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), macrophage colony-stimulating factor (M-CSF), platelet-derived growth factor (PDGF-AA), vascular endothelial growth factor (VEGF) and erythropoietin (EPO) (Barrientos et al., 2008). Each of these growth factors have overlapping functions in wound healing through the modulation of angiogenesis and cell migration (Barrientos et al., 2008; Lynch et al., 1989). Complications or imbalances with the wound healing process may lead to negative downstream effects such as chronic wounds or pathogenic infection (Aldridge, 2015). Bacterial infection may cause increased inflammation at or around the wound site causing excessive swelling, which may hinder the wound healing process (Aldridge, 2015). Adverse pathogenic infection of wounds may spread to deeper tissues of the body and may reach the bloodstream, causing bacteremia. Thereafter, pathogens may

travel to other tissues of the body which could potentially trigger a rapid inflammatory response throughout the affected tissues, leading to septicemia (Zielin,ska-Borkowska, 2012). Excessive swelling and inflammation restricts blood flow to vital organs, or may hinder normal functioning of cellular mechanisms, ultimately leading to organ failure and even death (Churpek et al., 2017). Due to the unpredictability and rapid progression of septicemia, it has been deemed difficult to treat, whereas prevention by maintenance of wound infection is more plausible and presumably easier to achieve (de Janerio, 2019). Furthermore, studies have shown that pathogenic bacteria such as Staphylococcus aureus (37%) and Pseudomonas aeruginosa (17%) are the predominating pathogens isolated from patients suffering from pathogenic wound infection preceding septicemia (Churpek et al., 2017; Cohen and Kurzrock, 2004; Micek et al., 2005). This highlights the importance of developing an affordable treatment with antibacterial and anti-inflammatory properties that simultaneously facilitates healthy wound healing, through the stimulation of growth factors and ensures efficient and rapid wound recovery.

The use of medicinal plants as a source for novel drug discovery is made evident by the incorporation of these plants into various traditional medicine practices throughout the world (Ekor, 2014). According to a survey conducted by the World Health Organization (2019), it is estimated that over 80 % of individuals in South Africa use medicinal plants as a primary source of healthcare due to the lack of availability and affordability of modern treatments.

Aloe arborescens Miller, also known as "Krantz aloe", is a perennial flowering succulent from the 'Xanthorrhoeaceae' family, has been used for its wound healing properties throughout South Africa. This succulent readily hybridizes and there are a large number of varieties found throughout South Africa which may provide a wide range of ethnomedicinal properties (Hankey and Notten, 2021). The leaves are utilised across several South African cultures (such as the Zulu, Xhosa and Khoisan) for various ailments, including the use of leaf decoctions for pain-relief and immune boosting properties to livestock. The raw leaves are bitter in taste and are thus ingested to alleviate stomach aches, nausea and constipation. Despite being indigenous to South Africa, the inner leaf gel of A. arborescens has been reported to have wound healing and anti-inflammatory properties and is thus utilised to treat and soothe minor cuts, irritations and burns throughout the world (Pellizzoni et al., 2012).

Despite the wide-spread use of this *Aloe* L. species, there is a lack of research conducted on the bioactivity of different *A. arborescens* varieties. Therefore, the aim of this study was to identify the potential wound healing activity of *A. arborescens* varieties by investigating the antibacterial, antioxidant and antiproliferative effects and the potential stimulatory effects on growth factors associated with wound healing. Furthermore, the aim was to determine whether there were any differences in bioactivity among the seven varieties of *A. arborescens* originating from the Kirstenbosch National Botanical Garden collection, namely: 'Eloff', 'Huntley', 'Jack Marais', 'John Winter', 'Le Roux', 'Matthews' and 'Rycroft'.

2. Methods and materials

2.1. Materials and reagents

Bacterial strains of *S. aureus* (ATCC 6538 and ATCC 25293) and *P. aeruginosa* (ATCC 9027) were purchased from Anatech Analytical Technology (Johannesburg, SA). Reagents used to conduct the antibacterial assay, such as Nutrient Agar/Broth, Tryptic Soy Agar/Broth, vancomycin (purity $\geq$ 90 %) and ciprofloxacin (purity $\geq$ 98%), were acquired from Sigma Chemicals Co. (St. Louis, MO, USA). PrestoBlue® was obtained from Life Technologies (Johannesburg, SA). The human

keratinocyte (HaCaT) cell line was donated by the University of Cape Town. Reagents used for cell culture maintenance, such as Dulbecco's Modified Eagle Medium (DMEM), foetal bovine serum (FBS), phosphate buffer saline (PBS), penicillin, streptomycin and amphotericin B, were supplied by ThermoFisher Scientific (Johannesburg, South Africa). Cell culture consumables, such as sterile cell culture flasks and microtiter plates were purchased from Lasec SA (Pty) Ltd (Midrand, South Africa). Other reagents, such as sodium nitroprusside, actinomycin D (purity $\geq$ 95%), ascorbic acid (purity $\geq$ 98%), and Griess-Ilosvay's nitrite reagent, were acquired from Sigma Chemicals Co. (St. Louis, MO, USA). The LEGENDPlex™ Growth Factor Panel Flow Cytometry Kit was purchased from Biocom Biotech (Pty) Ltd (Centurion, SA).

2.2. Plant material collection and extraction

Clonal plant material of seven *A. arborescens* varieties that were originally obtained from the Kirstenbosch National Botanical Garden collection, was collected in late January 2020 from the Manie van der Schijff Botanical Garden (-25.752406, 28.229718) at the University of Pretoria (Table 1). The voucher specimen

5

numbers were obtained from the Kirstenbosch National Botanical Garden. For each plant specimen, the gel was separated from the leaf epidermis, and the gel was subsequently homogenized by blending and thereafter filtered through a cheesecloth. The gel filtrate was then freeze-dried with a freeze-dryer (ICHR101530, Alpha 1-2 Ldplus (Lasec SA (Pty) Ltd)) to obtain dried gel extracts. The leaf epidermis material was oven-dried at 37 °C and subsequently ground to a powder using a mortar and pestle. The powdered plant material was extracted at room temperature (25 °C) with absolute ethanol (99 %) and placed on a shaker (Shaking Platform from Labdesign Engineering (Pty) Ltd.) for 48 h before being vacuum-filtered and freeze-dried to obtain ethanolic leaf extracts. The extracts were then stored at 4 °C until further use. See Table 1 for the percentage yield obtained for each extract.

Table 1. Accession numbers of the *A. arborescens* varieties and the percentage extraction yield obtained for each extract

Aloe arborescens Miller variety	Accession number	Plant part	Plant material weight collected (g)	Extract mass obtained (g)	Percentage extraction yield (%)
Eloff	KBG 1985/196	Gel	112.54	0.61	0.54
		Leaf epidermis	14.30	0.55	3.85
Huntley	KBG 1988/209	Gel	369.64	3.57	0.97
		Leaf epidermis	64.03	3.39	5.29
Jack Marais	KBG 1993/417	Gel	104.34	0.57	0.55
		Leaf epidermis	12.97	0.26	2.00
John Winter	KBG 2001/74	Gel	128.42	0.86	0.67
		Leaf epidermis	22.81	0.99	4.34
Le Roux	KBG 2001/93	Gel	224.47	1.83	0.82
		Leaf epidermis	42.76	1.03	2.41
Matthews	KBG 1985/214	Gel	135.43	0.86	0.64
		Leaf epidermis	13.32	0.53	3.98
Rycroft	KBG 1983/667	Gel	72.18	0.84	1.16
		Leaf epidermis	24.38	1.28	5.25

2.3. Antibacterial assay

Pure cultures of *S. aureus* (ATCC 6538 and ATCC 25293) and *P. aeruginosa* (ATCC 9027) were maintained on sterile tryptic soy and nutrient agar plates, respectively. The *A. arborescens* variety extracts were evaluated for their antibacterial activity according to the method described by Lall et al (2013). Briefly, 24 h subcultures of *S. aureus* and *P. aeruginosa* were inoculated in correspondence with the 0.5 McFarland standard (1.5×10^8 colony forming units per mL (CFU/ mL)) in the appropriate broth and the resulting solutions were diluted further for use in the assay (1:1000). Stock concentrations of the extracts were prepared (2 mg/mL in 10% DMSO) and serially diluted and tested in triplicate at a final concentration of 7.81 – 500 µg/mL. Vancomycin (at a stock concentration of 200 µg/mL) was used as a positive control against *S. aureus* (tested at a final concentration of 0.39 – 50 µg/mL), whereas ciprofloxacin (at a stock concentration of 200 µg/mL) was used as a positive control against P. aeruginosa (tested at a final concentration of 0.039 - 5 µg/mL). Other controls included in the assay were media without bacteria, bacteria only (negative control) and a DMSO vehicle control (tested at a final concentration of 0.02 - 2.5 % v/v). Bacteria were added to all the wells (excluding the media controls) and the plates were subsequently incubated at 37°C for 18 h. After incubation, 20 µL of PrestoBlue® reagent was added to all wells to act as an indicator of bacterial growth and plates were incubated for 20 min. PrestoBlue® is a reagent that acts as an indicator of bacterial viability as the reagent changes from blue to pink in colour in the presence of viable cells. This colour

change is due to the reduction of resazurin to resorufin by cellular oxidoreductase enzymes within viable cells (Lall et al., 2013). The minimum inhibitory concentration (MIC) for each extract was then determined by visual analysis based on the observed colour change, with blue indicating bacterial inhibition and pink indicating bacterial growth.

2.4. Cell culture

Human keratinocytes (HaCaT) were cultured in DMEM supplemented with 10 % heat-inactivated FBS, 1 % amphotericin B (250 mg/ mL), and 1 % antibiotics (100 µg/mL streptomycin and 100 U/mL penicillin) and were incubated at 37°C and 5 % CO_2. After an 80 % confluent monolayer formed, cells were subcultured using 0.25 % trypsin-0.01 % EDTA for 5 min before being resuspended in complete DMEM media.

2.5. Antiproliferative effects on HaCaT cells

The antiproliferative activity of the A. arborescens extracts was evaluated on HaCaT cells according to the method described by Lall et al (2013). Cells were plated in sterile 96-well microtitre plates at a concentration of 1 x 10_5 cells/mL (10,000 cells/well), followed by 24 h incubation at 37°C and 5 % CO_2, to allow for cell attachment. Controls included media with (100 % cell viability) and without cells (0 % cell viability) and a vehicle control (1 % DMSO) and a toxic inducer control (DMSO tested final concentration of 0.625 - 20 %). The extracts (at stock concentrations of 40 mg/mL in DMSO) were serially diluted and tested at a final concentration of 3.13 - 400 µg/mL. The plates were subsequently incubated further for 72 h, where after 20 µL PrestoBlue reagent was added to act as an indicator for cell viability and the plates were incubated for a further 2 h. The fluorescence of the colour complex was measured at an excitation of 560 nm and an emission of 590 nm using a VICTOR Nivo™ Multimode Microplate Reader (PerkinElmer South Africa (Pty) Ltd.). Percentage cell viability was calculated for each extract using the following formula;

$$\% \text{ Cell viability} = \left[\frac{(A_{\text{treatment}} - A_{\text{blank}})}{(A_{\text{control}} - A_{\text{blank}})} \right] \times 100\%$$

With, 'A' indicating Fluorescence, 'A_{blank}' indicating fluorescence of media without cells (0% cell viability control), 'A_{control}' indicating fluorescence of vehicle control (1 % DMSO) and '$A_{\text{treatment}}$' indicating fluorescence of treatment. Analysis was performed using the GraphPad Prism Version 4.0 (San Diego, California, USA) software to obtain the half maximal inhibitory concentration (IC_{50}) of the extracts and toxic inducer.

2.6.Nitric oxide scavenging assay

The nitric oxide scavenging assay was conducted according to the method described by Mayur et al (2010) to evaluate antioxidant activity. The extracts (at stock concentrations of 8 mg/mL) were first serially diluted in 96-well plates to be tested at a final concentration of 31.25 - 4000 µg/mL. The positive control, ascorbic acid (at a stock concentration 2 mg/mL), was tested at final concentration of 15.63 - 2000 µg/mL. The vehicle control (DMSO) was tested at a final concentration of 0.16 - 20 %. Fifty microliters of sodium nitroprusside (10 mM) was then added to all the wells before the plates were incubated at room temperature for 90 min. After incubation, Griess reagent was added to all the wells (100 µL), except the blank colour controls prepared for each extract, in which distilled water was added instead (100 µL). The plates were then incubated at room temperature for 5 min, and subsequently read at 546 nm using a BIO-TEK Power-Wave XS multi-well reader using the KC Junior software and the percentage NO inhibition (%) was calculated using the formula;

$$\% \text{ NO inhibition} = \left[\frac{A_{\text{blank}} - (A_t - A_{tb})}{A_{\text{blank}}} \right] \times 100\%$$

With 'A_{blank}' indicating average vehicle control absorbance, 'A_t' indicating absorbance of the treatment and 'A_{tb}' indicating the absorbance of the colour blank of the treatment. The obtained data were further analysed using the GraphPad Prism Version 4.0 software to determine the concentration needed to scavenge 50% NO (IC_{50}).

2.7.Wound healing assay and growth factor quantification

The scratch assay was conducted according to the method described by Liang et al. (2007), with slight deviations, to determine the effect of the gel and leaf extracts on the proliferation and migration of HaCaT cells. A monolayer culture of HaCaT cells were prepared by plating cells at a concentration of 1.5×10^5 cells/mL (150,000 cells/well) in 24-well microtitre plates, which were incubated for 24 h (at 37°C and 5 % CO_2). A wound was simulated by using a 1000 µL pipette tip to scratch a cross into the monolayer and cell debris was subsequently removed by replacing the media (1 mL) with fresh complete media. The simulated wounds were subsequently treated with gel and EtOH leaf extracts of 'Jack Marais' and 'Eloff' at two non-lethal concentrations, 50 and 100 µg/mL. Cell viability standard controls, such as sterile media, with and without cells, and a vehicle control (0.25 % v/v DMSO) were included in the experiment. Each treatment was evaluated in duplicate. Images were taken of the scratches at 0 h, immediately after addition of the extracts. The plates were then incubated for 18 h (at 37°C and 5 % CO_2) and images were taken again, thereafter. Two independent

experiments were performed. The 0 h and 18 h images were processed using the ImageJ Version 1.53e software to determine the area of the wound before and after incubation. The images were processed in the following manner: (1) The image was converted to 8-bit, (2) before the addition of a bandpass filter, to obtain a pixelated image. To intensify the appearance of the scratch border, (3) the threshold of the resulting image was automatically adjusted and (4) the radius of the pixels was adjusted to a size 7 or higher. Any unwanted or discrepant areas were (5) excluded manually with the paintbrush tool. To calculate the area of the scratch, (6) the scratch outline was selected with the wand tool and the (7) analyse-measure function was implemented to obtain the area value. The resulting areas at 0 h and 18 h were recorded and used to calculate percentage wound closure with the following formula:

;

$$\% \text{ Wound closure} = \left[\frac{(A_{0hr} - A_{18hr})}{A_{0hr}} \right] \times 100\%$$

With 'A' indicating area. In preparation for the quantification of growth factors, the plates were

subsequently centrifuged at 980 rpm and cell-free supernatant was obtained from all wells and frozen at -

80 °C until use. Cell viability of the cells comprising the simulated wound was determined by the addition of

PrestoBlue® cell viability reagent to the remaining media and the fluorescence was read as described in the

antiproliferative assay method (Lall et al., 2013). The quantification of growth factors was conducted using

the LEGENDPlex™ Human Growth Factor Panel Kit from BioLegend, and the cell-free supernatants which

were prepared according to the protocol provided by the manufacturer (Cat # 740180). The experiment was

conducted in a V-bottom 96-well microtitre plate provided with the LEGENDPlex™ Human Growth Factor

Panel Kit and flow cytometer setup was conducted according to the manufacturer's protocol. The

preparation included the addition of 25 µL of thawed supernatants, standards (tested at a final concentration

of 0 - 10,000 pg/mL for M-CSF and 0 – 50,000 pg/mL for ANGPT2, EPO, FGF, HGF, PDGF-AA and

VEGF) and blanks to the correlating wells in the 96-well microtitre plate. Thereafter, assay buffer (25 µL),

pre-mixed beads (25 µL) and 25 µL detection antibodies were added to all wells and the plate was incubated

for 2 hrs with consistent shaking (800 rpm). After the incubation time, without washing, 25 µL of

streptavidin-phycoerythrin (SA-PE) conjugate was added to the plate before a further incubation time of

30 min with shaking (800 rpm). Thereafter, the plate was centrifuged for 5 min (4000 rpm) and the

remaining supernatant was removed without disturbing the bead pellet. The beads were then washed and

resuspended in 200 μL wash buffer. The extracts were then read on a BD Accuri^{TM} C6 Plus Flow Cytometer BD Biosciences (San Diego, CA, USA) guided by the LEGENDPlex^{TM} protocol (Biolegend, 2021). The flow rate was set to slow and the number of beads to be acquired to 2000-2500 on Beads A gate and B gate.

2.8. Statistical analysis

Antibacterial assays were performed in duplicate with three independent experiments (n = 3) and results were reported based on visual MIC analysis. The scratch assay, and cell viability, were performed in duplicate with two independent experiments (n=2), whereas the antiproliferative assay and NO scavenging assay were performed in triplicate with three independent experiments (n = 3) and the results were reported as mean ± standard deviation. GraphPad Prism Version 4.0 software was used for calculation of the IC$_{50}$ to obtain the non-linear regression analysis of the sigmoidal dose-response curves (with constraints top and bottom constraints of 100 and 0, respectively). Results obtained from the growth factor quantification were analysed using the LEGENDPlexTM v8.0 data analysis software to obtain the specific growth factor concentrations (pg/mL), which were further analysed with the GraphPad Prism Version 4.0 software. The results for the scratch assay and the growth factor quantification were subjected to a one-way ANOVA in comparison to the vehicle control (0.25 % DMSO) and subsequent analysis by Dunnett's multiple comparison test or Bonferroni posttest (specified in the results section) using GraphPad Prism Version 4.0 software, and results with *$p < 0.05$ and **$p < 0.01$ were regarded as statistically significant.

3. Results

3.1. Antibacterial activity against wound associated bacteria

Visual MIC analysis identified that the EtOH leaf extracts of *A. arborescens* varieties 'Eloff' and 'Jack Marais' displayed antibacterial activity against strains of *S. aureus*. The ethanoic leaf extract of 'Eloff' displayed antibacterial activity against only one strain of *S. aureus* (ATCC 25293) with MIC value of

500 µg/mL, whereas the EtOH leaf extract of 'Jack Marais' exhibited antibacterial activity against *S. aureus* ATCC 25293 (MIC of 250 µg/mL) and ATCC 6538 (MIC of 500 µg/mL). All other extracts displayed no antibacterial activity against either strain of *S. aureus* at the highest tested concentrations (MIC > 500 µg/mL). The vancomycin positive control against both strains of *S. aureus* exhibited an MIC of 0.78 µg/mL. The ciprofloxacin positive control against *P. aeruginosa* exhibited an MIC of 0.08 µg/mL, however, none of the extracts displayed antibacterial activity against *P. aeruginosa* (ATCC 9027) at the highest tested concentrations (MIC > 500 µg/mL).

3.2. Antiproliferative activity on HaCaT cells

The antiproliferative effects of the extracts on HaCaT cells was tested to determine potential toxicity against normal human keratinocytes. Furthermore, the results would identify non-toxic concentrations at which to test the extracts in the scratch assay and for growth factor quantification. Cell viability, based on fluorescence readings of PrestoBlue® viability reagent, indicated that none of the extracts displayed antiproliferative activity against HaCaT cells at the highest tested concentrations in comparison to the media and vehicle controls ($IC_{50} > 400$ µg/mL). According to a literature study conducted by Kuete and Efferth (2015), the obtained values fall under the proposed cut-off IC_{50} value indicating no cytotoxicity ($IC_{50} > 400$ µg/mL) and so the extracts were deemed non-toxic.

3.3. Nitric oxide scavenging activity

The nitric oxide scavenging activity for the *A. arborescens* variety extracts were investigated to identify the potential antioxidant action within the wound site as a possible mechanism of action. The ascorbic acid positive control exhibited IC_{50} of 54.78 ± 7.83 µg/mL, however, only the gel extract of 'Le Roux' exhibited potential antioxidant activity by inhibition of nitric oxide with an IC_{50} value of 2696 ± 582.66 µg/mL, whereas the other extracts displayed $IC_{50} > 4000$ µg/mL.

3.4.Effect on wound closure

Extracts which showed antibacterial activity (EtOH leaf extracts of 'Eloff' and 'Jack Marais') were evaluated for potential wound healing activity using the scratch assay which provides insight into the effect of the extracts on the proliferation and migration of cells in a simulated wound. Even though none of the gel extracts exhibited antibacterial activity, the gel extracts of 'Eloff' and 'Jack Marais' were also included in the scratch assay due to the use of *A. arborescens* leaf gel in traditional wound healing practices (Koshioka et al., 1982). Gel and EtOH leaf extracts of the 'Eloff' and 'Jack Marais' varieties were tested at 50 and 100 µg/mL. The percentage wound closure (%) for each sample was determined and compared to the vehicle control (0.25 % DMSO) (Figure 1). Figures 2 and 3 present images obtained of the scratch assay which were further analysed for calculation of the percentage wound closure values for each sample. The 'Eloff' variety gel extract (at 100 µg/mL) showed a significant increase in wound closure ($54.20 \pm 1.11\%$ increase ($p < 0.05$)) (Figure 1, [A]), whereas the 'Jack Marais' variety gel extract (at 50 and 100 µg/mL) significantly stimulated wound closure (11.16 ± 1.97 % and 5.65 ± 1.11 % increase, respectively ($p < 0.01$) (Figure 1, [B]). Both 'Eloff' and 'Jack Marais' variety EtOH leaf extracts significantly stimulated wound closure at a concentration of 50 µg/mL ($3.98 \pm 1.47\%$ and $7.86 \pm 0.65\%$, respectively ($p < 0.01$)), however not at 100 µg/mL.
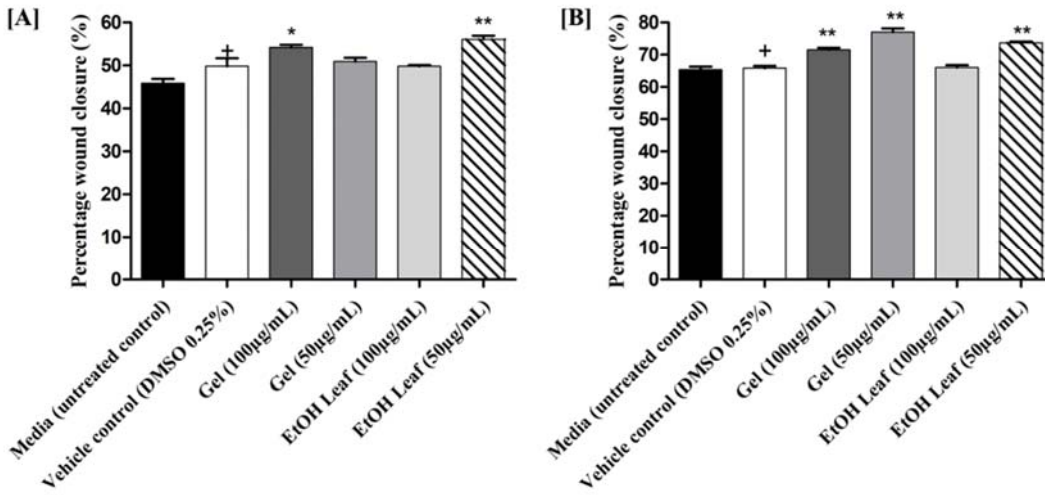
Figure 1. Percentage wound closure (%) of *A. arborescens* extracts on HaCaT cells. [A] Percentage wound closure (%) of *A. arborescens* 'Eloff' gel and EtOH leaf extract [B] Percentage wound closure (%) of *A. arborescens* 'Jack Marais" gel and EtOH leaf extract. With * and ** indicating $p < 0.05$ and $p < 0.01$, respectively.
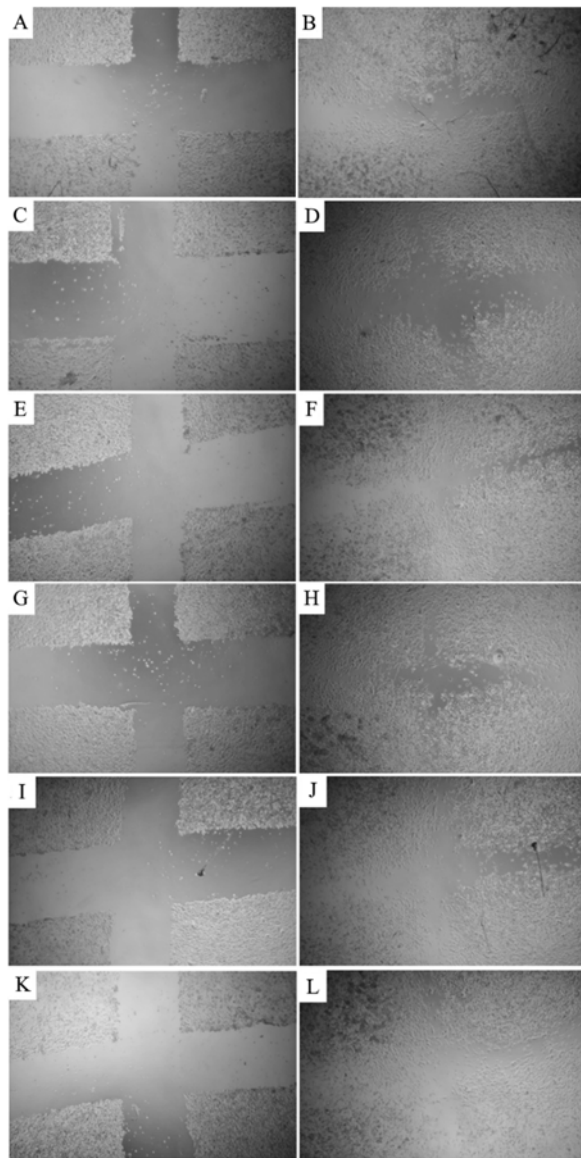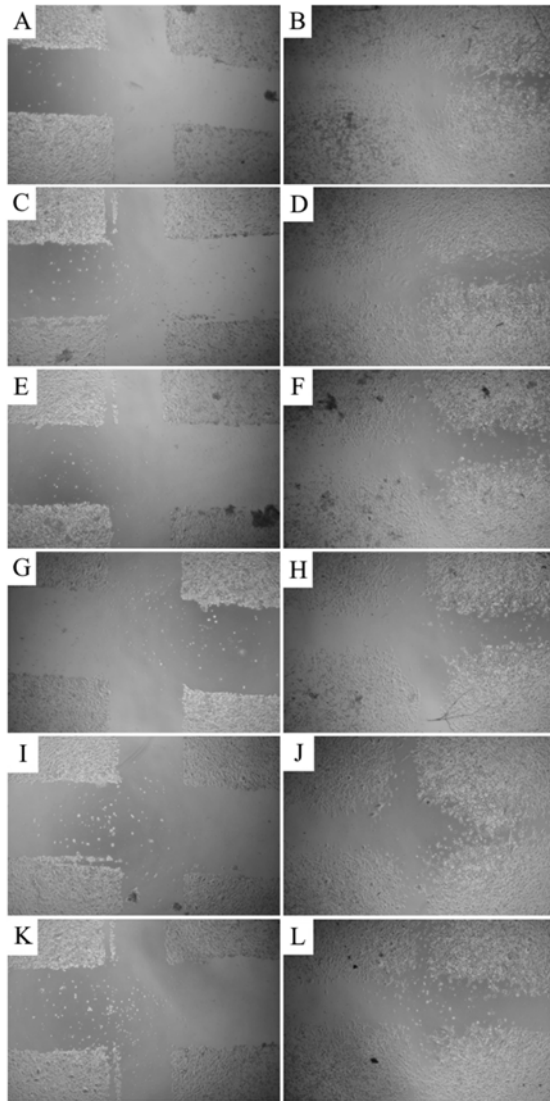
Figure 2. Scratch assay results (at 0 h and 18 h incubation time) of untreated media control, vehiclecontrol (0.25 % DMSO) and *A. arborescens* 'Jack Marais' gel and EtOH leaf extracts. [A] Untreated media control at 0 h and [B] after 18 h incubation [C] Vehicle control after 0 h and [D] after 18 h incubation. [E] *A. arborescens* 'Jack Marais' gel extract (50 μg/mL) after 0 h and [F] after 18 h incubation. [G] *A. arborescens* 'Jack Marais' gel extract (100 μg/mL) after 0 h and [H] after 18 h incubation. [I] *A. arborescens* 'Jack Marais' leaf extract (50 μg/mL) after 0 h and [J] after 18 h incubation. [K] *Aloe arborescens* 'Jack Marais' leaf extract (100 μg/mL) after 0 h and [L] after 18 h incubation.

Figure 3. Scratch assay results (at 0h and 18h incubation time) of untreated media control, vehicle control (0.25% DMSO) and *A. arborescens* 'Eloff' gel and EtOH leaf extract. [A] Untreated media control at 0 h and [B] after 18 h incubation. [C] Vehicle control after 0 h and [D] after 18 h incubation. [E] *A. arborescens* 'Eloff' gel extract (50 μg/mL) after 0 h and [F] after 18 h incubation. [G] *A. arborescens* 'Eloff' gel extract (100 μg/mL) after 0 h and [H] after 18 h incubation. [I] *A. arborescens* 'Eloff' leaf extract (50 μg/mL) after 0 h and [J] after 18 h incubation. [K] *A. arborescens* 'Eloff' leaf extract (100 μg/mL) after 0 h and [L] after 18 h incubation.

3.5. Effect on growth factor secretion in HaCaT cells

The quantification of growth factors secreted from HaCaT cells treated with both gel and EtOH leaf extracts of 'Jack Marais' and 'Eloff' was determined. Cell viability of HaCaT cells remaining after

16

collection of the cell free supernatant treated with 'Eloff' and 'Jack Marais' extracts indicated no toxicity at both 50 and 100 μg/mL after 18 hr of exposure (Figure 4A&B). The EtOH 'Eloff' leaf extract (at 100 μg/mL) and the 'Jack Marais' gel extract (at 100 μg/mL) significantly increased production of growth factor PDGF-AA compared to the vehicle (0.25 % DMSO) control (Figure 5, [B] & [C]). 'Eloff' gel (50 μg/mL) and EtOH leaf (100 μg/mL) exhibited an increase in human M-CSF, although these did not prove to be statistically significant (Figure 5, [A] & [B]). 'Jack Marais' gel extract (100 μg/mL) increased ANGPT2 and decreased M-CSF, whereas the EtOH leaf extract (50 and 100 μg/mL) was shown to decrease ANGPT2 and M-CSF (Figure 5, [C] & [D]), however, these results did not show statistical significance.

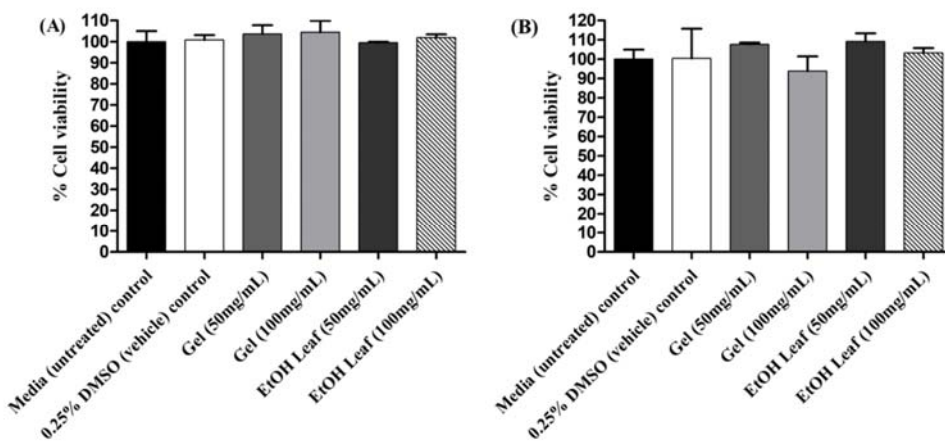**Figure 4.** Cell viability (%) of human keratinocytes (HaCaT) treated with gel and EtOH leaf extracts (at 50 and 100 μg/mL) of (A) *A. arborescens* 'Eloff' and (B) *A. arborescens* 'Jack Marais' after 18 h.
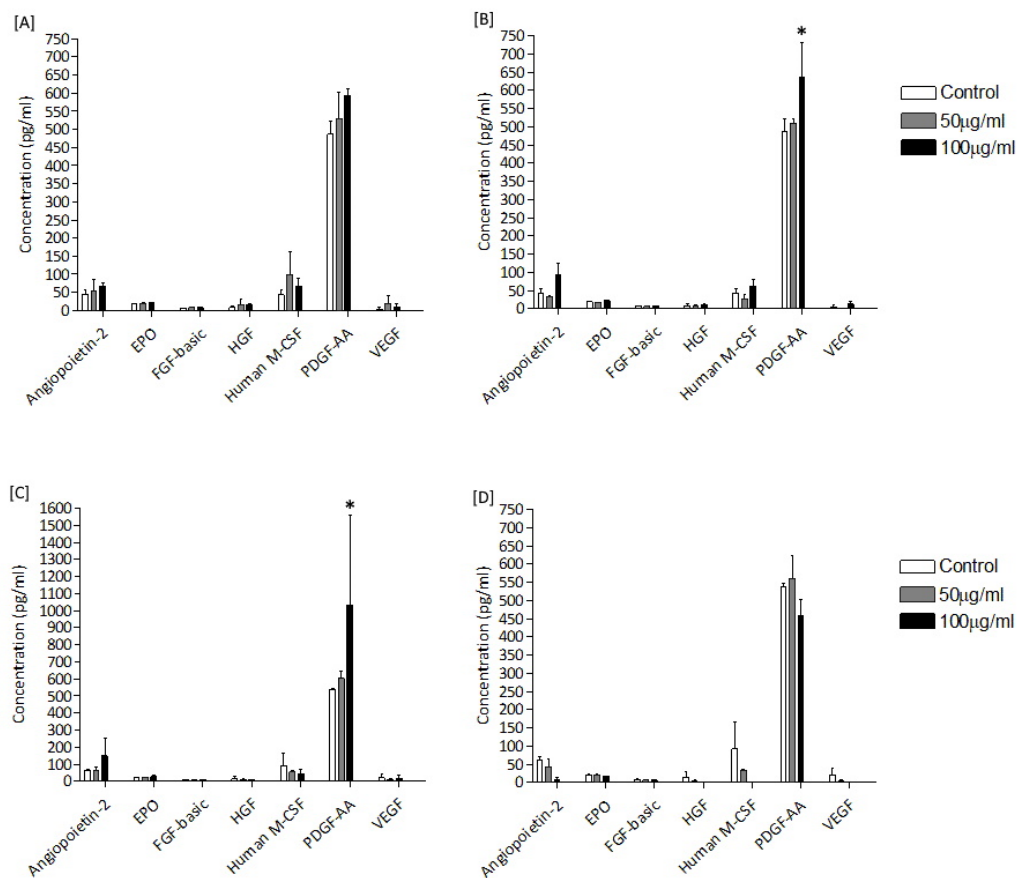
Figure 5. The effect of the vehicle control (0.25 % DMSO) and *A. arborescens* extracts (at 50 and 100 μg/mL) on the production (pg/mL) of human growth factors in HaCat cells. Growth factor quantification for [A] 'Eloff' gel extract, [B] 'Eloff' EtOH leaf extract, [C] 'Jack Marais' gel extract and [D] 'Jack Marais' EtOH leaf extract. * Indicating results statistically significant in comparison to the control ($p < 0.05$).

4. Discussion

Aloe arborescens has been successfully investigated for its use to treat skin irritations and dermal wounds in male Wistar rats and New Zealand white rabbits (Jia et al., 2008). Whole-leaf juice of *A. arborescens*, which was topically applied to scalpel-produced wounds showed significantly reduced wound severity compared to the control in both rat and rabbit models after 4 days of observation. Furthermore, no symptoms of irritation or inflammation were observed in any of the rats within the 14 day period.

A methanolic, warm-water and cold-water extracts of the leaf epidermis of *A. arborescens* showed antibacterial activity against *S. aureus* exhibiting zones of inhibition (ZOI) of 8, 4 and 5 mm respectively (Bisi-Johnson et al., 2011). There were no reports on the antibacterial activity of *A. arborescens* against *P. aeruginosa*. In a study by Lucini et al (2015), it was hypothesised that *A. arborescens* may accelerate wound healing by down-regulating inflammation via the elimination of oxidative stress in the wound site. In this study, dissolved powdered whole-leaf, leaf epidermis and gel extracts of *A. arborescens* showed 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity with trolox equivalent values of 71 ± 7 µM, 183 ± 37 µM and 83 ± 8 µM, respectively (Lucini et al., 2015). In a study conducted by Di Luccia et al (2013), the effect of *A. arborescens* on the differentiation of human keratinocytes was investigated. Differentiated human primary keratinocytes and HaCaT cells supplemented with whole-leaf cold-water extract of *A. arborescens* (1:10 v/v) were significantly shrunken and pluristratified (having several stratifications) when compared to the untreated control. Furthermore, protein differentiation biomarkers, such as Involucrin and Transglutaminase, were significantly expressed at higher levels in aloe-treated cells. These reports suggest that *A. arborescens* may accelerate the differentiation of human keratinocytes which may ultimately stimulate the wound healing process.

The potential wound healing activity of the *A. arborescens* extracts was evaluated by identifying their antibacterial, antioxidant activities and their stimulatory effects on growth factors associated with wound healing. Varieties that showed promising antibacterial activity were further selected to identify their effects on wound closure and stimulation of growth factors. The antibacterial assays conducted displayed that the ethanoic leaf extract of 'Eloff' exhibited an MIC of 500 µg/mL against *S. aureus* strain ATCC 25293, whereas the EtOH leaf extract of 'Jack Marais' exhibited MIC values of 250 µg/mL and 500 µg/mL against S. aureus strains ATCC 25293 and ATCC 6538, respectively. According to Shirinda et al. (2019), antibacterial activity displayed by plant extracts are considered note-worthy when exhibiting MIC values $\leq$ 160 µg/mL, whereas MIC values between 160 µg/mL and 1000 µg/mL are moderately active. Furthermore, MIC values larger than 1000 µg/mL are considered to display weak antibacterial activity. Therefore, the

ethanolic leaf extracts of "Eloff" and "Jack Marais" variety display moderate antibacterial activity against strains of *S. aureus.*

Only the EtOH leaf extracts of 'Eloff' and 'Jack Marais' varieties exhibited antibacterial activity against strains of *S. aureus* (Gram-positive bacteria), but not *P. aeruginosa* (Gram-negative bacteria). This corresponds with a study conducted by Bisi-Johnson et al (2011), in which EtOH leaf extracts of *A. arborescens* exhibited promising activity against Gram-positive bacteria as opposed to Gram-negative bacteria, where aqueous and EtOH leaf extracts exhibited antibacterial activity against *Escherichia coli* (Gram-negative bacteria) (10.08 ± 0.29 and 18.75 ± 3.96 mm, respectively) *Bacillus cereus* (Gram-positive bacteria) (22.75 ± 2.18 and 25.17 ± 5.67 mm, respectively), *Bacillus licheniformis* (Gram-positive bacteria) (14.83 ± 2.89 and 17.50 ± 4.60 mm, respectively) and *Staphylococcus epidermidis* (Gram-positive bacteria) (21.00 ± 5.69 and 17.08 ± 3.37 mm, respectively), (Bisi-Johnson et al., 2011). However, there are no previous reports on the antibacterial activity of *A. arborescens* against *S. aureus* and *P. aeruginosa.*

Further studies suggests that various *Aloe* L. species possess antibacterial activity against Gram-positive bacteria due to the presence of anthraquinononecompounds which react with and destabilize the peptidoglycan outer membrane of the bacterial cell wall (Malik and Christa, 2016; Malmir et al., 2017). In a study conducted by Koshioka et al. (1982), the anthraquinone content within the leaf gel of *A. arborescens* Mill. var. *natalensis* Berger was found to be 2.17 %. However, there is a lack of research that focusses on the isolation of anthraquinone-type compounds from *A. arborescens*.Antiproliferative activity of the extracts was conducted to determine whether the extracts were toxic to human keratinocytes and to determine non-toxic concentrations for subsequent testing in the scratch assay. The results displayed that the extracts of *A. arborescens* varieties were non-toxic in comparison to the media and vehicle controls ($IC_{50} > 400$ µg/mL). This corresponds with reports by Bisi-Johnson et al. (2011), where fractions of methanolic extracts of *A. arborescens* leaves displayed an IC_{50} value >1000 µg/mL against HaCaT cells. Furthermore, clinical trials conducted on 3069 patients (3-14 years old) exhibited that *A. arborescens* is safe as a concomitant treatment. The study evaluated the effects of *A. arborescens* on chronic and reoccurring respiratory tract illnesses,

which showed that *A. arborescens* treatment reduced symptoms and reoccurrences of respiratory tract illness in 71 % of the patients with no toxic side effects (Singab et al., 2015).

The *in vitro* NO scavenging activity of the *A. arborescens* varieties was evaluated to identify extracts which may exhibit antioxidant action by reduction of NO. Antioxidant activity against NO may provide insight to whether or not the extracts may show anti-inflammatory activity. All *A. arborescens* extracts were deemed non-active at the highest tested concentrations ($IC_{50} > 4000$ µg/mL), except for the 'Le Roux' gel extract (2696 ± 582.66 µg/mL). This suggests that *A. arborescens* varieties may not inhibit inflammation through NO scavenging, but possibly by an alternative mechanism, like targeting NO synthesis in macrophages through the inhibition of inducible nitric oxide synthase (iNOS).

Aloe arborescens EtOH extracts that displayed antibacterial activity were further selected to evaluate their effect on wound closure. Their respective gel extracts were also included in the scratch assay in order to determine differences in bioactivity between the different extract preparations.. Gel and EtOH leaf extracts of 'Eloff' and 'Jack Marais' (at 50 and 100 µg/mL) were evaluated using the scratch assay to determine their effect on the proliferation and migration of HaCaT cells. The results displayed that the gel and EtOH leaf extracts of 'Jack Marais' (at both 50 and 100 µg/mL) and the EtOH leaf extract of 'Eloff' (at 50 µg/mL) exhibited a significant increase in percentage wound closure in comparison to the vehicle control ($p < 0.05$) (Figure 1). These results suggest that extracts of 'Jack Marais' and 'Eloff' varieties may stimulate the proliferation and migration of HaCaT cells, especially at lower concentrations as opposed to higher concentrations. This may be due to bioactive compounds which may have a stimulatory effect on cellular proliferation at low concentrations but may exhibit inhibitory or modulatory properties when in excess at higher concentrations. To elucidate the possible mechanism of action, the supernatants from the scratch assay were further subjected to growth factor quantification to determine the effect of extract treatment on concentrations of growth factors involved in the wound healing process.

The 'Jack Marais' gel extract (at 100 µg/mL) and the 'Eloff' EtOH leaf extract (at 100 µg/mL) displayed significant stimulation in the production of the PDGF-AA growth factor ($p < 0.05$) (Figure 3, [B] and [C]). Human PDGF-AA is a growth factor that regulates cell growth and proliferation in various cells, including

21

human keratinocytes. Thus, the upregulation by treatment with these extracts corresponds to the significant increase in percentage wound closure compared to the control (Figure 1). However, these results still do not account for the larger increase in wound closure at 50 μg/mL compared to 100 μg/mL. Based on these results, it could be hypothesized that increasing the extract concentration may correlate with increased PDGF-AA concentration. However, this does not translate to higher stimulation of wound closure within the scratch assay where concentrations of 50 μg/mL resulted in increased wound closure compared to at 100 μg/mL. This is unexpected as PDGF-AA acts as a potent mitogen in keratinocytes. This may occur via alternative anti-migration mechanisms, such as the regulation of other cytokines involved in wound healing rather than growth factors involved in proliferation. Furthermore, the scratch assay gives insight to the effect of the extracts on both migration and proliferation of HaCaT cells without distinguishing between the two processes.

5. Conclusion

Ethanolic leaf extracts of 'Jack Marais' and 'Eloff' variety displayed promising antibacterial activity against strains of *S. aureus*, but not *P. aeruginosa*. The nitric oxide scavenging assay revealed low antioxidant activity by NO scavenging for all extracts. Extracts prepared from the 'Jack Marais' variety exhibited a significant increase in percentage wound closure compared to the control, whereas percentage wound closure of 'Eloff' variety extracts were statistically similar to the control. This suggested that the 'Jack Marais' extracts may have stimulatory effects on the proliferation and migration of HaCaT cells. However, further testing may be conducted to identify the effect of the extracts on other factors of wound healing, such as the effect of extract treatment on *in vivo* blood vessel formation (angiogenesis) by chick chorioallantoic membrane (CAM) assay (Aleksandrowicz and Herr, 2015). This may give insight to alternative pharmacological potential of aloes to stimulate wound healing. Growth factor quantification identified that the stimulation of wound closure did not correspond with the up-regulation of human growth factor PDGF-AA, and thus it was hypothesised that the stimulation of wound closure may occur via an alternative mechanism, such as the stimulation of cytokines involved in wound healing. Possible cytokines

to investigate in this regard includes tumour necrosis factor alpha (TNF-), interleukin 1, interleukin 6 and interleukin 7 which are pro-inflammatory cytokines involved in wound healing. Further testing may be conducted to identify potential anti-inflammatory activity of the *A. arborescens* varieties in wound healing, possibly by the inhibition of iNOS. The intracellular nitric oxide inhibition assay may be another way to potentially determine *in vitro* inhibition of iNOS activity of the extracts in stimulated murine macrophages (RAW 264.7). This assay aims to determine the effect of extract treatment on the amount of NO produced by iNOS enzyme in macrophage cells (Esposito et al., 2014). Furthermore, the effect of the extracts on cell migration alone may be evaluated by an alternative assay, such as the transwell migration assay, in order to exclude the potential effect of natural cell proliferation on wound closure (Pijuan et al., 2019). Based on the results obtained in this study, *A. arborescens* displayed varying wound healing potential among varieties and different extract preparations, which warrants further investigation towards potential clinical use.

Acknowledgements

References

Aleksandrowicz, E., Herr, I., 2015. Ethical euthanasia and short-term anesthesia of the chick embryo. Altex 32, 143–147. https://doi.org/10.14573/altex.1410031

Aldridge, P., 2015. Complications of wound healing: causes and prevention. Companion Anim. 20, 453–459.

https://doi.org/10.12968/coan.2015.20.8.453

Almine, J.F., Wise, S.G., Weiss, A.S., 2012. Elastin signaling in wound repair. Birth Defects Res. Part C - Embryo Today Rev. 96, 248–257. https://doi.org/10.1002/bdrc.21016

Barrientos, S., Stojadinovic, O., Golinko, M.S., Brem, H., Tomic-Canic, M., 2008. Growth factors and cytokines in wound healing. Wound Repair and Regeneration 16, 585–601. https://doi.org/10.1111/j.1524-475X.2008.00410.x

Biolegend, 2021. Setup Procedure for BD Accuri ™ C6 Tel : 858-768-5800.

Bisi-Johnson, M.A., Obi, C.L., Hattori, T., Oshima, Y., Li, S., Kambizi, L., Eloff, J.N., Vasaikar, S.D., 2011. Evaluation of the antibacterial and anticancer activities of some South African medicinal plants. BMC Complementary and Alternative Medicine 11. https://doi.org/10.1186/1472-6882-11-14

Churpek, M.M., Snyder, A., Han, X., Sokol, S., Pettit, N., Howell, M.D., Edelson, D.P., 2017. Quick sepsis-related organ failure assessment, systemic inflammatory response syndrome, and early warning scores for detecting clinical deterioration in infected patients outside theintensive care unit. American Journal of Respiratory and Critical Care Medicine 195, 906–911. https://doi.org/10.1164/rccm.201604-0854OC

Cohen, P.R., Kurzrock, R., 2004. Community-acquired methicillin-resistant Staphylococcus aureus skin infection: An emerging clinical problem. J. Am. Acad. Dermatol. 50, 277–280. https://doi.org/10.1016/j.jaad.2003.06.005

de Janerio, R., 2019. Sepsis 2019. Intensive Care Med. Exp. 7, 1–19. https://doi.org/10.1186/s40635-019-0254-1

Di Luccia, B., Manzo, N., Vivo, M., Galano, E., Amoresano, A., Crescenzi, E., Pollice, A., Tudisco, R., Infascelli, F., Calabrò, V., 2013. A biochemical and cellular approach to explore the antiproliferative and prodifferentiative activity of *Aloe arborescens* leaf extract. Phytotherapy Research 27, 1819–1828. https://doi.org/10.1002/ptr.4939

Ekor, M., 2014. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Neurology 1–10. https://doi.org/10.3389/fphar.2013.00177

Esposito, D., Chen, A., Grace, M.H., Komarnytsky, S., Lila, M.A., 2014. Inhibitory effects of wild blueberry anthocyanins and other flavonoids on biomarkers of acute and chronic inflammation in vitro. J. Agric. Food Chem. 62, 7022–7028. https://doi.org/10.1021/jf4051599

Fang, Y., Shen, J., Yao, M., Beagley, K.W., Hambly, B.D., Bao, S., 2010. Granulocyte-macrophage colony-stimulating factor enhances wound healing in diabetes via upregulation of proinflammatory cytokines. Br. J. Dermatol. 162, 478–486. https://doi.org/10.1111/j.1365-2133.2009.09528.x

Hankey, A., Notten, A., 2004. *Aloe arborescens* Mill. [WWW Document]. URL http://pza.sanbi.org/aloe-arborescens (accessed 3.3.20).

Haroon, Z.A., Amin, K., Jiang, X., Arcasoy, M.O., 2003. A novel role for erythropoietin during fibrin-induced wound-healing response. The American Journal of Pathology. 163, 993–1000. https://doi.org/10.1016/S0002-9440(10)63459-1

Halloran, C.M., Slavin, J.P., 2002. Pathophysiology of Wound Healing. Surg. 20, i–v. https://doi.org/10.1383/surg.20.5.0.14629

Jia, Y., Zhao, G., Jia, J., 2008. Preliminary evaluation: The effects of *Aloe ferox* Miller and *Aloe arborescens* Miller on wound healing. Journal of Ethnopharmacology 120, 181–189. https://doi.org/10.1016/j.jep.2008.08.008

Koshioka, Mayumi, Koshioka, Masaji, Takino, Y., Suzuki, M., 1982. Studies on the Evaluation of *Aloe arborescens* Mill. var. natalensis Berger and Aloe Extract (JP IX). International Journal of Crude Drug Research 20, 53–59.

Kuete, V., Efferth, T., 2015. African flora has the potential to fight multidrug resistance of cancer. Biomed Res. Int. 2015, 1–24. https://doi.org/10.1155/2015/914813

Lall, N., Henley-Smith, C.J., De Canha, M.N., Oosthuizen, C.B., Berrington, D., 2013. Viability reagent, prestoblue, in comparison with other available reagents, utilized in cytotoxicity and antimicrobial assays. International Journal of Microbiology 2013. https://doi.org/10.1155/2013/420601

Liang, C.C., Park, A.Y., Guan, J.L., 2007. In vitro scratch assay: A convenient and inexpensive method for analysis of cell migration in vitro. Nature Protocols 2, 329–333. https://doi.org/10.1038/nprot.2007.30

Li, J.F., Duan, H.F., Wu, C.T., Zhang, D.J., Deng, Y., Yin, H.L., Han, B., Gong, H.C., Wang, H.W., Wang, Y.L., 2013. HGF accelerates wound healing by promoting the dedifferentiation of epidermal cells

through β 1 -Integrin/ILK pathway. Biomed Res. Int. 2013, 1–9. https://doi.org/10.1155/2013/470418

Lucini, L., Pellizzoni, M., Pellegrino, R., Molinari, G. Pietro, Colla, G., 2015. Phytochemical constituents and in vitro radical scavenging activity of different *Aloe* species. Food Chemistry 170, 501–507. https://doi.org/10.1016/j.foodchem.2014.08.034

Lynch, S.E., Colvin, R.B., Antoniades, H.N., 1989. Growth factors in wound healing. Single and synergistic effects on partial thickness porcine skin wounds. Journal of Clinical Investigation 84, 640–646. https://doi.org/10.1172/JCI114210

Malik, E.M., Christa, E.M., 2016. Anthraquinones As Pharmacological Tools and Drugs. Medicinal Research Reviews 36, 705–748. https://doi.org/10.1002/med

Malmir, M., Serrano, R., Maria, O., Silva, D., 2017. Anthraquinones as potential antimicrobial agents-A review. BMC Microbiology 17(41). https://doi.org/10.1186/s12866-017-0954-1

Mayur, B., Sandesh, S., Shruti, S., Sung-yum, S., 2010. Analyses of the methanolic extract of the leaves of *Rhinacanthus nasutus*. Journal of Medicinal Plants Research 4, 1554–1560. https://doi.org/10.5897/JMPR10.210

Micek, S.T., Lloyd, A.E., Ritchie, D.J., Reichley, R.M., Fraser, V.J., Kollef, M.H., 2005. Pseudomonas aeruginosa Bloodstream Infection: Importance of Appropriate Initial Antimicrobial Treatment. Antimicrob. Agents Chemother. 49, 1306–1311. https://doi.org/10.1128/AAC.49.4.1306

Pellizzoni, M., Ruzickova, G., Kalhotka, L., Lucini, L., 2012. Antimicrobial activity of different *Aloe barbadensis* Mill. and *Aloe arborescens* Mill. leaf fractions 6(10), 1975–1981. https://doi.org/10.5897/JMPR11.1680

Pijuan, J., Barceló, C., Moreno, D.F., Maiques, O., Sisó, P., Marti, R.M., Macià, A., Panosa, A., 2019. In vitro cell migration, invasion, and adhesion assays: From cell imaging to data analysis. Front. Cell Dev. Biol. 7, 1–16. https://doi.org/10.3389/fcell.2019.00107

Shirinda, H., Leonard, C., Candy, G., van Vuuren, S., 2019. Antimicrobial activity and toxicity profile of selected southern African medicinal plants against neglected gut pathogens. S. Afr. J. Sci. 115, 1–10. https://doi.org/10.17159/sajs.2019/6199

Singab, A.N.B., El-Hefnawy, H.M., Esmat, A., Gad, H.A., Nazeam, J.A., 2015. A Systemic Review on *Aloe arborescens* Pharmacological Profile: Biological Activities and Pilot Clinical Trials. Phytotherapy Research 29, 1858–1867. https://doi.org/10.1002/ptr.5483

World Health Organization, 2019. WHO global report on traditional and complementary medicine 2019, World Health Organization.

Zielińska-Borkowska, U., 2015. Sepsis – 2015. Pielęgniarstwo w Anestezjologii i Intensywnej Opiece 1, 177–180. https://doi.org/10.15374/pwaiio2015010