Potential of succulents for eczema-associated symptoms

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Highlights

•Five succulent extracts displayed high tyrosinase inhibition

•XL significantly inhibited all six cytokines at 50 µg/mL

•AG inhibited IL-8 and IL-1β at 3.125 µg/mL

Abstract

Eczema is considered a worldwide concern as more people, particularly children, are affected each year. Side effects of eczema include hyperpigmentation and wrinkle formation. Due to the overexpression of interleukin (IL)-1β and IL-6, large quantities of the tyrosinase enzyme are released causing hyperpigmentation, whereas during wrinkle formation elastase is produced. This study examined the antityrosinase, anti-elastase and immune-modulatory potential of 22 succulent plant extracts. These extracts were prepared from the leaves and gels of 10 plant species, which included four aloes, *Bulbine frutescens* (L.) Willd. and five aloe hybrids. Five extracts displayed tyrosinase inhibition at a concentration of 200 µg/mL, which included *Aloe arborescens* gel (AG) (41.38 ± 0.94 %), *A. vera* leaf (VL) (26.64 ± 1.50 %), *Aloe X principis* (2) leaf (XL) (33.11 ± 1.29 %), (*A. chabaudii* x *A. petricola*) x *A. zubb* pure leaf (CPL) (18.70 ± 1.40 %) and *A. ferox* x *A. zubb* leaf (FZL) (21.82 ± 1.12 %), however, these extracts displayed no elastase inhibition at the highest tested concentration. Furthermore, these extracts displayed no antiproliferative activity on human keratinocyte (HaCaT) cells, with fifty percent inhibitory concentrations (IC_{50}) >400 µg/mL. The AG and XL extracts, which showed the highest tyrosinase inhibition, were selected for cytokine modulatory activity using peripheral blood mononuclear cells (PBMC's) to evaluate the effect on interleukin-8 (IL-8), 1β, 6, 10, 12p70 and TNF-α production. *Aloe arborescens* gel inhibited IL-8 (54.69 ± 3.42 pg/mL) and IL-1β (8.78 ± 0.80 pg/mL) production at a concentration of 3.125 µg/mL, while *Aloe X principis* (2) leaf reduced the production of all the cytokines at the highest concentration (50 µg/mL). In conclusion, this study showed that *Aloe X principis* (2) leaf extract, which inhibited tyrosinase and cytokine production, should be considered for further investigation as a potential treatment for side effects associated with eczema.

Keywords: Anti-elastase, antityrosinase, cytokine modulation, cytotoxicity, eczema, TNF-α

Abbreviations: IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; SPINK, serine protease inhibitor Kazal-type 1; IgE, immunoglobulin E; HaCaT, human keratinocytes; FIC index, Fractional inhibitory concentration index; PBMC, peripheral blood monocyte cells;

DMEM, Dulbecco's modified Eagle's Medium; RPMI, Roswell Park Memorial Institute; PMA, Phorbol 12-myristate 13-acetate; EDTA, ethylenediaminetetraacetic acid; DMSO, dimethyl sulfoxide; EGF, epidermal growth factor; ACK, ammonium-chloride-potassium lysis buffer; IC_{50}, 50% inhibitory concentration.

1. Introduction

Eczema, or atopic dermatitis, is an inflammatory skin condition, which is characterized by an itching sensation, the appearance of a rash and dry cracked skin. Eczema occurs in two phases, chronic or acute, depending on which T-helper (Th) lymphocytes are activated (Finberg, 2013; Medicine, 2008; Nedoszytko et al., 2014). Chronic eczema is characterized by the activation of Th-1 lymphocytes which increases the production of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin-8 (IL-8) and IL-12. Acute eczema occurs when macrophages, which are located within Langerhans cells, secrete high quantities of TNF-α, which stimulates the influx of Th-2 lymphocytes, thereby secreting IL-4, IL-5 and IL-13 (Nedoszytko et al., 2014). Furthermore, Th-2 lymphocytes influence the influx of IL-10 which is overexpressed during the chronic phase (Ohmen et al., 1995).

Eczema patients who experience stress may aggravate symptoms associated with the condition as stress hormones stimulate the release of IL-1 and IL-6, which induce inflammation and other conditions such as post-inflammatory hyperpigmentation (Callender et al., 2011; Elenkov et al., 2005). Hyperpigmentation occurs when there is an increase in melanin production, due to the overstimulation of tyrosinase (Narayanaswamy and Ismail, 2015; Parvez et al., 2007).

Nedoszytko et al. (2014) showed that the itchy sensation, associated with eczema, may be due to an increase in histamine and neuropeptides located near the skin. When there is an influx of pro-inflammatory cytokines, histamine and neuropeptides are secreted by the mast cells and act directly on nerve fibers leading to the damage of the skin barrier (Nedoszytko et al., 2014). Once the skin barrier is damaged, serine protease inhibitor Kazal-type 1 (SPINK) is released to prevent the overexpression of serine proteases. However, in eczema patients, this protein is absent resulting in the

overexpression of serine protease elastase-2, which leads to the formation of wrinkles (Agarwal et al., 2014).

Although there is no cure for eczema, several clinical treatments are available to relieve symptoms associated with eczema, however, tachyphylaxis is a common side effect that occurs when these types of creams and ointments are used over a prolonged period (Oakley, 2009; Puterman et al., 2014). Other adverse side effects include steroid acne and skin atrophy (Puterman et al., 2014). This has led to an increase in investigating medicinal plants and natural products for the potential to treat eczema or alleviate symptoms associated with eczema (Ahuja et al., 2021).

In South Africa, *Bulbine futescens* (L.) Willd is traditionally used for the treatment of eczema. A warm poultice is prepared from the leaf sap which is topically applied to treat eczema, wounds and arthritis (Abdissa et al., 2014; Hoffman, 2020). Furthermore, Aloes are well-known for their medicinal properties, such as *Aloe vera* (*Aloe barbadensis*) (L.) Burm.f. (Brien et al., 2011; Nema et al., 2013), which is used in numerous cosmetic products (Brien et al., 2011) due to its antioxidant, immune-boosting, wound healing and hydrating properties (Kumari and Sharmila, 2015; Radha and Laxmirpriya, 2015). *Aloe ferox* Mill, which is traditionally used to treat burns and arthritis, possesses similar properties to *A. vera*, however, differs in chemical composition (Radha and Laxmirpriya, 2015). Furthermore, *Aloe arborescens* Mill, has previously been reported to reduce pain associated with inflammation and thermal burns in mice (Singab et al., 2015).

In the present study, 22 extracts were prepared from ten plant species namely; *B. frutescens,* four pure aloe species and five aloe hybrids, were evaluated for their potential to reduce symptoms associated with chronic eczema, such as hyperpigmentation, wrinkle formation and inflammation, by determining their potential to inhibit tyrosinase, elastase and several inflammatory cytokines associated with the condition.

2. Materials and methods

2.1. Materials, chemicals and reagents

The human keratinocyte (HaCaT) cell line was donated by the University of Cape Town (Cape Town, South Africa). Dulbecco's modified Eagle's Medium (DMEM), Roswell Park Memorial Institute (RPMI-1640) medium, phosphate-buffered saline (PBS), fetal bovine serum (FBS), ammonium-chloride-potassium (ACK) lysing buffer, PrestoBlue™, amphotericin B, streptomycin and penicillin, were obtained from ThermoFisher Scientific (Johannesburg, South Africa). Cell culture plates and flasks were purchased from LasecSA (Pty) Ltd. (Midrand, South Africa). The human inflammatory cytokine kit (Cat # 551811) was sourced from BD Bioscience (Randburg, South Africa). Phorbol 12-myristate 13-acetate (PMA), Histopaque™-1077, ethylenediaminetetraacetic acid (EDTA), dimethyl sulfoxide (DMSO), actinomycin D (purity > 95 %), kojic acid (purity > 98 %), *N*-succinyl-Ala-Ala-Ala-ρ-nitroanilide, elastase, ursolic acid (purity > 90 %), *L*-tyrosine and mushroom tyrosinase were obtained from Sigma-Aldrich (Johannesburg, South Africa).

2.2. Plant collection and extraction

Ten plant species were collected at the Manie van der Schijff (University of Pretoria) botanical gardens and herbarium specimen vouchers were deposited at the H.G.W.J Schweickerdt Herbarium (University of Pretoria) (Table 1). Plant species names were validated using http://mpns.kew.org/mpns-portal. Fresh leaves were collected, dissected longitudinally and the parenchyma tissue/ gel was scraped and transferred into a clean container. The remaining leaf material was homogenized with absolute ethanol at a ratio of 1:5 (w: v) and left on a shaker to agitate overnight. Thereafter, the menstruum was filtered using a Büchner funnel (Whatman No. 3 filter paper) and concentrated using rotary evaporation (Büchi Rotavapor R-200). The leaf extracts and the parenchyma tissue/ gel were freeze-dried for two days and stored at 4ºC until further use. During the extraction process, the leaves of (*Aloe marlothii* x *Aloe ferox*) x *Aloe arborescence* were combined with the leaves of (*Aloe chabaudii* x *Aloe petricola*) x *Aloe zubb* at a ratio of 2:8 which was labeled as (*Aloe chabaudii* x *Aloe petricola*) x *Aloe zubb* leaf hybrid (CHL). This was repeated for the gel extract

of (*Aloe marlothii* x *Aloe ferox*) x *Aloe arborescence* and (*Aloe chabaudii* x *Aloe petricola*) x *Aloe zubb* and was labeled as (*Aloe chabaudii* x *Aloe petricola*) x *Aloe zubb* gel hybrid (CHL). Therefore, a total of 22 extracts were prepared; gel and leaf extracts of each of the species as well as the combined leaf and gel extracts of (*Aloe chabaudii* x *Aloe petricola*) x *Aloe zubb* and (*Aloe chabaudii* x *Aloe petricola*) x *Aloe zubb*.

Table 1
Herbarium specimen voucher numbers (PRU) as deposited at the H.G.W.J Schweickerdt Herbarium (University of Pretoria).

Plant extracts	PRU numbers
Aloe arborescens gel (AG)	128542
Aloe arborescens leaf (AL)	
Aloe elegans gel (EG)	128538
Aloe elegans leaf (EL)	
Aloe ferox gel (FG)	128544
Aloe ferox leaf (FL)	
(*Aloe marlothii* x *A. ferox*) x *A. arborescens* gel (MG)	128543
(*Aloe marlothii* x *A. ferox*) x *A. arborescens* leaf (ML)	
Aloe vera gel (VG)	128536
Aloe vera leaf (VL)	
Aloe X principis (2) (*Aloe ferox* x *A. arborescens*) gel (XG)	128539
Aloe X principis (2) (*Aloe ferox* x *A. arborescens*) leaf (XL)	
Bulbine frutescens gel (BFG)	122179
Bulbine frutescens leaf (BFL)	
(*Aloe chabaudii* x *A. petricola*) x *A. zubb* pure gel (CPG)	128541
(*Aloe chabaudii* x *A. petricola*) x *A. zubb* pure leaf (CPL)	
Aloe X principis (1) (*Aloe ferox* x *A. arborescens*) gel (FAG)	128537
Aloe X principis (1) (*Aloe ferox* x *A. arborescens*) leaf (FAL)	
Aloe ferox x *A. zubb* gel (FZG)	128540
Aloe ferox x *A. zubb* leaf (FZL)	

2.3. Tyrosinase inhibition

The method as described by (Lall et al., 2019) was used to determine tyrosinase inhibitory activity. Stock concentrations of the extracts and positive control, kojic acid, were prepared at 20 mg/mL (in DMSO). The final concentration at which kojic acid and the extracts were tested ranged between 1.56-200 µg/mL, with 1% DMSO as the vehicle control. Using a BIO-TEK Power-Wave XS plate reader (Analytical and Diagnostic Products CC, Roodepoort, South Africa), the absorbance values were kinetically measured at a wavelength of $OD_{492\ nm}$ for 30 min. The percentage inhibition was calculated using the following equation:

$$\%\ inhibition = 100 - \left(\frac{Absorbance\ sample\ at\ 30\ min\ -\ absorbance\ at\ 0\ min}{Absorbance\ control\ at\ 30\ min\ -\ absorbance\ at\ 0\ min}\right) \times 100$$

GraphPad Prism 4 was used to calculate the 50% inhibitory concentration (IC_{50}) of the samples.

A combinational study was further conducted, to determine the combined effect of kojic acid and *A. arborescens* gel (AG) extract on tyrosinase inhibition. Both kojic acid and AG were prepared at a stock concentration of 20 mg/mL (in DMSO) and combined in different ratios from kojic acid: AG (9:1) to kojic acid: AG (1:9). In a 96-well plate, these ratios as well as kojic acid and AG alone were serially diluted. The vehicle control (1 % DMSO) was diluted in the same manner. Thereafter, 20 µL of tyrosinase enzyme (333 units/mL in phosphate buffer) was added to 100 µL of serially diluted sample and incubated at 25ºC for 5 min, where after the reaction was initiated by the addition of 30 µL of *L*-tyrosine (2 mM). The final concentration of each sample ranged from 1.56-200 µg/mL. The absorbance was measured at a wavelength of $OD_{492\ nm}$ for 30 min using, BIO-TEK Power-Wave XS plate reader. The percentage inhibition was calculated using the same equation stated above. To calculate the fractional inhibitory concentration (FIC) index the following equation was used:

$$Fractional\ inhibitory\ concentration\ (FIC)\ index = \left(\frac{(D)1}{(Dx)1}\right) + \left(\frac{(D)2}{(Dx)2}\right)$$

Where (Dx) 1 and (Dx) 2 are the IC_{50} values of kojic acid and AG, respectively, while D1 and D2 are the IC_{50} values of the combined sample at the respected ratios. According to (Zhang et al., 2009), an FIC index < 0.5, indicates synergistic activity, FIC = 0.5-1, indicates an additive effect, FIC = 1-4, indicates indifference and FIC > 4, indicates antagonism.

2.4. Elastase inhibition

The method used to determine the elastase inhibition was described by (Lall et al., 2017), with modifications. Stock concentrations of the plant extracts and the positive control, ursolic acid were prepared at 20 mg/mL (in DMSO). From the ursolic stock solution, 4.8 µL was transferred into 35.2 µL of DMSO to obtain a final concentration range from 0.94-60 µg/mL. The extracts were serially diluted in DMSO to obtain a concentration ranging from 7.81-500 µg/mL. In a 96-well plate, 155 µL of 100 mM Trizma base (pH 8) was added to all the wells, where after, 5 µL of each extract dilutions was added (in triplicate). Thereafter, 20 µL of 4.942 mU elastase enzyme was added and incubated at 37ºC for 5 min. Following incubation, the reaction was initiated by adding 20 µL of 4.4 mM *N*-succinyl-Ala-Ala-Ala-*ρ*-nitroanilide substrate. A vehicle control (1 % DMSO) was prepared in the same manner as the extracts. A blank control was included that contained all the reagents including 5 µL of 1 % DMSO, however, no enzyme was added. The absorbance values were measured using a BIO-TEK Power-Wave XS plate reader at a wavelength of $OD_{405\ nm}$ for 15 min. The percentage inhibition was calculated using the following equation.

$$\%\ Inhibition = 100 - \left(\frac{Absorbance\ sample\ at\ 15\ min\ -\ absorbance\ at\ 0\ min}{Absorbance\ control\ at\ 15\ min\ -\ absorbance\ at\ 0\ min}\right) \times 100$$

GraphPad Prism 4 was used to determine the 50% inhibitory concentration (IC_{50}) for each sample.

2.5. Cell culture

Human keratinocytes (HaCaT) were used to determine the antiproliferative activity of the five samples that showed the highest tyrosinase inhibition. The HaCaT cells were maintained in DMEM, supplemented with 1 % antibiotics (penicillin (100 U/mL), streptomycin (100 µg/mL), 1 % amphotericin B (250 µg/mL) and 10 % fetal bovine serum. The cells were incubated at 5 % CO_2 and

37ºC until a confluent monolayer was obtained. The cells were sub-cultured using 0.25 % trypsin-EDTA once a monolayer had formed.

2.6. Antiproliferative activity

The method used to determine antiproliferative activity was described by (Lall et al., 2019). Cells were seeded into 96-well plates at a concentration of 1×10^5 cells/mL. Stock concentrations of the extracts were prepared at 20 mg/mL (in DMSO), whereas the positive control, actinomycin D was prepared at a concentration of 1 mg/mL (in distilled water). The extracts and actinomycin D were tested at final concentration ranges of 3.125 - 400 and 3.9 × 10-4 - 0.05 µg/mL respectively. A 100 % cell growth control, 0 % cell growth control (media only) and a 2 % DMSO vehicle control were included. After 72 h incubation, 20 µL Prestoblue was added and the plates were incubated for a further 2 h. The fluorescence was measured at an excitation/emission wavelength of 560/590 nm using a Victor Nivo plate reader (PerkinElmer, Midrand, South Africa). Cell viability was calculated using the following equation, which was used to determine the IC_{50} values using GraphPad Prism 4 software.

$$\%\, Viability = \frac{Fluorescence\ \ sample - Fluorescence\ \ 0\%\ control}{(Fluorescence\ \ vehicle\ control - Fluorescence\ \ 0\%\ control)} \times 100$$

2.7. Peripheral blood mononuclear cell isolation

Peripheral blood mononuclear cells (PBMC's) were isolated from whole blood using Histopaque®-1077. Blood samples were collected from healthy human volunteers, above the age of 21, with no history of major diseases, at the student health services, University of Pretoria. Permission to perform the isolation was approved by the ethics committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria, (EC120411-046). To isolate the PBMC's, a method described by (Oosthuizen et al., 2017) was used. Two plant extracts, *Aloe arborescens* and X principis clone 2, which showed the highest tyrosinase inhibition, were selected for further investigation for their effect on cytokine modulation in PBMCs, and therefore were tested for antiproliferative activity against PBMCs as described in section 2.2.5.

2.8. Quantification of human inflammatory cytokines

Inflammatory cytokines (IL-12p70, TNF-α, IL-10, IL-6, IL-1β and IL-8), from PBMC cell supernatants, were quantified using the CBA Human Inflammatory Cytokine Kit (BD Biosciences), according to the manufacturer's protocol. PBMC monocytes were differentiated into macrophages using 2 µg/mL phorbol 12-myristate 13-acetate (PMA). Cells were seeded in a 24-well plate at a concentration of 1×10^5 cells/mL and incubated for 1 h in 5 % CO_2 at 37ºC. Thereafter, extracts were added, in triplicate, at final concentrations of 3.125, 12.5 and 50 µg/mL. Cells stimulated with PMA (2 µg/mL) were used as the untreated control. After 24 h of incubation, the cells were centrifuged at 980 rpm for 5 min to collect 100 µL of the cell-free supernatant, which was transferred into a 96-well plate and stored at -80ºC until further use. The cell viability was measured by adding 40 µL of prestoblue reagent to the remaining cells. Cell viability was calculated, as described in section 2.2.5, to ascertain that the modulation of cytokines was not due to cell death. Quantification of the cytokines was done using the BD™ Accuri C6 flow cytometer and the FCAP Array™ Software V 3.0 (BD Biosciences, San Jose, CA, USA).

2.9. Statistical analysis

Results are reported as mean ± standard error (or standard deviation) as displayed in the results section. To obtain the IC_{50} values, a nonlinear regression analysis of the sigmoidal dose-response curves (4-parameter logistic) using GraphPad Prism 4 was conducted. Statistical analysis was done using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests (GraphPad, version 4), where $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) were considered statistically significant.

3. Results

3.1. Tyrosinase inhibition

Five extracts showed tyrosinase inhibition at a concentration of 200 µg/mL, which included *Aloe arborescens* gel (AG) (41.38 ± 0.94 %), *Aloe vera* leaf (VL) (26.64 ± 1.50 %), *Aloe X principis* (2)

leaf (XL) (33.11 ± 1.29 %), (*Aloe chabaudii* x *Aloe petricola*) x *Aloe zubb* leaf (CPL) (18.70 ± 1.40 %) and *Aloe ferox* x *A. zubb* leaf (FZL) (21.82 ± 1.12 %), however, each extract showed an IC_{50} >200 µg/mL, whereas the positive control (kojic acid) displayed an inhibition of 96.68 ± 1.21 % and an IC_{50} of 4.82 ± 1.31 µg/mL (Fig. 1).

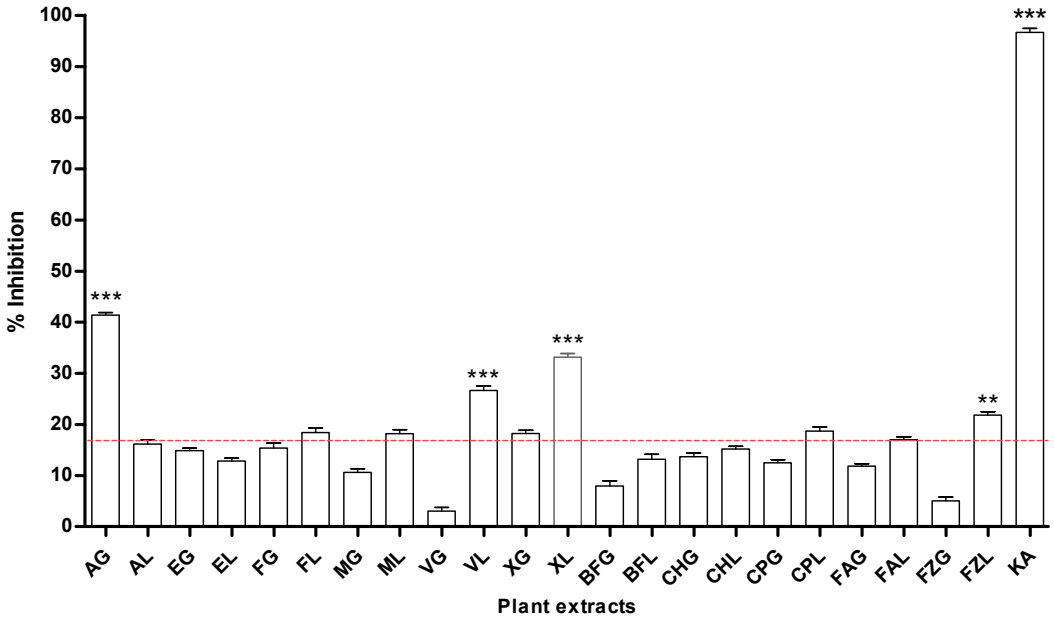

Fig. 1. Percentage tyrosinase inhibition of 22 plant extracts namely *Aloe arborescens* (AG and AL), *Aloe elegans* (EG and EL), *Aloe ferox* (FG and FL), (*Aloe marlothii* x *A. ferox*) x *A. arborescens* (MG and ML), *Aloe vera* (VG and VL), *Aloe X principis* (2) (*Aloe ferox* x *A. arborescens*) (XG and XL), *Bulbine frutescens* (BFG and BFL), (*Aloe chabaudii* x *A. petricola*) x *A. zubb* hybrid (CHG and CHL), (*Aloe chabaudii* x *A. petricola*) x *A. zubb* initial extract (CPG and CPL), *Aloe X principis* (1) (*Aloe ferox* x *A. arborescens*) (FAG and FAL), *Aloe ferox* x *A. zubb* (FZG and FZL) gel and leaf extracts and kojic acid, at a concentration of 200 µg/mL. Five plant extracts namely *Aloe arborescens* gel (AG), *Aloe vera* leaf (VL), X principis clone 2 leaf (XL), (*Aloe chabaudii* x *A. petricola*) x *A. zubb* pure leaf (CPL) and *Aloe ferox* x *A. zubb* leaf (FZL) extracts displayed the highest tyrosinase inhibition. Data shown are mean ± SEM (n=3). Statistical significance was determined using a one-way ANOVA followed by Dunnett's multiple comparison test against the cut-off (16.64 %). The cut-off value represented the average tyrosinase inhibition percentage across all samples. This was done to identify samples that were statistically more effective than the cut-off. A significant difference was represented as $p < 0.01$ (**) and $p < 0.001$ (***).

A combination study was conducted to determine whether AG in combination with kojic acid (at various ratios), was able to synergistically inhibit tyrosinase. The IC_{50} values decreased when AG and kojic acid were tested in combination when compared to the IC_{50} values of AG, however, the

calculated FIC values indicated that the combined AG and kojic acid ratios displayed no effect (Table 2).

Table 2
Fifty percent inhibition concentration (IC_{50}) and fractional inhibitory concentration (FIC) index calculated from a combination study using kojic acid and *Aloe arborescens* gel (AG) extract, combined in different ratios from kojic acid: AG (9:1) to kojic acid: AG (1:9).

Extract ratios	IC_{50} [a] ± SD [b] values in µg/mL	FIC [c]
Kojic acid (KA) [d]	4.92 ± 1.37	-
9:1 [e]	6.65 ± 1.51	1.35
8:2	6.59 ± 3.86	1.19
7:3	7.49 ± 1.69	1.19
6:4	9.75 ± 3.05	1.33
5:5	11.03 ± 1.73	1.26
4:6	16.22 ± 2.07	1.49
3:7	18.79 ± 1.64	1.31
2:8	29.49 ± 1.76	1.40
1:9	64.13 ± 1.89	1.62
AG [f]	>200	-

[a] Fifty percent inhibitory concentration, [b] Standard deviation (n=3), [c] Fractional inhibitory concentration index, [d] Positive control for tyrosinase inhibition, [e] Ratios from kojic acid: AG (9:1) to kojic acid: AG (1:9), [f] *Aloe arborescens* gel extract

3.2. Elastase inhibition

The five extracts which showed the highest tyrosinase inhibition, AG, XL, VL, CPL and FZL, were further evaluated for elastase inhibitory activity. However, the extracts showed IC_{50} values >500 µg/mL and did not inhibit elastase at the highest tested concentration, whereas ursolic acid (positive control) displayed an IC_{50} of 22.30 ± 2.79 µg/mL.

3.3. Antiproliferative activity

The antiproliferative activity of AG, VL, XL, CPL and FZL were evaluated against HaCaT cells. The extracts displayed no antiproliferative activity at the highest tested concentration (IC_{50} > 400 µg/mL), which was compared to actinomycin D, which showed an IC_{50} value of 0.02 ± 0.007 µg/mL. AG and

XL, which showed the highest tyrosinase inhibition, were further evaluated for antiproliferative activity on PMA stimulated PBMC's. AG displayed no antiproliferative activity at the highest concentration tested (IC_{50} > 400 µg/mL), however, *Aloe X principis* (2) leaf (XL) displayed an IC_{50} value of 219.5 ± 0.89 µg/mL. Furthermore, XL showed a significant decrease in cell viability, compared to the control, at a concentration of 100 µg/mL, with a percentage viability of 77.48 ± 2.74 %. This was compared to the positive control, actinomycin D, which showed an IC_{50} value of 0.06 ± 0.10 µg/mL.

3.4. Cytokine quantification

AG and XL were tested for their effect on inflammatory cytokines at non-toxic concentrations of 50, 12.5 and 3.125 µg/mL, as determined by the antiproliferative activity against PBMC's. To ensure that the modulation of cytokines was not due to a decrease in cell proliferation, the cell viability of the PBMC's treated with AG and XL, at each of the tested concentrations, were measured. No significant difference was observed at each of the tested concentrations compared to the untreated control (Fig. 2).

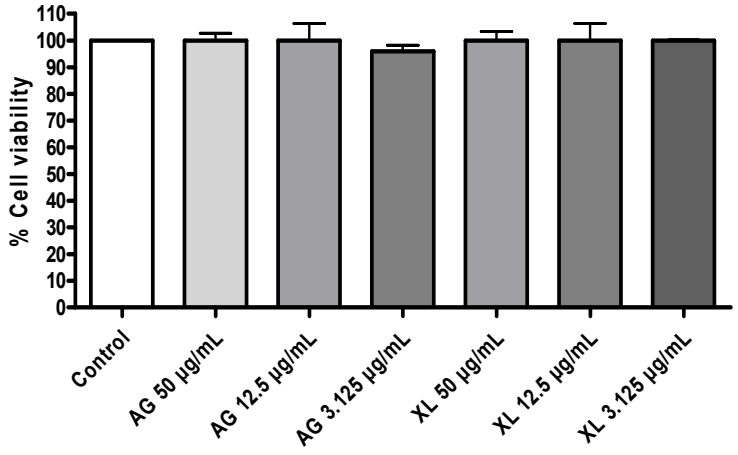

Fig. 2. Cell viability of *Aloe arborescens* gel (AG) and *Aloe X principis* (2) leaf (XL) at concentrations of 50, 12.5 and 3.125 µg/mL on phorbol 12-myristate 13-acetate (PMA) stimulated peripheral blood monocyte cells (PBMC's). Data shown are mean ± SD (n=3). No significant difference was determined using a one-way ANOVA followed by Dunnett's multiple comparison test when compared to the untreated control.

The AG extract enhanced TNF-α (20.70 ± 0.84 pg/mL), IL-10 (18.38 ± 0.07 pg/mL), IL-1β (19.47 ± 1.35 pg/mL) and IL-8 (164.50 ± 3.98 pg/mL) ($p < 0.001$) production at the highest testing concentration (50 µg/mL) while at 12.5 µg/mL the extract enhanced IL-1β (15.61 ± 0.54 pg/mL), IL-12p70 (25.11 ± 1.90 pg/mL) ($p < 0.05$), TNF-α (17.41 ± 0.16 pg/mL), IL-10 (15.31 ± 0.28 pg/mL) and IL-6 (20.12 ± 0.88 pg/mL) ($p < 0.01$) production. Furthermore, AG inhibited IL-8 (53.63 ± 1.25 pg/mL; 54.69 ± 3.42 pg/mL) ($p < 0.01$) at 12.5 and 3.125 µg/mL, respectively, whereas IL-1β (8.78 ± 0.80 pg/mL) ($p < 0.05$) was inhibited at a concentration of 3.125 µg/mL. No statistical difference in the production of IL-12p70 (21.08 ± 2.14 pg/mL) and IL-6 (16.10 ± 3.95 pg/mL) at 50 µg/mL and IL-12p70 (12.65 ± 0.58 pg/mL), TNF-α (11.02± 0.70 pg/mL), IL-10 (10.53 ± 1.50 pg/mL) and IL-6 (9.74 ± 1.34 pg/mL) at 3.125 µg/mL was observed compared to the control (IL-12p70 at 16.60 ± 3.66, TNF-α at 10.80 ± 1.21, IL-10 at 10.66 ± 0.92, IL-6 at 10.77 ± 0.43, IL-1β at 12.35 ± 0.31 and IL-8 at 72.67 ± 0.04 pg/mL) (Fig. 3).

Aloe X principis (2) leaf (XL) extract inhibited TNF-α (2.93 ± 0.16 pg/mL), IL-1β (4.72 ± 0.18 pg/mL), IL-12p70 (0.00 ± 0.00 pg/mL) ($p < 0.001$), IL-10 (5.87 ± 0.12 pg/mL), IL-8 (56.81 ± 0.20 pg/mL) ($p < 0.01$) and IL-6 (3.69 ± 1.53 pg/mL) ($p < 0.05$) at the highest testing concentration. At a concentration of 12.5 µg/mL, XL inhibited IL-8 (39.33 ± 4.10 pg/mL) ($p < 0.001$) production while at 3.125 µg/mL IL-1β (9.25 ± 1.05 pg/mL) and IL-8 (59.10 ± 1.92 pg/mL) ($p < 0.05$) production was reduced. Furthermore, XL enhanced TNF-α (15.43 ± 0.71, $p < 0.05$ and 17.84 ± 1.50 pg/mL, $p < 0.01$) production at concentration of 12.5 and 3.125 µg/mL. Lastly, XL displayed no effect towards IL-12p70 (20.14 ± 0.10 and 15.67 ± 0.74 pg/mL), IL-6 (14.79 ± 0.19 and 15.39 ± 0.08 pg/mL), IL-10 (12.54 ± 0.97 and 13.55 ± 0.52 pg/mL) and IL-1β (12.72 ± 0.17 pg/mL) production at 12.5 and 3.125 µg/mL compared to the control (IL-12p70 at 16.60 ± 3.66, TNF-α at 10.80 ± 1.21, IL-10 at 10.66 ± 0.92, IL-6 at 10.77 ± 0.43, IL-1β at 12.35 ± 0.31 and IL-8 at 72.67 ± 0.04 pg/mL).

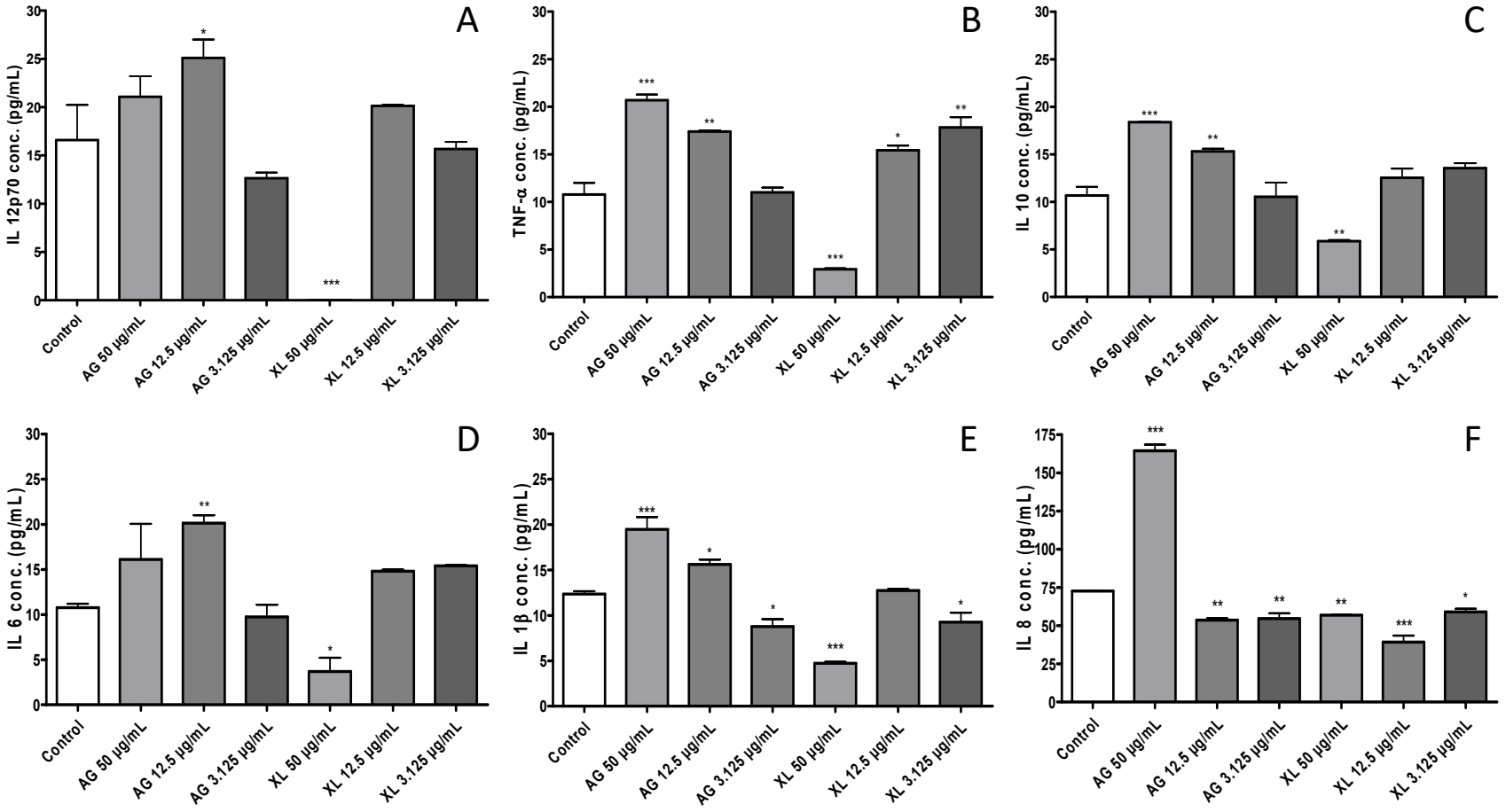

Fig. 3. Effects of *Aloe arborescens* gel (AG), *Aloe X principis* (2) leaf (XL) extracts at concentrations of 50, 12.5 and 3.125 µg/mL against human inflammatory cytokines; A: interleukin (IL) 12p70, B: tumor necrosis factor alpha (TNF-α), C: IL-10, D: IL-6, E: IL-1 beta (IL-1β) and F: IL-8. Data are represented as mean cytokine production ± SEM (n=2). Statistical significance was determined using one-way ANOVA followed by a Dunnett's multiple comparison test, where $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***), indicate significance when compared to the control.

4. Discussion

4.1 Tyrosinase inhibition

The results obtained in this study have not previously been reported. The leaf and gel extracts of the different succulent species were evaluated for tyrosinase inhibitory potential using mushroom tyrosinase, due to eczema patients experiencing post-inflammatory hyperpigmentation (Davis and Callender, 2010). The leaf extracts showed higher inhibition than the gel extracts, with exception of the *Aloe arborescens* extract (Fig. 1). The difference in activity between the leaf and the gel extracts could potentially be due to aloesin, which is present within the leaves of aloe species and has been reported to inhibit *L*- 3, 4- dihydroxyphenylalanine oxidation (64.7 ± 5.3 %) at a concentration of 1 g/L (Wang et al., 2008). The high percentage inhibition of aloesin may be due to its chemical structure as several tyrosinase inhibitors possess two aromatic rings linked by a 2 or 3-atom chain, which contain hydrogen bond acceptor groups (i.e. hydroxyl) in appropriate positions (Pillaiyar et al., 2017). *Aloe arborescens* gel, on the other hand, contains a unique compound known as 2'-*O*-feruloylaloesin, which has been shown to inhibit tyrosinase by 27 ± 0.57 % at 0.4 µM, which could indicate why AG displayed a higher tyrosinase inhibition in comparison to the leaf extracts (Dagne et al., 2000; Yagi et al., 1987). However, the chemical composition of AG in the present study requires further evaluation. Furthermore, there been no previous reports on the anti-tyrosinase activity of *A. arborescens*. Hybrid aloe species, used in this study, contained at least one of the three aloe species mentioned above. These hybrids are either related to aloes or contain DNA from species that are similar to aloes. As of current, no other reports on the biological activity of the hybrid species. Furthermore, the unique compound composition of these species has not yet been isolated or evaluated for biological activity.

4.2. Elastase inhibition

As AG, VL, XL, CPL and FZL showed the highest tyrosinase inhibition, these extracts were further evaluated for their elastase inhibitory activity. Patients with eczema form wrinkles on the affected area due to the absence of the serine protease inhibitor Kazal-type 1 (SPINK), which prevents the

overexpression of elastase once the skin barrier has been damaged (Agarwal et al., 2014). None of the selected samples displayed elastase inhibition at the highest tested concentration (500 µg/mL). Sacan et al. (2017) reported that the gel and leaf skin of *A. vera* displayed anti-elastase properties with IC_{50} values of 247.85 ± 35.19 and 0.07 ± 0.004 µg/mL, respectively. These authors concluded that this effect was potentially due to the high antioxidant properties of *A. vera* in both the gel and leaf skin (Sacan et al., 2017).

4.3. Antiproliferative activity

The antiproliferative activity of AG, VL, XL, CPL and FZL was evaluated against HaCaT cells, where IC_{50} values of > 400 µg/mL were obtained. The findings in this study correlate with Fox et al. (2017), where the whole leaf and gel of *Aloe vera, Aloe ferox* and *Aloe marlothii* were evaluated against HaCaT cells and showed half-maximal cytotoxic concentration (CC_{50}) above 1000 µg/mL (Fox et al., 2017). Furthermore, AG and XL, which showed the highest tyrosinase inhibition, were evaluated for antiproliferative activity against PBMC's, as this cell line was used to evaluate cytokine modulation. The AG extract, which has a chemical profile similar to *A. vera*, displayed no antiproliferative activity towards PBMC's (IC_{50} > 400 µg/mL) (Brien et al., 2011). A study done by Basak et al. (2017) showed that whole leaf extracts made from *A. vera* stimulated the growth of PBMC's at a concentration of 300 µg/mL (Basak et al., 2017). The XL extract displayed moderate antiproliferative activity (IC_{50} of 219.5 ± 0.89 µg/mL), and could potentially contain mannan, which has previously been shown to inhibit the growth of PBMC's by 29% at a concentration of 0.6 mg/mL (Sampedro et al., 2004).

4.4. Cytokine quantification

Cytokines were quantified in the supernatant of PMA stimulated PBMC's treated with AG and XL at a non-toxic concentration range of 3.125-50 µg/mL. The selected concentration range was chosen based on the antiproliferative effect and low cell viability displayed by XL at a concentration range above 50 µg/mL. Four of the measured cytokines play a role in the chronic phase of eczema, namely tumor necrosis factor-alpha (TNF-α), interleukin 12p70 (IL-12p70), IL-10 and IL-8 while IL-6 and

IL-1β initiate post-inflammatory hyperpigmentation (Elenkov et al., 2005; Nedoszytko et al., 2014; (Ohmen et al., 1995).

Aloe arborescens gel inhibited the production of IL-8 and IL-1β at the lowest concentration (3.125 µg/mL) and enhanced the production of TNF-α and IL-8 at 50 µg/mL and IL-6 and IL-12p70 at 12.5 µg/mL (Fig. 3). No previous study on the effect of AG on IL-8 production was found. According to Bastain et al. (2013), acemannan extracted from the leaves of *A. arborescens* were shown to stimulate the production of IL-6 and TNF-α at a concentration of 5 µL/mL, while Park et al. (2011) indicated that aloin and aloesin at 0.005% reduced the mRNA expression of IL-1β (Bastian et al., 2013; Park et al., 2011). Nazeam et al. (2017) found that *A. arborescens* inhibited IL-12p70 production due to the presence of water-soluble (3.5 ± 0.4 pg/mL) and alkaline soluble (19.6 ± 2.7 pg/mL) polysaccharides at a concentration of 200 µg/mL (Nazeam et al., 2017).

Aloe X principis (2) leaf extract inhibited TNF-α, IL-8, IL-12p70, IL-6 and IL-1β at the highest testing concentration (50 µg/mL). During the study, it was noted that AG inhibited cytokine modulation at the lowest concentration while XL displayed an opposite effect. Aloe-emodin found in *A. ferox* was previously shown to decrease mRNA expression of IL-1β, IL-6 and TNF-α at a concentration of 100 µM (Chen et al., 2020; Kambizi et al., 2005). Aloin A, which reduced the mRNA expression of IL-1β in a study conducted by Park et al (2011) has been found in both *A. arborescens* and *A. ferox*. Thus, since XL was a cross between *A. ferox* and *A. arborescens*, the effects displayed by the extract could be due to a combination of compounds present in the parent plants, which may support the cytokine inhibitory effects of XL.

5. Conclusion

This study focused on evaluating selected South African succulents for their potential to reduce symptoms associated with eczema, which included tyrosinase and elastase production, and anti-inflammatory activity. Of the 22 plant extracts that were evaluated, five aloes namely *Aloe arborescens* gel (AG), *Aloe X principis* (2) leaf (XL), *A. vera* leaf (VL), (*A. chabaudii* x *A. petricola*) x *A. zubb* pure leaf (CPL) and *A. ferox* x *A. zubb* leaf (FZL) extracts displayed tyrosinase inhibition

with no anti-proliferative activity against HaCaT cells at the highest testing concentration (400 µg/mL). Of the five extracts, AG (41.38 ± 0.94 %) and XL (33.11 ± 1.29 %) were further evaluated for their anti-inflammatory activity. *Aloe arborescens* gel (AG) significantly inhibited IL-8 and IL-1β at the lowest testing concentration (3.125 µg/mL), while XL significantly reduced the production of all six cytokines at 50 µg/mL. This contrast in activity between AG and XL could be related to the chemical composition of XL as this hybrid species is a cross between *A. arborescens* and *A. ferox*. Other differences in biological activity between the pure and hybrid species included tyrosinase inhibitory potential and antiproliferative against PBMC's. In conclusion, both pure and hybrid species of aloes showed inhibitory activity against tyrosinase with XL displaying the most promising inhibitory activity against cytokines associated with chronic eczema. Further investigation into the formulation of a topical application of XL should be evaluated for cosmetic safety and efficacy studies in patients with chronic eczema.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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