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Medicinal Mascarene Aloe: An audit of their phytotherapeutic potential

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Abstract
A phytochemical and biological investigation of the endemic Mascarene Aloe (Aloe spp.), including A. tormentorii (Marais) L.E.Newton & G.D.Rowley, A. purpurea Lam, A. macra Haw., A. lomatophylloides Balf.f and A. vera (synonym A. barbadensis Mill.), which are used in the traditional folk medicine of the Mascarene Islands, was initiated. Methanolic extracts of the Aloe species under study were analysed using high resolution LC-UV-MS/MS and compounds belonging to the class of anthraquinones, anthrones, chromones and flavone C-glycosides were detected. The Mascarene Aloe could be distinguished from A. vera by the absence of 2’’-O-feruloylaloesin and 7-O-methylaloeresin. GC-MS analysis of monosaccharides revealed the presence of arabinose, fucose, xylose, mannose and galactose in all the Mascarene Aloe species and in A. vera. The crude extracts of all Aloe species analysed displayed antimicrobial activity against Bacillus cereus, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Only extracts of A. macra were active against P. aeruginosa and Klebsiella pneumoniae, while none of the Aloe extracts inhibited Propionibacterium acnes. A. macra displayed anti-tyrosinase activity, exhibiting 50% inhibition at 0.95 mg/ml, and extracts of A. purpurea (Mauritius) and A. vera displayed activity in a wound healing-scratch assay. In vitro cytotoxicity screening of
crude methanolic extracts of the Aloe, using the MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-
diphenyltetrazolium bromide) showed that only A. purpurea (Réunion) elicited a modest toxic
effect against HL60 cells, with a percentage toxicity of 8.2% (A. purpurea -Réunion) and none
of the Aloe extracts elicited a toxic effect against MRC 5 fibroblast cells at a concentration of
0.1 mg/ml. Mascarene Aloe species possess noteworthy pharmacological attributes associated
with their rich phytochemical profiles.

Keywords: Mascarene Aloe biological activity, LC MS, GC MS, monosaccharides
1. Introduction

The genus *Aloe* (Xanthorrhoeaceae) has been traditionally used in the medicinal practice for thousands of years in many cultures of the world. Today, *Aloe vera* (L.) Burm.f. in particular has become a popular household remedy, reputed to exhibit a range of beneficial health properties. Some of the most widely known *Aloe* species adopted for their medicinal properties include *A. vera* (synonym *A. barbadensis* Mill.), *A. ferox* Mill. (vernacular name: Cape *Aloe*), *A. arborescens* Mill. (vernacular name: Candelabra *Aloe*), *A. perryi* Baker (vernacular name: Perry's *Aloe*), *A. succotrina* Weston and *A. maculata* All. *A. vera* is the most widely studied species, and has been evaluated for clinical efficacy against various diseases (Jia *et al*., 2008; Chinchilla *et al*., 2013). Leaves from *Aloe* species yield two known medicinal products: a gel obtained from parenchymal tissue of the leaf, and a bitter exudate known as 'bitter aloes' or 'drug aloes', derived from pericycle cells beneath the epidermis. The bitter leaf exudate has been used worldwide as a laxative. Indeed, a monograph for the concentrated dried leaf juice of ‘Cape *Aloe*’ is included in the current European and British Pharmacopoeias (British Pharmacopoeia Commission, 2017). Whilst the leaves from many other *Aloe* species have been documented as traditional medicinal remedies (Grace *et al*., 2008), there is a comparative lack of scientific evidence that documents the chemistry and biological activities of the less widely known *Aloe* species, to validate their reputed medicinal effects.

The Mascarene Islands, which comprise Mauritius, Rodrigues and Réunion, have a rich and diverse flora and many indigenous and endemic plant species of these Islands have been used in folk medicine to treat various illnesses. However, even with this well-documented traditional knowledge, few medicinal plant species, including Mascarene *Aloe* species, have been scientifically validated for their medicinal uses. The Mascarene *Aloes* which belong to the former *Aloe* section *lomatophyllum* include *Aloe tormentorii* and *A. purpurea*, commonly known as ‘Mazambron marron’, are species endemic to Mauritius (Bosser *et al*., 1976; Gurib-Fakim, 2003); *Aloe macra* (Bosser *et al*., 1976; Pailler *et al*., 2000) is endemic to Réunion Island and *A. lomatophylloides* (Govinden-Soulange, 2014) is native to Rodrigues Island. The Mascarene *Aloes* are documented to have a range of medicinal properties. In Rodrigues, the crushed leaves of *A. lomatophylloides* Balf.f. have been applied as a poultice to relieve muscle pain, whilst a decoction of the leaves is taken to increase menstrual flow (Gurib-Fakim, 2003). The leaves of *A. macra* Haw. are used to alleviate minor infections, boils, constipation and as a general healing substance for external use (Govinden-Soulange, 2014), whilst the leaf sap of...
A. purpurea Lam. is applied to the breast to encourage weaning. The Mascarene Aloes are also used internally as antispasmodics and to relieve discomfort associated with menstruation (Gurib-Fakim., 2003). It is widely known that the hydroxyanthracene glycoside derivatives that occur in Aloe spp. explain their use as laxatives, while the polysaccharide gel of A. vera has been associated with the biological activities relevant to skin disorders and cosmetic use (Chinchilla et al., 2013).

The chemical constituents and biological activities that might explain the traditional and potential uses of the Mascarene Aloes, particularly any mechanisms relevant to dermatological uses, are largely unexplored to date. Previous studies investigated the morphological characteristics of A. macra, A. tormentorii (Marais) L.E.Newton & G.D.Rowley, A. purpurea, and the genetic differences between these Aloe species and A. vera, were compared, and flavone glycosides were identified (Ranghoo-Sanmukhiya et al., 2010). The uniqueness of Mascarene Aloes as compared to A. vera was confirmed using phylogenetic analysis of sequence data and the superior antioxidant activity and neuroprotective property of these species has been previously reported (Govinden-Soulange et al., 2017; Lobine et al., 2017). In the present report, further studies have been initiated to validate their traditional use and to evaluate their potential as sustainable phyto-medicines. Five different endemic Mascarene Aloe species were investigated using different approaches, with direct comparison of their biological activities with A. vera.

2. Materials and Methods
2.1. Plant material collection
Leaves from five-year old plants of A. purpurea Lam., A. tormentorii (Marais) L.E.Newton & G.D.Rowley, A. lomatophylloides Balf.f., A. macra Haw. and A. vera (L.) Burm.f. were obtained from the National Parks and Conservation Service (Mauritius) and Mauritius Herbarium garden, MSIRI and Conservatoire Botanique National de Mascarin (Réunion Island). A voucher specimen of each Mascarene Aloe species: A. purpurea (Mauritius) [MAU 0014447]; A. tormentorii [MAU 0014094]; A. lomatophylloides [MAU 0014095]; A. macra [WV 99110 and WS990130]; A. purpurea (Réunion Island) [WS 99067], was deposited at the Herbarium of the Mauritius Sugar Industry Research Institute (Réduit, Mauritius). The leaves were lyophilised and stored in air-tight bottles. A. purpurea from Réunion has been reported as a putative hybrid of A. macra and A. tormentorii (Ranghoo-Sanmukhiya et al., 2010). A. macra [WV 990110] and A. macra [WS990130] are morphologically different from each other, and
are thus suspected to be two different varieties. The leaves of *A. macra* [WV 990110] are green, while *A. macra* [WS990130] has prominent red-toned leaves, with both growing in the same habitat. A potential new *Aloe* species [WS 98002], which is undergoing evaluation to determine its taxonomic status, is also reported. We emphasize that with respect to species conservation strategies, only limited quantities of the *Aloe* spp. [WS 98002] and *A. macra* (‘forme rouge’) [WS 98 0130] were collected but were not subjected to bioassay studies, although their chemistry was evaluated using gas chromatography-mass spectrometry (GC-MS) and high resolution liquid chromatography-mass spectrometry coupled with UV detection (LC-UV-MS/MS).

### 2.2. LC-UV-MS/MS analysis

Analyses were performed on a Thermo Scientific system consisting of an ‘Accela’ U-HPLC unit with a photodiode array detector and an ‘LTQ Orbitrap XL’ mass spectrometer fitted with an electrospray source (Thermo Scientific, Waltham, MA, USA). Chromatography was performed on 5 μL samples (70% methanol extracts; as described in 2.4.) injected onto a 150 mm x 3 mm, 3 μm Luna C-18 column (Phenomenex, Torrance, CA, USA) using the following 400 μL/min mobile phase gradient of H₂O/CH₃OH/CH₃CN +1% HCOOH: 90:0:10 (0 min), 90:0:10 (5 min), 0:90:10 (60 min), 0:90:10 (65 min), 90:0:10 (67 min), 90:0:10 (70 min) followed by a return to start conditions and equilibration in start conditions for 5 min before the next injection. The electrospray ionization (ESI) source was operated with polarity switching and the mass spectrometer was set to record high resolution (30 k resolution) MS1 spectra (*m/z* 125–2000) in positive mode using the orbitrap and low resolution MS1 spectra (*m/z* 125–2000) in negative mode and data dependent MS2 and MS3 spectra in both modes using the linear ion trap. Detected compounds were assigned by comparison with accurate mass data (based on ppm), and by available MS/MS data, with reference to the published compound assignment system (*Schymanski et al.*, 2014) and supportive UV spectra; aloin A was also assigned by comparison with a reference standard (≥97%; Sigma-Aldrich, UK).

### 2.3. Determination of monosaccharide composition using GC-MS

Lyophilised mesophyll tissue (10 mg) of each Mascarene *Aloe* species was re-suspended in 500 μL of sterile distilled water and allowed to rehydrate in a sonicating water bath for 1 h. The material was acidified to 2 M trifluoroacetic acid (TFA) and 100 μg internal standard (inositol) was added, followed by incubation at 110 °C for 2 h in sealed glass sample tubes,
prior to centrifugation at 14,000 xg for 30 min and the supernatants were dried under nitrogen at 40°C. 400 µL of methanolic 1N HCl was added to the dried residue and incubated at 80°C overnight then dried under nitrogen at 40°C after addition of 100 µL tert-butanol. 1-(Trimethylsilyl) imidazole-pyridine (400 µL) was added to each sample, which were then incubated at 80°C for 30 min, dried under nitrogen at 40°C prior to re-suspension in 1 mL hexane for GC-MS analysis.

The GC-MS analyses were performed using a single-quadrupole Shimadzu QP-2010-Plus system fitted with a Restek Rxi-5Sil column (30 m × 0.25 mm × 0.25 µm). 2 µL of samples were introduced by split injection at a ratio of 1:20 and the carrier gas (helium) was set to a flow rate of 40 cm/sec. The injector temperature was 250 ºC and the initial oven temperature was 140 ºC, increasing at 2 ºC/minute to 180 ºC and held at this temperature for 5 minutes before increasing to 275 ºC at 10 ºC/minute, held for 10 minutes. The scan range was m/z 45 - 1000. Seven monosaccharides (arabinose, fucose, galactose, glucose, mannose, xylose and inositol obtained from Supelco and Sigma-Aldrich) were used as reference standards, based on a previous study (Grace et al., 2011).

2.4. Preparation of extracts for bioassays

Lyophilised leaf samples (100 mg) of each Mascarene Aloe species were extracted in 10 mL of cold 70% (v/v) methanol, heated under reflux for 1 h, sonicated for 15 min and centrifuged for 10 min at 5000 rpm. The supernatant was filtered (0.45 µm filter) and analysed. For bioassays, the extracts were concentrated to dryness and the residues were re-suspended in water and stored in aliquots at -20°C. For all the experiments, dilutions of extracts were performed fresh on the day of the bioassay.

2.5. Antimicrobial assay

The serial dilution technique described by Eloff (1998) was used to determine the minimum inhibitory concentration (MIC) for antibacterial activity of the Aloe extracts. 2 mL cultures of five bacterial strains [two Gram-positive, Staphylococcus aureus (ATCC No. 12600) and Bacillus cereus (ATCC No: 11778); and three Gram-negative, Escherichia coli (ATCC No. 11775), Pseudomonas aeruginosa (ATCC No: 27853) and Klebsiella pneumoniae (ATCC No. 13883)] were prepared and incubated overnight at 37 ºC. The overnight cultures were diluted with sterile MH (Mueller-Hinton) broth (1 mL bacteria/100 mL MH) to an absorbance of 0.4–
0.6 at 600 nm. For each bacterial strain used, 100 µL of the Aloe extracts (50mg/mL) was serially diluted two-fold with 100 µL sterile distilled water in a sterile 96-well microplate. A similar two-fold serial dilution of streptomycin (Sigma-Aldrich; 0.01 mg/mL) was used as a positive control against each bacterium. Sterile distilled water was used as a negative control and 100 µL of bacterial culture was added to each well. The plates were covered, sealed and incubated overnight at 37 °C. Bacterial growth was determined by addition of 50 µL of 0.2 mg/mL p-iodonitrotetrazolium violet (INT) to each well after incubation at 37 °C for 30 min. Bacterial growth in the wells was indicated by a red colour, and colourless wells indicated inhibition by the tested extracts.

2.6. Anti-acne bioassay

The Aloe extracts were tested against Propionibacterium acnes (ATCC 11827) by determining the MIC values obtained by a microdilution method as described by Sharma et al., (2014) with slight modifications. P. acnes (ATCC 11827) was cultured from a Kwik-Stick on mouse brain and heart agar and incubated under anaerobic conditions at 37°C for 72 h. The 72 h culture was suspended in nutrient broth and adjusted to an absorbance (A600nm) of 0.132. In a sterile 96-well plate, 100 µL of the plant extracts [8 mg/mL in 10% dimethyl sulphoxide, (DMSO)] and the positive control tetracycline was diluted with nutrient broth (100 µL). Two-fold serial dilutions were made in the nutrient broth and the bacterial suspension (100 µL) was added to all the wells of the microtiter plate. The final concentrations ranged from 2000-15.6 µg/mL for the plant extracts and 100-0.78 µg/mL for the positive control, tetracycline. To the control wells, 2.5% DMSO and bacterial suspension without additions served as the negative and bacterial controls, respectively. The plates were incubated for 72 h at 37 °C under anaerobic conditions. The MIC was determined by observation after addition of PrestoBlue reagent (20 µL).

2.7. Tyrosinase enzyme inhibition assay

The Aloe extracts were dissolved in dimethyl sulfoxide (DMSO) to a final stock concentration of 20 mg/mL. The stock sample solutions were two-fold serially diluted in 50 mM potassium phosphate buffer (pH 6.5) to a starting concentration of 3 mg/mL. In a 96-well plate, the sample solutions (70 µL) were combined with 30 µL of tyrosinase (48 Units/mL) in triplicate. The plate was incubated for 5 minutes at room temperature before the addition of 2 mM L-tyrosine (110µL) to each well. The final concentration of the extracts ranged between 1000-7.8 µg/mL.
Kojic acid served as positive control with a final concentration ranging between 400-3.1 µg/mL. The absorbance of the wells was analysed at 492 nm for 30 minutes at room temperature with a BIO-TEK PowerWave XS multi-well plate reader. The 50% inhibitory concentration (IC₅₀) value was determined by using Graph Pad Prism 4 software (Momtaz et al., 2008).

2.8. Wound healing (scratch) assay
The spreading and migration capabilities of the human keratinocyte HaCaT cell line were assessed using a scratch wound assay, which measured the expansion of a cell population on surfaces. HaCaT cells were seeded into 24-well tissue culture plates for 24 h, at a concentration of 2 x 10⁴ cells/mL, cultured in modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS) and incubated for 24 h at 37 °C with 5% CO₂, to almost confluent cell monolayers. A linear wound was then generated in the monolayer with a sterile 100 µl plastic pipette tip. Any cellular debris was removed by washing the well gently with phosphate buffer saline (PBS) (136.9 mM 2.68 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, pH 7.4) and fresh DMEM medium was added and replaced with 2 mL of DMEM containing the Aloe extracts (0.1 mg/mL); DMEM without sample served as a control. Cell migration was monitored by collecting digitised images using a Leica SP5 confocal microscope at 5 min intervals for 20 h. The microscope stage was heated to 37 °C and the cells were incubated at 5% CO₂ through the experiment. The data were analysed using Tscratch software (Gebäck et al., 2009). The percentage closed area was measured and compared with the value obtained before treatment. An increase in the percentage of the closed area indicated cell migration. The experiments were performed in replicates (n=6).

2.9. Human promyelocytic leukaemia cell assay
The Human promyelocytic leukaemia cells (HL60; kindly provided by Dr Nicholas Hole, Durham University) were cultured at 37°C in 5% CO₂ on 75 cm² tissue culture flasks (Sarstedt, Newton, NC) in Dulbecco's modified Eagles' medium DMEM-F-12 Media - GlutaMAX™-I (GIBCO, Grand Island, NY), supplemented with 10% foetal bovine serum (FBS; Sigma, St. Louis, MO, USA). Cells were passaged every 3-4 days at a 1:4 dilution. For the assay, cell pellets were re-suspended in fresh medium at a concentration of 0.30 x 10⁶ cells per mL and immediately plated in 24-well plates and incubated at 37°C with 5% CO₂. Cells were exposed
to 0.1 mg/mL of the *Aloe* extracts with appropriate controls for 24 h before the cytotoxicity assay was performed (Pilarski *et al.*, 2007).

### 2.10. MRC 5 fibroblast assay

The MRC 5 fibroblasts (ATCC CCL-171) were cultured via serial passage at 37°C in a humidified 5% CO₂ atmosphere in Dulbecco’s Modified Eagle’s Medium (Gibco, Paisley, UK) supplemented with 1% (v/v) penicillin-streptomycin and 10% foetal bovine serum (LabTech). Once cultures reached 90% confluence, they were washed twice in warm PBS prior to incubation in trypsin for 5 min at 37 °C. When passaging into flasks, a 1:2 split ratio was maintained consistently. Cells were diluted in fresh medium and seeded into 24-well plates at a density of 2 x 10⁴ per well. After incubation for 24 h, the cells were exposed to the *Aloe* extracts (0.1 mg/mL) and controls for 24 h before the cytotoxicity assay was performed.

### 2.11. MTT cell viability assay

The tetrazolium dye MTT (Sigma, UK) was used to assess the viability of HL60 and MRC 5 fibroblasts after 24 h pre-treatment with *Aloe* extracts as described by Abuhamdah *et al.*, (2015). 50 µL of PBS containing a final concentration of 5 mg/mL MTT was added to the cultures treated with the *Aloe* extracts (0.1 mg/mL) for 24 h and incubated at 37°C in 5% CO₂ for 2.5 h. The MTT-containing medium was then removed and the well surfaces were rinsed gently with 300 µL PBS prior to addition of 250 µL isopropanol. The absorbance of 100 µL samples was measured spectrophotometrically at 595 nm (Thermo Labsystems Multiskan Ascent, V1.3).

### 2.12. Statistical Analysis

All the experiments were performed in replicates (n = 3-6) and statistical analysis was carried out using one-way ANOVA followed by Tukey’s test, performed using SPSS 16.0. The values of *p* < 0.05 were considered to be statistically significant.
3. Results and Discussion

3.1. LC-UV-MS/MS analysis

The LC-MS chromatograms (positive ESI) for each Aloe extract, with the main detected compounds and their assignments, are shown in Figure 1. Hydroxycinnamic acids occurring as esters of quinic acid conjugated with caffeic (C₁₆H₁₈O₉. 1) or coumaric acids were detected in all the investigated Aloe species, except for A. tormentorii, in which only 4-O-p-coumaroylquinic acid (C₁₆H₁₈O₈. 3) was detected, suggesting that this compound may be useful to differentiate A. tormentorii from other Mascarene Aloes. A compound with m/z 395.1328 (C₁⁹H₂₂O₉. 2) was assigned as aloesin (formerly known as aloeresin B), and was detected in all the Aloe species investigated except in A. lomatophylloides. This suggests that a lack of aloesin may be useful to distinguish A. lomatophylloides from the other Mascarene Aloes. The thin leaves of A. lomatophylloides contain less exudate compared to other investigated Aloes, which may account for the absence of aloesin, which is a significant constituent of exudates of other Aloe species (Dagne et al., 2000; Cock, 2015).

A compound (C₂₈H₂₈O₁₁. 17) with m/z 541.1705 corresponded to aloeresin A or isomer. Compounds assigned from the observed [M+H]+ ions as C₂₉H₃₀O₁₂ [the molecular formula of 2”-O-feuloylaloesin (12)], and as C₂₉H₃₀O₁₁ [the molecular formula of 7-O-methylaloeresin A (14) were only detected in the A. vera extract (m/z 571.1812 and 555.1873, respectively). 2”-O-Feuloylaloesin and 7-O-methylaloeresin A were not detected in any of the Mascarene Aloes, so these might be useful marker compounds to distinguish these species from A. vera. 7-O-Methylaloeresin A was first reported in Aloe marlothii A. Berger (Bisrat et al., 2000) and to our knowledge, has not been previously reported in any species from the former section Lomatophyllum. The phenyl pyrones have a restricted distribution in the genus Aloe, with aloenin occurring in only 16 out of the 380 Aloe species (Viljoen and Van Wyk, 2000). In this study, a compound (13) assigned as 2”-O-trans-p-coumaroylaloenin (C₂₈H₂₈O₁₂ or isomer; m/z 557.1657) was detected in all the Mascarene Aloes except in A. lomatophylloides and A. vera. Previously, flavonoids were detected in only 31 of 380 Aloe species as major compounds, with the flavone isovitexin (apigenin 6-C-glucoside) considered as a chemotaxonomic marker restricted to the grass-like Aloes (Graminialoe and Leptopaloe), the Macrifolia and the Lomatophyllum section (Viljoen et al., 1998). In this present study, flavone C-glycosides, including vitexin (apigenin 8-C-glucoside) or isovitexin (C₂₁H₂₆O₁₀. 9), and isoorientin
(luteolin 6-C-glucoside) (C$_{21}$H$_{20}$O$_{11}$; 6), were detected. Compounds assigned as vitexin or isovitexin glycosides were also detected, including hexosides (C$_{27}$H$_{30}$O$_{15}$; 4 and 7) and a pentoside (C$_{26}$H$_{28}$O$_{14}$; 8). Other flavone C-glycosides included a compound assigned as isoorientin pentoside (C$_{26}$H$_{28}$O$_{15}$; 5), assigned from the observed [M+H]$^+$ ion and from MS2 and MS3 and supportive UV spectra. Isoorientin has been previously reported in A. macra (Ranghoo-Sanmukhiya et al., 2010).

Aloin A (C$_{21}$H$_{22}$O$_{9}$; 16) and an isomer assigned as aloin B (C$_{21}$H$_{22}$O$_{9}$; 15) were observed as their [M+H]$^+$ ions (m/z 419.1345). Additional isomers were detected with the molecular formulae C$_{21}$H$_{22}$O$_{9}$, corresponding to aloin, nataloin, or their isomers (10 and 11); whilst compounds assigned as 6'-O-malonylnataloin and isomer (C$_{24}$H$_{24}$O$_{12}$; 18 and 19) were also detected. Anthraquinones and pre-anthraquinones, which are known characteristic constituents of the subterranean Aloes of the former genus Lomatophyllum (now included in the Aloe genus) have been reported (Van Wyk et al., 1995). In this present study, anthraquinones were detected, with qualitative differences observed. A compound (C$_{42}$H$_{42}$O$_{18}$; 20) with m/z 835.2468, assigned as an aloemodin dianthrone di-O-hexoside, was only detected in A. purpurea, A. lomatophylloides and A. tormentorii; whilst a compound (C$_{30}$H$_{28}$O$_{11}$; 21) assigned as either microdontin A or B, or an isomer from the observed [M+H]$^+$ ion (m/z 565.1724) was only detected in A. tormentorii and A. vera. Aloe-emodin dianthrone di-O-hexosides were not detected in A. macra or A. vera, which is in agreement with a previous conclusion that A. macra anthraquinone levels are lower compared to other Aloe species from Mauritius and Réunion Island (Ranghoo-Sanmukhiya et al., 2010). Thus, the non-prevalence of aloe-emodin dianthrone di-O-hexosides in A. macra might be useful to distinguish A. macra from other Mascarene Aloes.

In summary, A. vera could be distinguished from the Mascarene Aloes by the detection of compounds assigned as 2''-O-feruloylaloesin (12) and 7-O-methylaloeresin A (14), which were not detected in the Mascarene Aloes, thus are potentially useful chemotaxonomic markers to distinguish A. vera from the Mascarene Aloes.

3.2. GC-MS analysis of monosaccharides
All species from the genus *Aloe* have succulent leaves but exhibit substantial variation in the thickness of the leaf mesophyll layer; the leaves are barely succulent in some species, whereas the leaf mesophyll is well developed in others (Grace *et al.*, 2013). The present study was conducted to determine the Mascarene *Aloes* monosaccharides content. GC-MS analysis revealed the presence of arabinose, fucose, xylose, mannose and galactose in all the Mascarene *Aloes* and *A. vera* (Table 1). Arabinose was the main monosaccharide in four species, *A. purpurea*, *A. tormentorii*, *A. lomatophylloides* and *Aloe* spp. (WS 98002), with the highest concentration detected in the latter. Mannose was the primary saccharide constituent in *A. macra* (WS 980130) and *A. vera*, while glucose was the predominant monosaccharide in *A. macra* (WV 990110) and *A. purpurea* from Réunion Island. A relatively low concentration of fucose was detected in the *Aloes*, relative to the other monosaccharides detected. The concentration of saccharides was variable among all the species; however following a general trend, a lower concentration of saccharides was observed in the two *A. macra* samples, followed by *A. lomatophylloides*. Both *A. macra* and *A. lomatophylloides* have comparatively thinner leaves and the texture of the inner mesophyll ranges from firm to dry, or slightly mucilaginous, which may account for their restricted monosaccharide content. Previously Govinden-Soulange *et al.* (2017) differentiated the Mascarene *Aloes* from *A. vera* using NMR fingerprinting and PCA analysis of the sugar content of Mascarene *Aloes*.

Table 1. Monosaccharides detected by GC-MS in the Mascarene *Aloes* and *A. vera*
<table>
<thead>
<tr>
<th>Species</th>
<th>Monosaccharide (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ara</td>
</tr>
<tr>
<td>A. purpurea (Mauritius)</td>
<td>204.8</td>
</tr>
<tr>
<td>A. tormentorii</td>
<td>538.57</td>
</tr>
<tr>
<td>A. lomatophylloides</td>
<td>106.31</td>
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<tr>
<td>A. macra WV 990110</td>
<td>29.29</td>
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<td>A. purpurea (Réunion)</td>
<td>100.75</td>
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<td>Aloe spp.</td>
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<tr>
<td>A. vera</td>
<td>130.11</td>
</tr>
</tbody>
</table>

Figure 1. LC-MS chromatograms (positive mode) of the Aloe extracts. Assigned compounds (or isomer): (1) 3-caffeoylquinic acid; (2) aloesin; (3) 4-O-p-coumaroylquinic acid; (4) vitexin/isorvitexin hexoside; (5) isoorientin pentoside; (6) isoorientin; (7) vitexin/isorvitexin hexoside; (8) vitexin/isorvitexin pentoside; (9) vitexin/isorvitexin; (10) and (11) aloin or nataloin isomer; (12) 2''-O-feruloylaloesin; (13) 2''-O-trans-p-coumaroylaloesin; (14) 7-O-methylaloeresin A; (15) aloin B; (16) aloin A; (17) aloeresin A; (18) and (19) malonylnataloin; (20) aloemodin dianthrone di-O-hexoside; (21) microdontin A or B.
3.3. Antimicrobial activity

The use of Aloe species to treat infections is perhaps the most popular medicinal application for the leaves in this genus. The antibacterial activity of the methanolic leaf extracts of the Mascarene Aloes and A. vera, using a micro-dilution assay, is presented in Table 2. The similar MIC values obtained with the Gram-negative and Gram-positive species tested in this study suggest that the Aloe extracts possess a broad antibacterial activity. Several studies have demonstrated the antibacterial activity of isolated anthraquinones from Aloes. The lack of aloe-emodin dianthrone di-O-hexosides in the A. vera sample may account for the low antimicrobial activity observed, which is in agreement with the findings of Ranghoo-Sanmukhiya et al. (2010).

Acne is an inflammatory disease of the sebaceous glands caused by the Gram-positive bacterium Propionibacterium acnes. In the present study, none of the Aloe extracts tested displayed significant inhibitory activity against P. acnes (Table 2) at the highest test concentration (2 mg/mL). This indicates that while the Mascarene Aloes have been used in traditional medicine for skin infections, they appear to lack sufficient efficacy against P. acnes and are therefore unlikely to prove useful in mediating antibacterial effects as a topical application against acne.

3.4. Anti-tyrosinase activity

Hyper-pigmentation of the skin, caused by the over production of melanin, is a common problem that is prevalent in middle aged and elderly people (Mapunya et al., 2011). With the potential use of A. vera in cosmetic products to target hyperpigmentation, and in view of the chemical variation observed between A. vera and the Mascarene Aloes, the tyrosinase inhibitory activity of the Mascarene Aloes was evaluated.

We found that, among all the Aloe extracts tested, only A. macra (WV 990110) showed inhibition of the enzyme, exhibiting 50% tyrosinase inhibition at 0.95 mg/mL, as compared to the positive control kojic acid, 0.003 mg/mL (Table 2). A number of flavonoids such as quercetin, luteolin, apigenin, taxifolin have also been associated with tyrosinase inhibition (Xie et al., 2003; An et al., 2008). Chromones and flavonoids may therefore have contributed to the anti-tyrosinase activity of the A. macra extract in this study, and our results suggest that this
species may be explored further for its ability to inhibit melanin production and potential for its sustainable cultivation for use in cosmetics.

Table 2: Antibacterial and anti-tyrosinase activity of crude extracts of A. vera and Mascarene Aloe species

<table>
<thead>
<tr>
<th>Test samples</th>
<th>Antibacterial (MIC) (mg/mL)</th>
<th>Tyrosinase (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BC</td>
<td>SA</td>
</tr>
<tr>
<td>A. purpurea (Mauritius)</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>A. tormentorii</td>
<td>6.25</td>
<td>3.13</td>
</tr>
<tr>
<td>A. lomatophyloides</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>A. macra (WV 990110)</td>
<td>12.5</td>
<td>3.13</td>
</tr>
<tr>
<td>A. purpurea (Réunion)</td>
<td>6.25</td>
<td>1.56</td>
</tr>
<tr>
<td>A. vera</td>
<td>12.5</td>
<td>25*</td>
</tr>
<tr>
<td>Positive control</td>
<td>3.13</td>
<td>0.78</td>
</tr>
</tbody>
</table>

*: streptomycin; b:tetracycline; c: Kojic acid; *weak activity; -: no activity; ** highest concentration tested: 1 mg/mL; *** highest concentration tested: 2mg/mL. BC: Bacillus cereus; SA: Staphylococcus aureus; P. ac: Propionibacterium acnes; EC: Escherichia coli; PA: Pseudomonas aeruginosa; KP: Klebsiella pneumoniae

3.5. Wound healing properties

The wound healing activity of A. vera has been studied extensively. To investigate the influence of the Aloe extracts on fibroblast migration and proliferation into the wounded monolayer, a scratch assay was employed with HaCaT cells. All extracts were tested at a concentration of 0.1 mg/mL and stimulation rate was expressed as a percentage of the closed area. The closed area with extracts of A. purpurea (Mauritius) was 74.1 %, and for A. vera, 64.2 % (Figure 2) compared to the controls, with 64.9 % and 56.4 % closed area respectively. These results indicate that A. purpurea (Mauritius) and A. vera have modest activity to reduce the damaged area after physical injury. To date, the relationship between various Aloe components and their wound healing effects has not been sufficiently elucidated. Polysaccharides such as acemannan from Aloe species have been associated with wound healing effects (Barbul, 1990; Jia et al., 2008; Chantarawaratit et al., 2013).
Anthraquinone and derivatives are also known to promote wound healing via multiple mechanistic effects, such as anti-inflammatory and antioxidant activities. However, aloe-emodin exhibits contradictory activities on cell growth and proliferation and it has been reported to produce a 2.5-fold increase in DNA synthesis of primary hepatocytes with a corresponding increase in cell growth (Wölfle et al., 1990). In contrast, aloe-emodin results in apoptosis-inducing effects in human squamous cell carcinoma (Lee, 2001; Lee et al., 2001). Such contradictory activities of aloe-emodin may be due to its antioxidant/prooxidant behavior, differences in concentration and the conditions of testing. Aloesin and aloin also appear to accelerate skin wound healing both in vitro and in vivo (Wahedi et al., 2017; Li et al., 2017), and may therefore have contributed to the moderate wound healing activity observed with A. purpurea (Mauritius) and A. vera in this present study, although other constituents potentially responsible for the observed effects require further investigation.

Figure 2. Effect of Mascarene Aloe extracts on the migratory and proliferative activity of HaCaT cells in the scratch assay after 24 h of incubation. Data are expressed as a percentage of closed area relative to the control. * p < 0.05 compared to control. APM: A. purpurea (Mauritius); AT: A. tormentorii; AL: A. lomatophyloides; AM: A. macra; APR: A. purpurea (Réunion); AV: A. vera

3.6. Cytotoxic activity

Cytotoxicity is an important factor to consider when evaluating the therapeutic potential of medicinal plants. The cytotoxic activity of the Mascarene Aloes and A. vera was analysed in vitro against human promyelocytic leukaemia cells (HL60) cells and MRC 5 fibroblast cells. The results showed that only A. purpurea (Réunion; 0.1 mg/mL) elicited a modest toxic effect
against HL60 cells, with toxicity observed at 8.2% (Figure 3). None of the other Aloe extracts produced a toxic effect upon MRC 5 fibroblast cells at a concentration of 0.1 mg/mL. A. purpurea (Réunion), followed by A. purpurea (Mauritius) and A. vera, displayed an apparent proliferative activity upon the MRC 5 fibroblast cells (Figure 3). The presence of anthraquinone derivatives such as aloin A and B, aloesin and aloe-emodin dianthrone dihexosides in the investigated Aloes may have contributed to the toxicity observed with the HL60 cells. Indeed, the toxic activity of aloe-emodin upon HL60 cells has previously been reported, concluding that it shows potential as an anti-cancer lead (Chen et al., 2002).

The MRC-5 human fibroblast cell line is a well-characterised cell culture system to investigate ageing and senescence, and it is known that ROS play a critical role during the course of senescence (Nelson et al., 2012). As some of the Mascarene Aloes have demonstrated antioxidant capability (Govinden-Soulange et al., 2017), and since these were not associated with cytotoxicity or negative effects on the process of ageing and senescence in the MRC-5 cell line, it may be hypothesised that extracts from the Mascarene Aloes have potential for use in topical cosmetic formulations. In addition, the tyrosinase inhibitory activity seen with A. macra may offer an additional advantage for application in cosmetic products.

Figure 3. Treatment of HL60 cells and MRC5 Fibroblast cells with Aloe extracts for 24 h. APM: A. purpurea (Mauritius); AT: A. tormentorii; AL: A. lomatophylloides; AM: A. macra; APR: A. purpurea (Réunion) and AV: A. vera. * p < 0.05 compared to control.
4. Conclusions

Evaluation of the literature provides support for the therapeutic value of the genus *Aloe*, particularly in medicinal applications for 25% of its species (Grace *et al.*, 2009). The present study provides fresh evidence for the potential value of the Mascarene *Aloes*, to validate their traditional uses, whilst also suggesting new uses for both medicinal and cosmetic applications. Some of the species investigated have been shown to have a broader spectrum of antimicrobial activity, particularly *A. purpurea* from Réunion; whereas others show potential for use in cosmetics, considering their previously reported antioxidant capacity, reported to be superior to that of *A. vera* (Govinden-Soulange *et al.*, 2017) and for *A. macra* (WV 990110), which possesses tyrosinase inhibitory activity. *A. purpurea* from Mauritius has been shown to have wound healing activity *in vitro*. Phytochemical studies concluded that species from Réunion Island contain higher concentrations of saccharides compared to other Mascarene *Aloes*, while more than twenty other compounds were characterised in the *Aloe* extracts.

We therefore conclude that the Mascarene *Aloes* are a potential source of new medicinal or cosmetic natural products, whilst further research on these species is justified to unveil their chemical diversity, which is not only relevant for their therapeutic potential, but may also to encourage their sustainable cultivation. The trade of many *Aloe* species is restricted under the Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2015), therefore the results from this study will further advocate the conservation of these Mascarene *Aloes*, which has been a key focus in previous work (Lobine *et al.*, 2015) and which remains essential for the preservation of the floral biodiversity of these Islands.

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