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Medicinal Mascarene *Aloes*: An audit of their phytotherapeutic potential

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ABSTRACT

A phytochemical and biological investigation of the endemic Mascarene *Aloes* (*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 *Aloes* 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 *Aloes* 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 *Aloes* and in *A. vera*. The crude extracts of all *Aloes* 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 *Aloes*, 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.

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 [9,22]. 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 [6]. Whilst the leaves from many other *Aloes* have been documented as traditional medicinal remedies [19], 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

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Aloe species, have been scientifically validated for their medicinal uses. The Mascarene *Aloes ()* include *Aloe tormentorii* and *A. purpurea*, commonly known as 'Mazambron marron', species endemic to Mauritius [5,21]; *Aloe macra* [5,31] endemic to Réunion Island and *A. lomatophylloides* [16] 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 [21]. The leaves of *A. macra* Haw. are used to alleviate minor infections, boils, constipation and as a general healing substance for external use [16], 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 [21]. 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 [9].

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 [33]. 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 [15,27]. In the present report, further studies have been initiated to validate their traditional use and to evaluate their potential as sustainable phytomedicines. 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. lomatophyllloides* 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 WS 990130]; *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*[33]. *A. macra* [WV 990110] and *A. macra* [WS 990130] 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* [WS 990130] 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 980130] 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 × 3 mm, 3 µm Luna C-18 column (Phenomenex, Torrance, CA, USA) using the following 400 µL/min mobile phase gradient of $H_2O/CH_3OH/CH_3CN$ + 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 [34] 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 × *g* for 30 min and the supernatants were dried under nitrogen at 40 °C. 400 µL of methanolic 1 N 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/s. The injector temperature was 250 °C and the initial oven temperature was 140 °C, increasing at 2 °C/min to 180 °C and held at this temperature for 5 min before increasing to 275 °C at 10 °C/min, held for 10 min. 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 [17].

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 [13] was used to determine the minimum inhibitory concentration (MIC) for