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Metabolomic and molecular signatures of Mascarene Aloes using a multidisciplinary approach

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ABSTRACT

In this research a multidisciplinary approach was used to unveil the genetic, metabolic uniqueness and relationships of endemic Mascarene Aloes (*Aloe macra, Aloe purpurea, Aloe tormentorii*) with respect to *Aloe vera*. Nuclear magnetic resonance spectroscopy, DNA sequencing and antioxidant profiles of these Aloes were studied. Principal component analysis following ^{1}H NMR revealed the specificity of the Mascarene Aloes relative to *Aloe vera*. The superior free radical scavenging ability of *A. purpurea, A. macra* and *A. tormentorii* as compared to other Aloes was also unveiled. Phylogenetic analyses of chloroplast genes and ITS region sequences of these Mascarene Aloes were done using maximum parsimony and Bayesian analysis. Mascarene Aloes clustered within one clade separate from *Aloe vera* confirming their relative recent emergence in this genus. Results from this study showed that there is sufficient evidence at the metabolomic and molecular level to distinguish between *Aloe purpurea* from Mauritius and that of Reunion.

1. Introduction

The Mascarene Islands situated in the south west of the Indian Ocean comprise three countries: Reunion, Mauritius and Rodrigues and share a rich indigenous biodiversity. The use of plants to treat various ailments forms a fundamental component of the lifestyle of the Mascarene people. *Aloe tormentorii* (Marais) Newton and Rowley and *Aloe purpurea* Lam. are species endemic to Mauritius (Bosser et al., 1976; Gurib-Fakim, 2003) and are commonly known as 'Mazambron marron' in this country. *Aloe macra* Haw. is endemic to Réunion Island (Bosser et al., 1976; Pailler et al., 2000) and phenotypically resembles *A. purpurea* but it has slender leaves of about 70 × 10 cm and smaller inflorescences (Ranghoo-Sanmukhiya et al., 2010). The leaves of *A. purpurea* are traditionally used to treat cutaneous bacterial infections and boils and as well as an antispasmodic to relieve stomach pains (Gurib Fakim et al., 2003; Govinden-Soulange, 2014; Lobine et al., 2015). *A. macra, A. purpurea* and *A. tormentorii,* in spite of having morphological differences, are often confused with other *Aloe* species particularly with the local Mazambron (*A. vera*/*A. barbadensis* Miller) (Guého, 1988). In this study, a multidisciplinary approach was used to resolve the affinities of the Mascarene Aloes including nuclear magnetic resonance spectroscopy, DNA sequencing and antioxidant profiling.

Chase et al. (2000) were the first to investigate phylogenetic relationships in Asphodelaceae using chloroplast DNA (cpDNA) sequence data and Alooideae were recovered as monophyletic with respect to other taxa studied (Chase et al., 2000). Recently, Grace et al. (2015) investigated the evolutionary history of Aloes and correlated leaves succulence to their medicinal use. Other phylogenetic work within the Asphodelaceae was concentrated on the subfamily Alooideae, using cpDNA sequences and genomic fingerprinting inter simple sequence repeat markers (ISSRs) (Treutlein et al., 2003). Ramdhani et al. (2011) have elucidated the monophyly of *Haworthia* species using both chloroplast trnL-trnF, psbA-trnH and nuclear internal transcribed sequence one (ITSI) markers.

Recent studies using chromatographic techniques have correlated the phytochemical and antimicrobial attributes of *A. macra, A. purpurea* and *A. tormentorii* and validated their use in the folk medicine of the Mascarene Islands and the anthraquinone profile of Mascarene Aloes has also been described (Ranghoo-Sanmukhiya et al., 2010) hence highlighting their prospective antioxidant attributes. Van Wyk et al. (2008) reviewed the various traditional uses of African Aloes highlighting *Aloe ferox* as the most renowned and widely used medicinal plant in Southern Africa. Likewise, several authors have described the antioxidant capacity of the reputed *Aloe vera* (Hu et al., 2003;

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Saritha et al., 2010; Lee et al., 2012; Padmanabhan et al., 2012). The leaf gel of *A. ferox* and *A. greatheadii* var. *davyana* (birtes) have been reported to exhibit antioxidant activities (Loots et al., 2007). Dagne et al. (2000) have reviewed the chemistry and chemotaxonomy of *Aloe* species and described the major classes of compounds present in Aloes. However, the antioxidant attributes of Mascarene Aloes have not yet been reported.

In addition to the above methods, NMR spectroscopic profiles of the Mascarene Aloes were compared in this study. NMR spectroscopy represents a reproducible, wide-spectrum chemical analysis technique and stable in time requiring very simple material preparation (Choi et al., 2004). NMR with PCA has been applied in the metabolic profiling of wines, juices, beers and many plant species (Choi et al., 2005). Jiao et al. (2010) have used ^{1}H NMR spectrometry to validate the purity of *Aloe vera* products and Campestrini et al. (2013) have used ^{13}C and ^{1}H NMR to establish the acetylation pattern of the polysaccharide, glucomannan, present in *Aloe barbadensis* Miller (*A. vera*).

The focus of this study was to determine the suitability of using both chloroplast DNA sequences and nuclear ITS I sequences for deriving the phylogeny of the Mascarene Aloe species. Additionally, the chemosystematics of Aloes from the Mascarene Islands is unveiled using a multidisciplinary approach through the integration of metabolomic and molecular data. The metabolic and genetic signatures of these Aloes are deciphered by using a chemometric approach and DNA sequences thus representing a critical method by which the Mascarene Aloes can be differentiated.

2. Materials and method

2.1. Plant material

Leaves from three year old plants of indigenous Mascarene *Aloe* plants were harvested from the Conservatoire Botanique National de Mascarin, Reunion Island and Mauritius Herbarium garden, MSIRI (Table 1). Samples were immediately lyophilized and ground into a fine powder. Fresh leaves were kept at −40 °C for molecular work. Voucher specimens were deposited at the Mauritius Herbarium.

2.2. NMR-analysis

2.2.1. Extraction and sample preparation

Deuterated methanol CD_3OD (99.9%), water D_2O (99.0%) and Sodium 3-Trimethylsilylpropionate) TMSP, were purchased from Eurisotop, France. The dry powdered plant material (50.0 mg) was treated with 2.0 ml of a 70:30 mixture of CD_3OD and D_2O (buffered at pH 7.2) containing TMS 0.01% in 2 ml Eppendorf tube. The extract was vortexed for 1 min at room temperature and then ultrasonicated for 10 min at room temperature. The mixture was then centrifuged at 13,000 rpm for 10 min and the supernatant was transferred to 2.0 ml microtube. The mixture was centrifuged once again for 1 min at room temperature and the supernatant was then distributed into 5 mm-NMR tubes for measurements.

2.2.2. NMR measurements

^{1}H-NMR spectra were recorded at 300 K on a Bruker Avance 500 MHz NMR spectrometer (Bruker, Karlsruhe, Germany) operating at a proton NMR frequency of 500.13 MHz for ^{1}H, using a triple BBI probe. Deuterated methanol was used as the internal lock. 128 scans of 32 K data points with a spectral width of 10,330 Hz were recorded with the following parameters: pulse width (PW) = 30°, and relaxation delay (RD) = 1.0 s. The acquisition time was 3.17 s. FIDs were Fourier transformed with LB = 0.3 Hz. The resulting spectra were manually phased and calibrated to 0.00, using the internal standard TMSP and topspin software (version 3.9, Bruker). Two replicates were measured for each plant material studied. The optimised ^{1}H-NMR spectra were automatically baseline corrected and reduced to ASCII files using AMIX software (version 3.9, Bruker). Spectral intensities were normalised to total intensity and reduced to integrated regions of equal width (0.04 ppm) corresponding to the region of δ 0.40 – δ 10.00. The regions of δ 4.50 – δ 5.24 and δ 3.30 – δ 3.35 were removed prior to further analysis because of the residual signals of water and methanol, respectively. The matrix size consisted then of 220 variables. Principal component analysis (PCA) was performed with AMIX (version 3.9, Bruker).

2.3. DNA isolation

Genomic DNA extraction was done using a modified CTAB protocol described by Govinden-Soulange et al. (2007).

2.4. PCR amplification

The *trnH-psbA* intergenic spacer, maturase K (*mat*K) and Ribulose bisphosphate carboxylase large chain (*rbcL*) genes that reside on the chloroplast genome and the ITS internal transcribed sequence (ITS) region located on the nuclear genome were utilised for DNA based studies. A region of the *trnH-psbA* gene was amplified using primer pairs trnH (5′-CGCGCATGGTGGATTCACAAATC-3′) and psbA (5-GTTATGCATGAACG TAATGCTC-3′) (Sang et al., 1997). The *matK* region was amplified using primer pairs 1R (5′-ACCCAGTCCATCTGGAAATCTGGTTC-3′) and 3F (5′ CGTACAGTACTTTTGTGTTTACGAG-3′) (CBOL Plant Working Group, 2009). The *rbcL* region was amplified using primer pairs rbcl-N (5′-ATGTCACCACAAACAGARACKAAAGC-3′) and rbcl-1R (5′-GGGTGCCCTAA AGTTCCTCC-3′) (Treutlein et al., 2003). The ITS region was amplified using primer ITS 1 (5′TCCGTAGGTGAACCTGCGG3′) and Chromo 5.8R (5′GATTCTGCAATTCAC3′) (Ramdhani et al., 2011). Each PCR reaction consisted of 4 U of DreamTaq™ DNA Polymerase, 100 mM KCl, 20 mM Tris–HCl pH 8.8, 20 mM $MgCL_2$, 80 mM dNTP, 0.4 mM primer and approximately 20 ng of template DNA. Thirty amplification cycles were performed on a Biorad minicycler. An initial denaturation step was performed at 95 °C for 5 min followed by a denaturation step at 94 °C for 1 min, annealing at 55 °C for 1 min and primer extension at 72 °C for 1 min. The final primer elongation was done for 10 min at 72 °C. PCR products were separated on a 1.5% (*w*/*v*) ethidium bromide-stained agarose gel and the bands were visualised under UV illumination.

2.5. DNA sequencing

PCR amplicons were purified using Fermentas PCR purification kits following the manufacturers' instructions. DNA sequencing reactions

Table 1
List of all specimens collected.

Taxon	Voucher (Herbarium)	Place of collection
Aloe purpurea Lam	MAU 0014447	Mauritius Herbarium garden, MSIRI
Aloe tormentorii (Marais) Newton & Rowley	MAU 0014094	Mauritius Herbarium garden, MSIRI
Aloe lomatophylloides Balf. f	MAU 0014095	Mauritius Herbarium garden, MSIRI
Aloe macra Haw	WS 92–729	Jean Bernard Castillon Residence, Le Tampon, Réunion Island
Aloe purpurea Lam. (Réunion)	WV 99067	Conservatoire Botanique National de Mascarin Réunion Island