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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 ¹H 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 ‘Mazambon marron’ in this country. *Aloe macra* Haw. is endemic to Réunion Island (Bosser et al., 1976; Paillet et al., 2000) and phenotypically resembles *A. purpurea* but it has slender leaves of about 70 × 10 cm and smaller inflorescences (Raghoo-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 Mazambon (*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 Aloioideae 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 Aloioideae, 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 (ITS1) 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 (Raghoo-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 ^1H NMR spectrometry to validate the purity of *Aloe vera* products and Campestrini et al. (2013) have used ^{13}C and ^1H 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) TMS, 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

^1H -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 ^1H , 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 TMS and topspin software (version 3.9, Bruker). Two replicates were measured for each plant material studied. The optimised ^1H -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 (*matK*) and Ribulose biphosphate 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'-CGCGCATGGTGGATTCAAAATC-3') and *psbA* (5-GTTATGCATGAACGTAATGCTC-3') (Sang et al., 1997). The *matK* region was amplified using primer pairs 1R (5'-ACCCAGTCCATCTGAAATCTGGTTC-3') and 3F (5'-CGTACAGTACTTTGTGTTTACGAG-3') (CBOL Plant Working Group, 2009). The *rbcl* region was amplified using primer pairs *rbcl*-N (5'-ATGTCACCACAAACAGARACKAAAGC-3') and *rbcl*-1R (5'-GGGTGCCCTAAAGTTCCTCC-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
<i>Aloe purpurea</i> Lam	MAU 0014447	Mauritius Herbarium garden, MSIRI
<i>Aloe tormentorii</i> (Marais) Newton & Rowley	MAU 0014094	Mauritius Herbarium garden, MSIRI
<i>Aloe lomatophylloides</i> Balf. f	MAU 0014095	Mauritius Herbarium garden, MSIRI
<i>Aloe macra</i> Haw	WS 92–729	Jean Bernard Castillon Residence, Le Tampon, Réunion Island
<i>Aloe purpurea</i> Lam. (Réunion)	WV 99067	Conservatoire Botanique National de Mascarin Réunion Island

were done using a Big Dye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) following the protocol outlined by the manufacturers. Sequencing products were purified by ExoSAP method (Werle et al., 1994). All DNA sequences were determined with an ABI 3500 DNA sequencer (Applied Biosystems) at Inqaba Biotechnical Industries (Pty) Ltd., South Africa.

2.6. Phylogenetic analysis

2.6.1. Sequence editing and alignment

Forward and reverse sequences were assembled and edited using CLC Main Workbench Version 7.6 (<https://www.qiagenbioinformatics.com/>). All sequences were deposited in GenBank (Table 2). DNA sequences were aligned using the online version of MAFFT 5.66 (Katoh et al., 2005) using the iterative refinement method and the following settings: Needleman–Wunsch algorithm active, 2 tree rebuilding steps, 1000 iterations and the program's default values for gap opening and gap extension penalties. No further manual manipulation of the alignment was performed. Sequences from *Aloe*, *Haworthia* and *Gasteria* species were retrieved from the GenBank and used in the phylogenetic analysis (Table 2).

2.6.2. Phylogenetic analyses

Maximum parsimony (MP) and Bayesian inference (BI) methods were used in phylogenetic reconstruction. For each analysis, the cpDNA (trnH-psbA, *matK* and *rbcl*) and nrITS1 sequence matrices were treated separately first and then combined. Congruence of the cpDNA and ITS1 datasets was assessed using the partition homogeneity (ILD) test (Farris et al., 1994) implemented in PAUP* v.4.0b10 (Swofford, 2002) and using 1000 replicates. Maximum parsimony searches were performed with PAUP*. Gaps were treated as missing characters and cladograms were generated using heuristic searches based on 1000 random sequence additions, TBR branch swapping and MULPARS effective. Bootstrap analyses were done using the same settings with a 1000 replicates. Bayesian inference was performed using MrBayes v.3.1.1 (Huelsenbeck and Ronquist, 2001). The nucleotide substitution model that best fit each sequence matrix was determined with MrModeltest v.2.2. (Nylander, 2004) with model selection based on the Akaike information criterion (AIC). The MCMC run in MrBayes was set to 5×10^6 generations with tree sampling every 100th generations. The log-likelihood scores for the sampled tree were analysed to determine the number of generations that could be excluded before stationary has been reached (i.e. the “burn-in” value). Effective sampling size (ESS) values were assessed in Tracer version v1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>) as a further means to determine convergence. The trees generated were viewed in FigTree v.1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>) to obtain tree topologies and the posterior probability values for their nodes.

Table 2
Genbank accession number of *Aloe*, *Haworthia* and *Gasteria* species used in this study.

Species	Accession Number (NCBI)			
	<i>Matk</i>	<i>psbA-trnH</i>	<i>rbcl</i>	<i>ITS</i>
<i>A. arborescens</i>	AY323722	GQ434898	AY323645	AY32368
<i>A. vera</i>	AJ511390	GQ434896	AY323649	AY323684
<i>H. cymbiformis</i>	AY323700	HQ646866	JQ273909	AY323666
<i>H. fasciata</i>	AY323708	HQ646883	AY323629	HQ646935
<i>H. glauca</i>	AY323712	HQ646885	AY323633	HQ646938
<i>H. angustifolia</i>	AJ511372	HQ646849	AJ512295	HQ646908
<i>G. glomerata</i>	JQ024193	JQ024884	AJ512281	JQ025283
Mascarene Aloes				
<i>A. macra</i>	KX270415	KX270423	KX270419	KX270427
<i>A. purpurea</i> (Mauritius)	KX270415	KX270424	KX270420	KX270428
<i>A. tormentorii</i>	KX270415	KX270425	KX270421	KX270429
<i>A. purpurea</i> (Reunion)	KX270415	KX270426	KX270422	KX270430

Table 3
DPPH radical scavenging activity of Mascarene Aloes.

Samples	DPPH radical scavenging activity IC ₅₀ value (mg/ml)*
Standard (Quercetin)	0.110 ± 0.009
<i>A. purpurea</i> (Mauritius)	0.441 ± 0.034
<i>A. tormentorii</i>	0.413 ± 0.014
<i>A. macra</i>	0.389 ± 0.007
<i>A. purpurea</i> (Reunion)	0.334 ± 0.009
<i>A. vera</i>	0.469 ± 0.010

* Mean ± SD of three determinations.

2.7. DPPH radical scavenging assay

Radical scavenging activity of plant extracts against stable 2, 2-diphenyl 2-picrylhydrazyl hydrate (DPPH) was determined using a modified method of Wong et al. (2006). The control was composed of methanol and DPPH solution while blanks contained methanol instead of DPPH solution. Quercetin was used as the standard.

Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

$$\% \text{Inhibition} = [1 - (A_0 - A_1) / A_0] \times 100$$

where:

A_0 absorption of control
 A_1 absorption of test samples

3. Results

3.1. NMR fingerprinting

The chemical profiling of indigenous Mascarene Aloe was performed by ¹H NMR. The ¹H NMR data were subjected to PCA aiming to highlight the metabolic differences among the species. PCA analysis clearly distinguished *Aloe vera* from the Mascarene Aloes bases on their sugar profile (3.5–4.2 ppm) (Fig. 1). Fig. 2 indicates 3 principal clusters: *A. macra*, *A. vera* and *A. purpurea/A. tormentorii* group.

3.2. PCR amplification of the chloroplast and ITS 1 regions

The chloroplast DNA (cpDNA) and ITS 1 regions yielded single amplicons for all taxa. The PCR amplification of the cpDNA yielded amplicons of sizes 1000 bp for *matK*, 700 bp for *psbA-trnH* and 1000 bp for *rbcl*. Smaller amplicons of 500 bp were obtained for the ITS 1 region.

3.3. Phylogenetic reconstruction

Statistics from the MP analysis of individual chloroplast genes, ITS1, combined chloroplast only genes (*matK*, *psbA-trnH* and *rbcl*), and combined chloroplast and ITS datasets are shown in Table 4. Combining the chloroplast genes and ITS yielded a $P = 0.0100$ using the PHT test in PAUP, indicating that some of the genomic regions are phylogenetically incongruent. Through a process of elimination, the *psbA-trnH* gene was discovered causing incongruence in the combined data set. A PHT test of the combined *matK*, *rbcl* and ITS1 sequence matrices yielded a $P = 0.2300$, indicating congruence and a matrix containing these genomic regions were subsequently used in phylogenetic analyses.

Parsimony analysis of the combined dataset yielded 68 most parsimonious trees with tree length = 68 steps, consistency index (CI) = 0.8971 and retention index (RI) = 0.9300 (Fig. 4). Phylogenetic trees generated from parsimony and Bayesian analysis clustered the *Aloe* species in a monophyletic group (bootstrap = 100%, posterior probability (PP) = 1) (Fig. 3). On this tree the Mascarene Aloes formed

Table 4
Statistics from JModel Test and MP analyses obtained from separate and combined datasets.

	<i>matK</i>	<i>psbA-trnH</i>	<i>rbcl</i>	Combined cpDNA	ITS1	Combined cpDNA + ITS1	Combined <i>matK</i> , <i>rbcl</i> & ITS 1
No. of samples	11	11	11	11	11	11	11
No. of bp in alignment	848	556	1078	2482	256	2738	2182
No. of variable characters (%)	27 (3.2)	14 (2.5)	9 (0.8)	50(2.0)	23 (9.0)	73 (2.7)	59(2.7)
No. of PI characters (%)	17 (2.0)	36 (6.5)	7 (0.6)	60 (2.4)	33 (12.9)	93 (3.4)	57 (2.6)
CI, RI, tree length	0.9000, 0.9310, 20	0.7755, 0.7660, 49	1.0, 1.0, 7	0.7711, 0.7957, 83	0.9000, 0.9310, 40	0.8049, 0.8367, 123	0.8971, 0.9300, 68
ILD test P value	–	–	–	0.0500	–	0.0100	0.2300
BI models	TIM1	TPM3uf + G	TPM1uf	<i>matK</i> : TIM1 + G <i>psbA-trnH</i> : TPM3uf + G <i>rbcl</i> : TPM1uf	HKY	<i>matK</i> : TIM1 <i>psbA-trnH</i> : TPM3uf + G <i>rbcl</i> : TPM1uf ITS 1:HKY	<i>matK</i> : TIM1 <i>rbcl</i> : TPM1uf ITS 1:HKY
No. of samples	5	5	5	5	5	5	5
No. of bp in alignment	850	575	1076	2472	249	2472	2147
No. of variable characters (%)	5(0.6)	12 (2.4)	3 (0.3)	20 (0.8)	15(6.0)	35(1.4)	23 (1.1)
No. of PI characters (%)	0 (0)	0 (0)	0 (0)	0 (0)	5(2.0)	5(0.2)	5 (0.2)

a monophyletic group with strong nodal support (bootstrap = 92%, PP = 1). *A. tormentorii* grouped with *A. macra* in a monophyletic clade with 75% bootstrap support and PP = 1. *A. purpurea* from Reunion Island grouped sister with this group with strong support from the Bayesian analysis (PP = 0.98), but was not supported with bootstrap (54%). Contrary to what was expected, *A. purpurea* from Mauritius did not form a monophyletic group with *A. purpurea* from Reunion Island, but branched off separately from the *A. macra*, *A. tormentorii* and *A. purpurea* (from Reunion) clade.

Phylogenetic analysis of the ITS region was conducted to determine the phylogenetic signal of this region in comparison to the chloroplast genes. The MP analysis of the ITS I sequences yielded 39 parsimonious trees with tree length = 40 steps, CI = 0.900 and RI = 0.9310. (Fig. 3). The topology of the trees generated from parsimony and Bayesian analyses reflected the trees obtained from the combined data matrix (Fig. 4).

3.4. Antioxidant assay

Methanolic extracts of the Mascarene *Aloe* species studied show superior free radical scavenging activities (0.334 ± 0.009 to $0.441 \pm$

0.034) ($p < 0.05$) than *A. vera* (0.469 ± 0.010) (Table 3). However, the superior radical scavenging was weaker than the positive control Quercetin with an IC_{50} of 0.110 ± 0.009 . Results from the assay showed that *A. purpurea* (Reunion) exhibited highest radical scavenging potentiality, followed by *A. macra*, *A. tormentorii*, *A. purpurea* (Mauritius) and *A. vera* (reference standard) (Table 3).

4. Discussion

In this study we highlight the uniqueness of the Mascarene Aloes and their phylogenetic relationships with respect to *Aloe vera* and the *Haworthia* species from the Asphodelaceae family. A previous study (Ranghoo-Sanmukhiya et al., 2010) reported the use of RAPD markers to make species delineations and have validated the use of the targeted Aloes in the folk medicine of Mascarene Islands. In this study, we used DNA sequences from chloroplast genes and the ITS region of the nuclear genome, ¹H-NMR fingerprinting and antioxidant assays to characterise the Mascarene Aloes. Results from this study highlighted the distinctiveness of Mascarene Aloes with respect to *Aloe vera*.

Maximum parsimony analysis of the chloroplast genes (tree not shown) showed that genes from this genome and used in this study

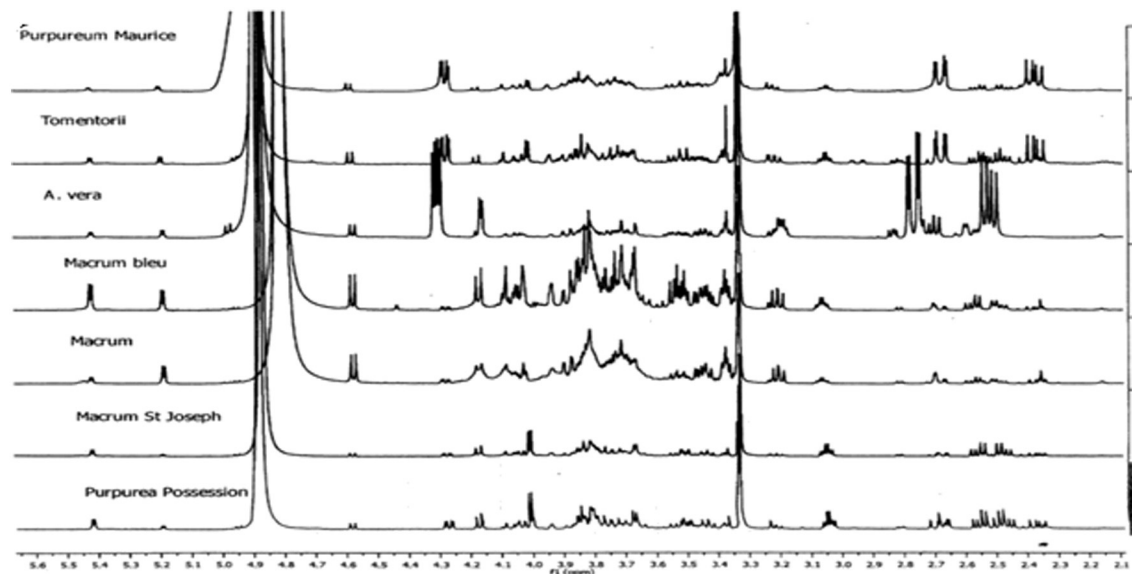


Fig. 1. ¹H NMR profile of Mascarene Aloes. Purpureum Maurice: *Aloe purpurea* Mauritius; Tormentorii: *Aloe tormentorii*; A. vera *Aloe vera*; Macrum Bleu, Macrum, Macrum St Joseph: *Aloe macra*; *Aloe purpurea* Reunion: Purpurea Possession.

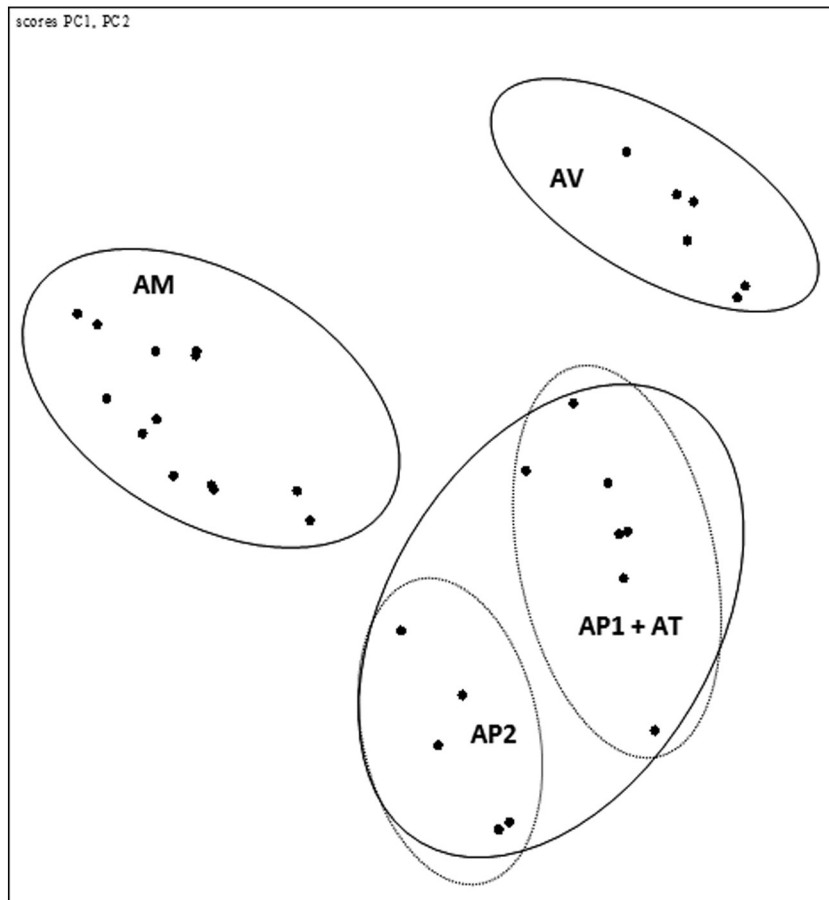


Fig. 2. Score plot of PCA results obtained from all 1H NMR data showing PC1 and PC2. AV: *Aloe vera*; AM: *Aloe macra*, AT: *Aloe tormentorii*, AP1: *Aloe purpurea* Mauritius, AP2: *Aloe purpurea* Reunion.

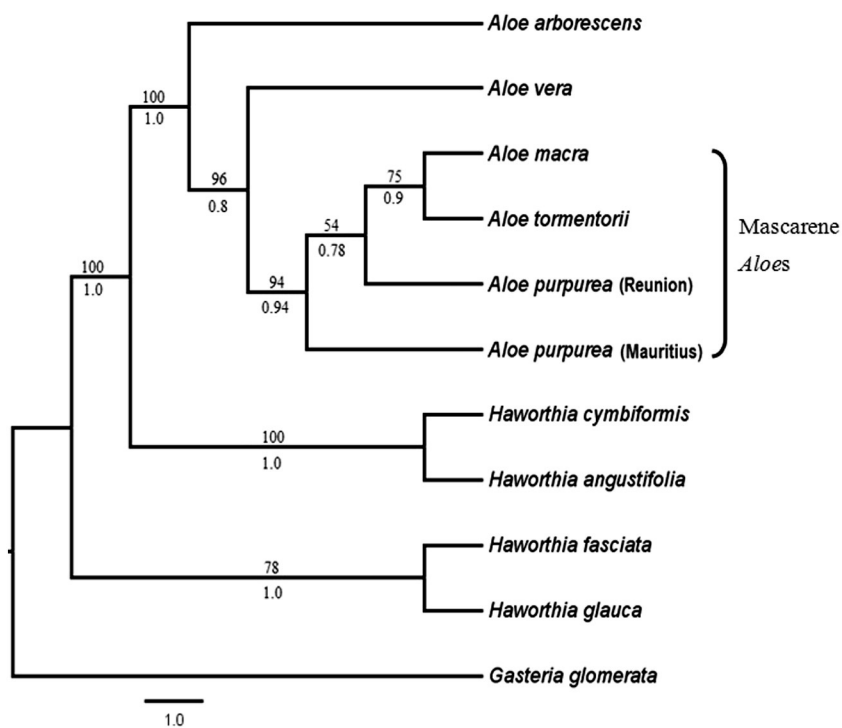


Fig. 3. One of the 39 most parsimonious tree obtained from the maximum parsimony (MP) analyses of the ITS 1 dataset. *Gasteria glomerata* was used as outgroup. Numbers above branches indicate MP bootstrap values, while numbers below the branches indicated the posterior probability values obtained from a Bayesian analysis. Scale bar: Number of steps.

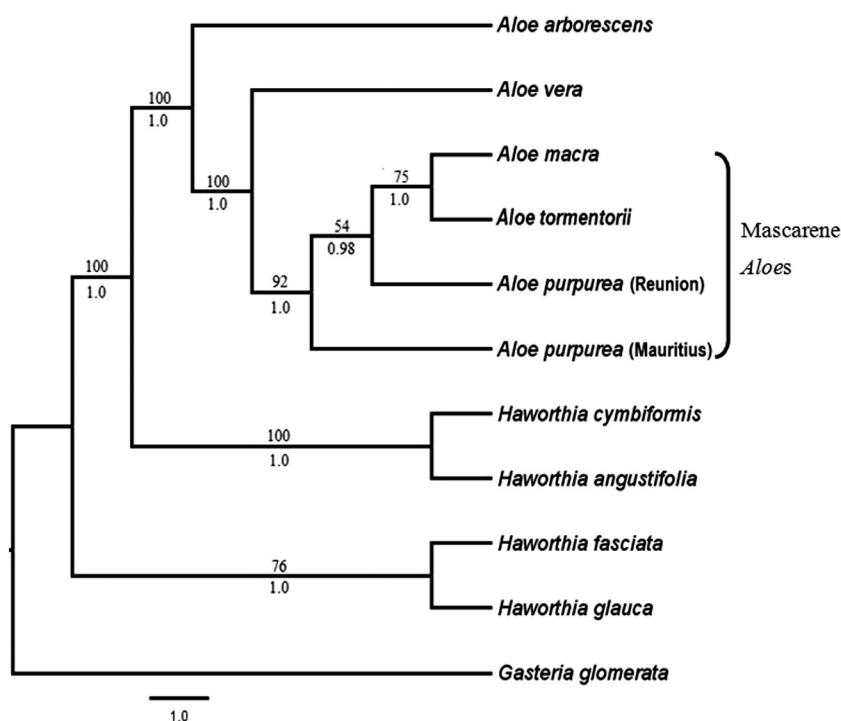


Fig. 4. One of the 68 most parsimonious trees obtained from the maximum parsimony (MP) analyses combined *matK*, *rbcl* and ITS 1 matrix. *Gasteria glomerata* was used as outgroup to root the tree. Numbers above branches are MP bootstrap values, while numbers below the branches are posterior probability values obtained from a Bayesian analysis. Scale bar: Number of steps.

are less informative compared to the ITS I region to elucidate the close relationships which exist among the Mascarene Aloes. Parsimony analysis of the ITS and combined datasets separately revealed a polytomy at the node that is shared by *A. purpurea* from Reunion and Mauritius, respectively, and *A. macra* and *A. tormentorii* formed a monophyletic group. The non-informative characteristic of the chloroplast DNA *rbcl* and *matK* has been previously reported (Gielly and Taberlet, 1994; Muller et al., 2006). However, the variable nature of the *psbA-trnH* intergenic spacer has also been reported previously (Ramdhani et al., 2011), and therefore it was expected that this region would be more variable than the other genes from the chloroplast genome. A combined cpDNA (*psbA-trnH*, *matk* and *rbcl*) and ITS1 phylogeny was thus used to obtain a finer resolution of the taxonomy of the Mascarene Aloes.

In this study the combined dataset including the chloroplast genes and ITS1 yielded a phylogeny that separated most of the Mascarene *Aloe* species with strong support. The trees generated in this study clustered the Mascarene Aloes in a strongly supported monophyletic group. Within this group, *A. tormentorii* formed a monophyletic group with *A. macra* and *A. purpurea* from Reunion Island being a sister species to this group as supported by the Bayesian posterior probability for the node. Interestingly, *A. purpurea* from Reunion Island was placed paraphyletic to *A. purpurea* from Mauritius and basal to *A. macra* and *A. tomentorii*. *A. purpurea* from Reunion Island is morphologically distinct from the *A. purpurea* of Mauritius (Ranghoo-Sanmukhiya et al., 2010) and we postulate that this *Aloe* could possibly be a hybrid of *A. macra* and *A. tormentorii*, formed during the migration of species associated with the history of the Mascarenes described in the introduction. The possibility of *A. purpurea* from Reunion being a hybrid of *A. macra* and *A. tomentorii* has been proposed in a previous study (Ranghoo-Sanmukhiya et al., 2010).

The NMR profile and score plot of PCA results, based on expression of genes clearly differentiated the three indigenous Mascarene species from *A. vera*. The discrimination, in the NMR analysis, between *A. macra* and other *Aloe* species, was correlated with sugar signals (3.5–4.2 ppm), indicating a higher sugar content in *A. macra*. PCA is an

unsupervised clustering method which necessitates no knowledge of the data set and aims at reducing the dimensionality of multivariate data but preserves most of the variance within it (Eriksson et al., 2001). In the present study, the two origins of *A. purpurea* were clearly discriminated in their NMR profile based on their sugar content. The presence of saccharides such as arabinose, galactose, glucose, mannose and xylose were previously reported to occur in *Aloe* species (Davis, 1997). From PCA analysis based on sugar content, *A. purpurea* and *A. tormentorii* from Mauritius seem to have produced the same metabolites probably due to their exposure to similar environmental conditions.

The trends observed with the NMR fingerprinting were also reflected in the antioxidant capacity of the studied Aloes. More similar lower IC₅₀ values were observed between *A. purpurea* and *A. macra* from Reunion than between Mauritian Aloes and *A. vera*. Results from this study showed that *A. purpurea* from Mauritius had less important antioxidant activity than Reunion Island Aloes, the IC₅₀ value was still lower than that of *A. vera*.

The correlation between the polyphenolic profile and antioxidant capacity of natural products is well established (Mihai et al., 2011). The present findings agree with an earlier study (Ranghoo-Sanmukhiya et al., 2010) in which the HPLC and TLC profiles of *A. vera* were completely different from that of the Mascarene Aloes, especially with respect to the flavonoid content. The increasing interest in the search for natural antioxidants has focused on those containing flavonoids and phenolic acids reputed to fight free radical damage (Gulcin et al., 2010). The antioxidant activity of phenolics is mainly due to their redox properties, thereby acting as reducing agents with free radicals scavenging abilities (Mihai et al., 2011). The 2, 2 diphenyl 2 picryl hydrazyl hydrate (DPPH) is a stable and commercially available source of organic nitrogen radicals and is widely used to assess the radical scavenging activity of antioxidant compounds. In a previous study (Ko et al., 1998; Wang et al., 2012), the renowned antioxidant, isoorientin, was only detected in the putative hybrid *A. purpurea* (IC₅₀ 0.334 ± 0.009) and in *A. macra* (IC 50: 0.389 ± 0.007) from Reunion Island and not in the other Mascarene Aloes (Ranghoo-Sanmukhiya

et al., 2010). This could explain the higher antioxidant capacity of the Aloes of Reunion Island as compared to their other Mascarene counterparts.

5. Conclusion

The multidisciplinary approach followed in this study by integrating ¹H NMR/PCA, antioxidant and phylogenetic analysis was complementary and has proven a very promising tool for discriminating between *Aloe* species of common origin. Simple PCA used for the reduction of the NMR data set obtained from the metabolites corroborated with the antioxidant assay. The superior antioxidant capacity of the Mascarene Aloes over *A. vera* is pioneering. Furthermore, phylogenetic studies have confirmed the uniqueness of the Mascarene Aloes relative to *Aloe vera*. Results, based on antioxidant profile from this study validated their medicinal virtues. The Mascarene Aloes warrant further research as they represent an untapped source of natural products with potential for pharmaceutical application.

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References

- Bosser, J., Cadet, T., Gueho, J., Julien, H.R., Marais, W., 1976. Flore des Mascareignes: La Reunion, Maurice, Rodrigues. Mauritius Sugar Industry Research Institute, Port Louis, Mauritius L'Office de la Recherche Scientifique et Technique Outremer: Paris and the Royal Botanic Gardens: Kew.
- Campestrini, L.H., Silveira, J.L.M., Duarte, M.E.R., Koop, H.S., Nosedá, M.D., 2013. NMR and rheological study of *Aloe barbadensis* partially acetylated glucomannan. *Carbohydrate Polymers* 94, 511–519.
- CBOL Plant Working Group, 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America* 106, 12794–12797.
- Chase, M.W., Bruijn, D.E., Cox, A.V., Reeves, G., Rudall, P.J., Johnson, M.A., Eguiarte, L.E., 2000. Phylogenetics of Asphodelaceae (Asparagales): an analysis of plastid *rbcl* and *trn-L-F* DNA sequences. *Annals of Botany* 86, 935–951.
- Choi, H.K., Choi, Y.H., Verberne, M., Lefeber, A.W.M., Erkelens, C., Verpoorte, R., 2004. Metabolic fingerprinting of wild type and transgenic tobacco plants by H NMR and multivariate analysis technique. *Phytochemistry* 65, 857–864.
- Choi, Y.H., Sertic, S., Wilson, E.G., Michopoulos, F., Lefeber, A.W.M., Erkelens, C., Kricun, S.P.D., Verpoorte, R., 2005. Classification of *Ilex* species based on metabolomic fingerprinting using nuclear magnetic resonance and multivariate data analysis. *Journal of Agricultural and Food Chemistry* 53, 1237–1245.
- Dagne, E., Bisrat, D., Viljoen, A., Van-Wyck, V.E., 2000. Chemistry of aloe species. *Current Organic Chemistry* 4, 1055–1078.
- Davis, R.H., 1997. *Aloe Vera: A Scientific Approach*. Vantage Press Inc., New York, pp. 1–321.
- Eriksson, E., Johansson, N., Kettaneh-Wold, N., Wold, S., 2001. Multi- and megavariable data analysis. Principles and Applications, Umetrics Academy, Umeå, 2001 ISBN 91-973730-1-X, 533 pp.
- Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of congruence. *Cladistics* 10, 315–319.
- Gielly, L., Taberlet, P., 1994. The use of chloroplast DNA to resolve plant phylogenies: non-coding versus *rbcl* sequences. *Molecular Biology and Evolution* 11, 769–777.
- Govinden-Soulange, J., 2014. Healing aloes from the Mascarenes. In: Gurib-Fakim, A. (Ed.), *Novel Plant Bioresources: Applications in Food, Medicine and Cosmetics*, First Edition John Wiley & Sons, Ltd., UK, pp. 205–214.
- Govinden-Soulange, J., Ranghoo-Sanmukhiya, M., Seeburrin, S.D., 2007. Tissue culture and RAPD analysis of *Cinnamomum camphora* and *Cinnamomum verum*. *Biotechnology* 6, 239–244.
- Grace, I.M., Buerki, S., Symonds, M.R.E., Forest, F., Van Wyk, A.E., Smith, G.F., Klopper, R.R., Biora, C.S., Demissew, S., Simmonds, M.S.J., Ronsted, N., 2015. Evolutionary history and leaf succulence as explanations for medicinal use in aloes and the global popularity of *Aloe vera*. *BMC Evolutionary Biology* 1–21.
- Guého, J., 1988. *La Végétation de l'île Maurice*. Editions de l'Océan Indien, Rose-Hill, Mauritius.
- Gulcin, I., Huyut, Z., Elmastas, M., Aboul-Enein, H.Y., 2010. Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry* 3, 43–53.
- Gurib-Fakim, A., 2003. An Illustrated Guide to the Flora of Mauritius and the Indian Ocean Islands. Baie du Tombeau, Mauritius, Caractère Ltée.
- Hu, Y., Xu, J., Hu, Q., 2003. Evaluation of antioxidant potential of *Aloe vera* (*Aloe barbadensis* Miller) extracts. *Journal of Agricultural and Food Chemistry* 51, 7788–7791.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17, 754–755.
- Jiao, P., Jia, Q., Randel, J., Diehl, B., Weaver, S., Milligan, G., 2010. Quantitative ¹H-NMR spectrometry method for quality control of *Aloe vera* products. *Journal of AOAC International* 93, 842–848.
- Katoh, K., Kuma, K., Toh, H., Miyata, T., 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* 33, 511–518.
- Ko, F.N., Chu, C.C., Lin, C.N., Chang, C.C., Teng, C.M., 1998. Isoorientin-6''-O-glucoside, a water-soluble antioxidant isolated from *Gentiana arisanensis*. *Acta Biochimica et Biophysica* 1389, 81–90.
- Lee, S., Do, S.G., Kim, S.Y., Kim, J., Lee, C.H., 2012. Mass spectrometry-bases metabolite profiling and antioxidant activity of *Aloe vera* (*Aloe barbadensis* Miller) in different growth stages. *Journal of Agricultural and Food Chemistry* 60, 11222–11228.
- Lobine, M., Govinden-Soulange, J., Ranghoo-Sanmukhiya, V.M., Lavergne, C., 2015. A tissue culture strategy towards the rescue of endangered Mascarene Aloes. *ARPN Journal of Agricultural and Biological Science* 10, 28–38.
- Loots, D.T., Van der Westhuizen, F.H., Botes, L., 2007. *Aloe ferox* leaf gel phytochemical content, antioxidant capacity and possible health benefits. *Journal of Agricultural and Food Chemistry* 55, 6891–6896.
- Mihai, C.M., Marghitas, A.L., Dezmirian, D.S., Barnitui, L., 2011. Correlation between polyphenolic profile and antioxidant activity of Propolis from Transylvania. *Animal Science and Biotechnologies* 44, 100–103.
- Muller, K.F., Borsch, T., Hilu, K.W., 2006. Phylogenetic utility of rapidly evolving DNA at high taxonomic levels: contrasting *matK*, *trnT-F* and *rbcl* in basal angiosperms. *Molecular Phylogenetics and Evolution* 41, 99–117.
- Nylander, J.A.A., 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Padmanabhan, P., Suresh, N., Jangle, S.M., 2012. *In-vitro* antioxidant potential of a herbal preparation containing four selected medicinal plants. *Journal of Krishna Institute of Medical Sciences University* 1, 52–63.
- Pailler, T., Picot, F., Lavergne, C., Strasberg, D., 2000. Statut démographique et biologie de la reproduction d'une espèce endémique menacée de l'île de La Réunion: *Lomatophyllum macrum* (Haw.) Salm-Dyck (Liliacées). *Revue Écologie (Terre et Vie)* 55, 201–214.
- Ramdhani, S., Barker, N.P., Cowling, R.P., 2011. Revisiting monophyly in *Haworthia duvali* (Asphodelaceae): incongruence, hybridization and contemporary speciation. *Taxon* 60, 1001–1014.
- Ranghoo-Sanmukhiya, V.M., Govinden-Soulange, J., Lavergne, C., Khoyratty, S., Da Silva, D., Frederich, M., Kodja, H., 2010. Molecular biology, phytochemistry and bioactivity of three endemic *Aloe* species from Mauritius and Réunion Islands. *Phytochemical Analysis* 21, 566–574.
- Sang, T., Crawford, D., Stuessy, T., 1997. Chloroplast DNA phylogeny, reticulate evolution and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84, 11120–11136.
- Saritha, V., Anilakumar, K.R., Khanum, F., 2010. Antioxidant and antibacterial activity of *Aloe vera* gel extracts. *International Journal of Pharmaceutical and Biological Archives* 1, 376–384.
- Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.
- Treutlein, J., Smith, G.F., Van Wyk, B.E., Wink, M., 2003. Evidence for the polyphyly of *Haworthia* (Asphodelaceae subfamily Alooideae; Asparagales) inferred from nucleotide sequences of *rbcl*, *matK*, *ITS1* and genomic fingerprinting with ISSR-PCR. *Plant Biology* 5, 513–521.
- Van Wyk, B.E., de Wet, H., Van Heerden, F.R., 2008. An ethnobotanical survey of medicinal plants in the southeastern Karoo, South Africa. *South African Journal of Botany* 74, 696–704.
- Wang, J., Yue, Y.D., Tang, F., Sun, J., 2012. TLC screening for antioxidant activity of extracts from fifteen bamboo species and identification of antioxidant flavone glycosides from leaves of *Bambusa textilis* McClure. *Molecules* 19, 12297–122311.
- Werle, E., Schneider, C., Renner, M., Volker, M., Fiehn, W., 1994. Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research* 22, 4354–4355.
- Wong, S.P., Leong, L.P., Koh, J.H.W., 2006. Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry* 99 (4), 775–783.