Bio-prospecting endemic Mascarene *Aloes* for potential neuroprotectants

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Abstract

The Mascarene Aloes are used in the traditional pharmacopoeia against various ailments including cutaneous diseases and as antispasmodics. Scientific evidence to support these claims is non-existent and mainly based on the scientific repute of *A. vera*. The antioxidant profile of methanolic leaf extracts 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. was studied using the Total Antioxidant Capacity, Copper Equivalent and Superoxide Dismutase assays. In vitro cytotoxicity was evaluated on CAD (Cath. - differentiated) neuronal cells by the MTT (methyl tetrazolium) assay and the neuroprotective profile was assessed using hydrogen peroxide-induced neurotoxicity with the CAD cells. The aloin and vitexin content were determined by High-performance liquid chromatography with diode-array detection . *A. purpurea* had the highest aloin content (546.6 nmol/g), while *A. tormentorii* had the highest vitexin content (67.3 nmol/g). *A. macra* (concentration < 0.1 mg/ml) elicited a 10% cytotoxicity effect on CAD cells while other Mascarene *Aloe*s were not cytotoxic. This study validates the antioxidant and neuroprotective potential of Mascarene *Aloe*s focusing on their aloin and vitexin content which are also present in other reputed medicinal *Aloe*s.

Keywords: Mascarene Aloes, antioxidant, neuroprotection, aloin, vitexin.

Introduction

The Mascarene *Aloe*s are comprised of *A. tormentorii* (Marais) L.E Newton & G.D Rowley and *A. purpurea* Lam. which are endemic to Mauritius (Marais *et al.,* 1978; Gurib-Fakim, 2003), as well as *A. macra* Haw. endemic to Réunion Island and *A. lomatophylloides* Balf.f native to Rodrigues (Bosser *et al.,* 1976; Marais *et al.,* 1978; Pailler *et al.,* 2000). *A. purpurea* from Reunion has been reported as being a putative hybrid of *A. macra* and *A. tormentorii* (Ranghoo-Sanmukhiya *et al.,* 2010). The indigenous Mascarene *Aloe*s are extensively used in the Mauritian pharmacopoeia to treat cutaneous bacterial infections and boils, and as antispasmodic medications to relieve stomach pains (Gurib-Fakim, 2003). Various extracts of *Aloe* species are traditionally and commercially used for both cosmetic and medicinal purposes with very little scientific evidence to support these claims, since they are mostly based on research performed on *A. vera* (L.) Burm. f.

Mauritius is experiencing a high prevalence of chronic diseases of the circulatory system and non-communicable diseases among its population. Biochemical, physiological and pharmacological data have revealed that oxidative stress is implicated in the pathology of many diseases, for example cardiovascular tissue injury (Neergheen *et al.,* 2010), diabetes and dementia (Williams *et al.,* 2011; Howes and Simmonds, 2014). There has been an increased interest and engagement of researchers in evaluating local herbs and medicinal plants in the quest for novel sources of antioxidants and neuroprotectants.

The anthraquinone profiles of Mascarene *Aloe*s have been previously described (Ranghoo-Sanmukhiya *et al*., 2010. In addition several authors have described the anti-oxidant capacity of *A. vera* (Hu *et al.,* 2003; Saada *et al.,* 2003; Saritha *et al.,* 2010; Lee *et al.,* 2012; Padmanabhan and Jangle, 2012).

Several plants already have a proven track record for central nervous system activities, including lemon balm (*Melissa officinalis* L.), lavender *(Lavandula angustifolia* Mill.), ginseng (*Panax ginseng* C.A.Mey.), saffron (*Crocus sativus* L.) and valerian (*Valeriana officinalis* L.) (Abuhamdah and Chazot, 2008; Perry and Howes, 2010) and it is possible that there are further such neuroactives still to be found from nature. A survey carried out by (Grace *et al.,* 2008) referred to the traditional use of an *Aloe* species against a nervous system disorder namely the use of a leaf decoction of *Aloe asperifolia* A. Berger as an ailment for epilepsy. *A. vera* has also been reported to be a therapeutic against Alzheimer's disease and depression, glaucoma,

among others (Foster *et al.*, 2011). Such traditional uses indicate prospective antioxidant and neuroprotective properties in the *Aloe* family of plants. However, to date, the neuroprotective attributes of Mascarene *Aloes* have not yet been reported. There are many challenges with the use of plants for medicinal applications and a major issue is the correct authentication of species medicinally, whilst ensuring that the correct plant part is also used. Furthermore, it is important to chemically characterise the plant preparations used pharmacologically and clinically, since considerable variation can occur in phytochemical profiles. Whilst these issues have been addressed for many herbal medicine preparations (Howes and Simmonds, 2015), there remain many issues with the identity and composition of plant preparations used as medicines. An additional concern is the use or overexploitation of species that are endangered. This is a particular issue for the genus *Aloe*, and species are included in the Appendices for the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2015) and the International Union for Conservation of Nature (IUCN, 2015) Red List of Threatened Species.

This study aims to evaluate the antioxidant activity, cytotoxicity and neuroprotective properties of Mascarene *Aloes* in an attempt to validate, for the first time, their potential as a source therapeutics for neuropharmacological applications (as well as epilepsy), setting the scene for their future sustainable cultivation and use.

Material and Methods

Plant material

Leaves from five-year old plants of *A. purpurea*, *A. tormentorii*, *A. lomatophylloides*, *A. macra* and *A. vera* were obtained from the National Parks and Conservation Service (Mauritius) and Mauritius Herbarium garden, MSIRI. 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; *A. purpurea* (originating from 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. Genetic data (not shown) and chemical profiles were determined to support the botanical identification of the respective *Aloe* species (Govinden-Soulange et *al.,* 2017).

Methods

Metabolite extraction

Lyophilised leaf samples (100 mg) of each Mascarene *Aloe* species were suspended in 10 mL of cold 70% (v/v) methanol, heated under reflux at 60 ºC for 1 hr, sonicated for 15 min and centrifuged for 10 min at 5000 resolutions per min (rpm). The supernatant was filtered (0.45 mm filter) and analysed. For bioassays, the extracts were concentrated by leaving to air dry and the residues were resuspended in 10% (v/v) ethanol and stored at -20°C in aliquots. For all the experiments, dilutions of extracts were performed fresh using a starting concentration of 1mg/ml on the day of the bioassay.

High Performance-Liquid Chromatography (HPLC) Analysis

The HPLC analyses were performed using a Waters Acquity H-Class UPLC system. The HPLC operating conditions were as follows: The column was a Waters Acquity BEH C18 (1.7 µm, 100 mm x 2.1mm) and the solvent system constituted of (A) 10 mM ammonium phosphate (pH 3.0) with methanesulfonic acid and (B) acetonitrile at a flow rate of 0.4 mL/min; the column temperature was 40°C. Initial mobile phase conditions were 5% B, increased to 15% B over 3 min, with a linear increase to 45% B after 12 min. The column was washed with 100% B for 1 min and allowed to re-equilibrate to the starting conditions, giving a run time of 20 min. Detection was achieved by diode array detector and data were extracted at 260 and 340 nm. Data analysis was performed using Empower 3 software (Waters). The HPLC profiles of *A. purpurea* (Mauritius and Réunion), *A. tormentorii*, *A. lomatophylloides* and *A. macra* were compared with that of *A. vera*. Compounds were assigned by comparison with the reference compounds vitexin (AApin Chemicals Limited, UK) and aloin (AApin Chemicals Limited, UK), and by comparison with published UV spectra (Da Graça Campos and Markham, 2007). Calibration curves were constructed at a concentration ranging from 0.1mg to 10mg/ml following HPLC analysis (as described above) of the reference compounds (aloin and vitexin) to determine the quantities of these compounds in each of the *Aloe* samples, based on peak area.

Total Antioxidant Capacity (TAC) Assay

The OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit (Cell Biolabs, USA) measures the total antioxidant capacity of biomolecules from a variety of samples via a SET mechanism. In the presence of anti-oxidants, copper (II) is reduced to copper(I). In turn, the copper(I) ions react with a chromogen to produce a colour with maximum absorbance at 490nm. Various

concentrations of *Aloe* crude extracts (0.1, 0.01, 0.001 and 0.0001 mg/ml) were evaluated for the total anti-oxidant capacity. The anti-oxidant capacity is determined by comparison with uric acid standards and expressed in millimoles.

Total anti-oxidant capacity (Trolox) assay

This assay measures both reactive oxygen and nitrogen species. The Cayman's (Cayman Chemical Company, USA) anti-oxidant assay relies on the ability of anti-oxidant to inhibit oxidation of ABTS and is quantified as millimolar TROLOX equivalents. The amount of ABTS+ is monitored by absorbance at 405nm. The tested concentrations of the *Aloe* extracts are 0.1, 0.01, 0.001 and 0.0001 mg/ml, respectively.

CAD cell culture

CAD (Cath.-a-differentiated) cultures were grown at 37°C and in 5% CO_2 on 75 cm^2 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 6–7 days at a 1:4 dilution (Abuhamdah *et al.*, 2015).

Superoxide Dimutase Assay on CAD cells

Undifferentiated and 6 days differentiated CAD cells seeded in 6-well plates were treated with 0.1 mg/ml extract for 24 h and incubated at 37°C and 5% CO2. After centrifugation at 200 × g for 5 min at 4 °C, the pellet was re-suspended in 100µl of 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.2 containing 1mM ethylene glycol tetraacetic acid (EGTA), 210 mM Mannitol and 70 mM sucrose and frozen until further use. The supernatant was evaluated for SOD1 activity using a SOD activity assay kit (Cayman Chemicals Inc., Ann Arbor, Michigan) according to the manufacturer's protocol. Nitro blue tetrazolium (NBT) salt was used to quantify superoxide radicals generated by xanthine oxidase and hypoxanthine. All treatments were performed in triplicates.

Treatment of cell cultures with hydrogen peroxide and *Aloe* extracts

Hydrogen peroxide (H_2O_2)-induced insult with a series of increasing concentrations was performed to determine the dose-dependent effects on CAD cell survival as described by (Abuhamdah *et al.*, 2015). A series of concentrations of H_2O_2 were prepared from a fresh stock (2500µm) into Dulbecco's modified eagles' medium DMEM/F-12 Media - GlutaMAX™-I

supplemented with 10% FBS to achieve final concentrations of 250 µM, 200 µM, 150 µM, 100 µM and 50 µM. Subconfluent cultures (70–80%) were dislodged by gentle pipetting; transferred and centrifuged at 200 × g for 5 min at 4 °C. The pellet was then re-suspended in DMEM/F-12 Media GlutaMAX-I supplemented with 10% FBS and immediately plated in 24-well plates and incubated at 37°C and 5% CO_2. After 24 h, the media were replaced with media containing the different H_2O_2 concentrations prepared earlier and grown in culture for further 24 h after which an 3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay was performed. The effect of *Aloe* extracts concentrations was initially investigated by applying different concentrations (0.0001, 0.001, 0.01, 0.1mg/ml) for 24h to CAD cell cultures. The cell viability was assayed using a standard MTT assay (Freshney, 2001; Abuhamdah *et al.*, 2015) .

Preconditioning and Neuroprotection study

Survival of CAD cells was assessed after 24 h pretreatment with *Aloe* extracts (0.01 and 0.1 mg/ml (w/v) followed by 24 h post-treatment with 250 µm H_2O_2 (i.e. *Aloe* extracts containing media removed before addition of H_2O_2 - preconditioning protocol). A second set of experiments were performed when the *Aloe* extracts containing media was not removed prior to exposure to the H_2O_2 – neuroprotection method). All the treatments were performed in triplicates.

MTT Cell Proliferation Assay

The tetrazolium dye MTT (Sigma, UK) was used to assess cell viability as described by Abuhamdah *et al.,* (2015). 50 microliters Phosphate buffered saline (PBS) (PBS) (136.9 mm 2.68 mm KCl, 4.3 mm Na_2HPO_4, 1.4 mm KH_2PO_4, pH 7.4) containing a final concentration of 5 mg/ml MTT was added to the cultures and incubated at 37°C and in 5% CO_2 for 2.5 h. The MTT containing medium was then removed and the surface of the wells was rinsed gently with 300 µl PBS before the application of 250 µL isopropanol. The optical density of 100 µL samples was spectrophotometrically read at 595 nm (Thermo Labsystems Multiskan Ascent, V1.3).

Statistical Analysis

All the experiments were performed in replicates (n= 3 - 4) and statistical analysis was carried out using one-way ANOVA as the data was normal. Tukey's test was performed using SPSS 16.0. The values of $p < 0.05$ were considered to be statistically significant.

Results

HPLC analysis

Evaluation of the aloin content showed that *A. purpurea* originating from Réunion Island was approximately three-fold higher in aloin content (546.6 nmol/g) compared to the other *Aloe* species, except for *A. lomatophylloides*, in which the aloin content was almost ten-fold lower and *A. macra*, in which aloin was not detected. Vitexin was detected in all the *Aloe* species under study, with the highest concentration in *A. tormentorii* (67.3 nmol/g) and the lowest (12.6 nmol/g) in *A. vera* (Table 1).

Antioxidant capacity of the *Aloe* extracts

The antioxidant activities of the methanolic *Aloe* extracts based on Trolox and TAC (copper reducing equivalent) are shown in Table 2. The scavenging potential of the *Aloe* extracts assayed by the TEAC method varied between 0.35mM and 0.55mM compared to Trolox equivalents, with *A. vera* exhibiting the highest activity (0.55 mM), followed by *A. lomatophylloides*. *A. purpurea* (Réunion Island) and *A. macra* were characterised with high copper-reducing activity with a value of 0.643 ± 0.025 mM and 0.456± 0.033 mM respectively, compared to *A. tormentorii*, *A. purpurea* (Mauritius), *A. lomatophylloides* and *A. vera*, which showed lower reducing potencies, ranging from 0.311 to 0.117 mM.

Superoxide dimutase assay

One unit (U) of activity of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Treatment with 0.1 mg/ml of the *Aloe*s extracts increased the SOD activities in the CAD cells (Figure 2). *Aloe purpurea* and *A. tormentorii* moderately increased the SOD activity by 26.6% and 15.4 % in 1 day-treated differentiated cells and 22.5% and 34.3 % in 6 days-treated differentiated cells. For *A. vera*, a higher increase (31.6 %) was observed in 1 day treated differentiated cells as compared to 6 days-treated differentiated cells. A low increase in SOD activity, varying from 5.3% to 6.3 % was observed in undifferentiated cells for all the *Aloe* extracts.

Neuroprotective activity of *Aloe* extracts v/s H_2O_2- induced neurotoxicity in CAD cells

Using the preconditioning protocol, no significant protection against H_2O_2-induced cytotoxicity was achieved with the *Aloe* extracts tested (not shown), but using the neuroprotection protocol, where the *Aloe* extracts were present throughout the exposure to H_2O_2, dose-dependent neuroprotection was observed. Interestingly, the *A. macra* extract elicited a significant delayed cytotoxicity (not observed at 24h, Figure 3b) over the extended 48 h period, which is comparable to the level of cytotoxicity elicited by 24h H_2O_2 alone (Figure 3), and neuroprotection was not observed (0.01 and 0.1 mg/ml). In contrast, the other *Aloe* extracts elicited differential significant levels of neuroprotection (Figure 4), ranging from 30-97%.

Discussion

Ever since Mauritius has been colonized, many exotic plants have been used for their various medicinal properties. Surveys have revealed that indigenous and endemic species have gradually started to become incorporated in the Mauritian Pharmacopeia for various uses. Mascarene Islands harbour a rich angiosperm flora with Mauritius leading with 273 out of 691 being single island endemics and 150 being Mascarene endemics (Baider *et al.,* 2016). Several studies have been carried out to evaluate the antioxidant potential of *Aloe* species. *A. ferox,* a species indigenous to southern Africa has been reported to have an EC_{50} of 10 µg/ml (Fawole *et al.,* 2010) and 86 µg/ml (Wintola, *et al.,* 2011) for methanol extracts in the DPPH assay. In a study carried out by Yoo *et al.,* (2008), *A. saponaria* (Aiton) Haw. (a synonym for *A. maculata* subsp. *maculata*) exhibited a potential radical scavenging activity with an IC_{50} value of 85 µg/ml. The leaf gel of *A. greatheadii* var. *davyana*, native to South Africa has also been shown to exhibit antioxidant activities (Loots *et al.,* 2007). Furthermore, Asamenew *et al.,* (2011) reported the antioxidant activity of the latex from *A. harlana* Reynolds and its two constituents; an anthrone (aloin) and a chromone (7-*O*- methylaloeresin A). According to a report by Tian and Hua, (2005), aloin and aloe-emodin which are major components in some *Aloe* species exhibited antioxidant or pro-oxidant effects on plasmid DNA.

In this study, three different antioxidant assays, which included evaluation for neuroprotection, were used in order to provide a comprehensive prediction of the antioxidant efficacy of the tested *Aloe* extracts, primarily because the mechanism of action of naturally occurring anti-oxidants can be diverse. The results revealed that all the *Aloe* species investigated displayed

significant free radical scavenging and reducing activities with a similar range of potencies (Table 2). As aloin has been previously reported for its antioxidant activity (Tian and Hua, 2005), the high aloin content in *A. purpurea* (Réunion) may account for the high antioxidant potency observed and possible synergistic effect with other phytochemicals is not to be excluded. SOD being an effective defence against ROS- mediated oxidative damage, catalyses the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) that can be degraded into H_2O and O_2 by catalase. We evaluated the influence of the selected *Aloe*s extracts on the level of serum SOD activity in CAD cells. All samples (0.1 mg/ml) increase the SOD activity in the CAD with highest rises in 1 day treated differentiated cells for *A. purpurea* and *A. tormentorii* and in 6 days treated differentiated cells for *A. vera.* The differences of activity among the different Mauritian *Aloe*s are probably due to their different antioxidative mechanisms. A study carried by El-Shemy *et al.,* (2009) has revealed a significant increase in serum SOD activity of EAC tumor-transplanted experimental animals, in response to the *A. vera* isolated compounds barbaloin, aloe-emodin and aloesin. Yu *et al.,* (2009) reported that *A. vera* polysaccharides are effective at enhancing SOD activity in the plasma and polysaccharides present in *A. vera* have been associated with many of phytotherapeutic properties (Grace *et al*., 2013; Reynolds and Dweck, 1999; Ni and Tizard, 2004) Henceforth, it can be hypothesised that along with aloin, there are probably other constituents such as polysaccharides and flavonoids acting synergistically to exhibit the elevated SOD activity in the neuronal cells. Our results further support the view that the medicinal *Aloe*s are promising sources of potential antioxidants.

Interestingly, despite this similarity in antioxidant potency, the *Aloe* species displayed a marked variation in their neuroprotective properties with *A. vera* and *A. lomatophylloides* being the most and least effective, respectively, which correlated with having the highest and lowest aloin: vitexin ratio (Table 1). *A. vera*, in which the lowest vitexin concentration was observed compared to the other species investigated, displayed the most potent neuroprotective effect in this study. It is, therefore, postulated that the neuroprotection observed could be due to the presence of other chemical constituents. A range of phytochemicals from various other plant species have been associated with antioxidant properties and are suggested to modulate factors associated with neurodegeneration. These have been investigated for their effects on cognitive functions and mechanisms associated with dementia pathology. Such phytochemicals are chemically diverse and evidence to support their therapeutic relevance for CNS disorders is variable, although emerging data are promising (Williams *et al.,* 2011). It is in this context that

we report and compare the antioxidant and neuroprotective potency of the selected Mascarene *Aloe*s.

Earlier literature described some of the effects of *A. vera* on neuronal activity. Wang *et al.,* (2010) reported that *A. vera* at a concentration of 10mg/L had protective effects on mitochondria integrity of PC12 neuronal cells and rat brain. Parihar *et al.,* (2004) stated that *A. vera* improves antioxidant activity within the hippocampus and cerebral cortex leading to improvement of motor and memory behavioural tasks in diabetic mice. Moreover, a recent study reported that *A. vera* leaf (extract) powder possessed significant antioxidant activity anti-epileptic (Rathor, 2014), consistent with a traditional *Aloe* use. Since vitexin has previously been reported as neuroprotective (Chandrashekara and Shakarad, 2011; Abbasi *et al.,* 2012; Yang *et al.,* 2014), the low concentration detected in *A. vera* (compared to the other *Aloe* species) suggests that other constituents of *A. vera* may also be important to mediate the neuroprotective effects. Furthermore, with the exception of *A. vera,* there are limited published data on the chemical constituents of the studied *Aloe* species; thus this study reports novel chemical data for these species, which may be correlated with their pharmacological profiles. It should also be noted that from a conservation perspective, the discovery that *A. vera* showed the highest potency for neuroprotection is of particular relevance to investigate further for its pharmacological potential for neurodegenerative diseases, since this species is not regulated by CITES (2015).

In this study, *A. tormentorii* and *A. purpurea* (Mauritius) Réunion also showed neuroprotective characteristics, ranging from approximately 59.6 % to 74%, representing a promising source of compounds with potential relevance for use in a range of neurological diseases (Table A.1). Interestingly, *A. macra* displayed the most notable differences in the observed pharmacological properties, including delayed cytotoxic effects and no detectable neuroprotection across the range of concentrations tested (0.001, 0.01, 0.01 and 0.1 mg/ml). *A. macra and A. vera* are often misidentified in the field and, subsequently, may be traded or used interchangeably by some cosmetic companies in their products. These present results highlight the importance of correct identification of medicinal plants as these species clearly display very different neuropharmacological effects, with *A. macra* being potentially hazardous.

Ranghoo-Sanmukhiya *et al.,* (2010) reported that *A. tormentorii* and *A. purpurea* from Mauritius were richer in anthraquinones than *A. macra* and that *A. vera* was almost four-fold

richer in anthraquinones than other Mascarene *Aloe* species. Vitexin was not detected in any of the Mascarene *Aloe* species in the latter study. Differences in chemical components between the previous report and this study may be due to many factors such as the site of plant collection, environmental factors during cultivation, age of the plants and the extraction protocols. *A. tormentorii* and *A. purpurea* (Mauritius and Réunion) elicited differential significant levels of neuroprotection with a percentage of 74.0, 58.3 and 59.6 respectively. The evaluation of the aloin content revealed that *A. purpurea* from Réunion Island is three-fold richer in aloin compared to the other *Aloes* studied.

Conclusion

This study demonstrated that the endemic Mascarene *Aloes* (*Aloe lomatophylloides, A. macra, A. purpurea and A. tormentorii)* possess antioxidant and significant neuroprotective activities, all of which may be of relevance for the development of new neurodegenerative disease therapeutics. HPLC results from the above study confirmed the presence of aloin and which is a major compound reported in many Aloes. However as Aloes are well known for harbouring other important phytochemicals, it can be postulated that along with aloin and vitexin, there could be other constituents acting synergistically to exhibit the potent bioactivity of these Mascarene Aloes. Future research will endeavour at elucidating these phytochemicals. However, a sustainable source of these threatened endemics is critical for further pharmacological studies. The use of plants for medicinal applications must therefore be underpinned by scientific evidence for their use without undermining the conservation of biodiversity.

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References

1. Abbasi E, Nassiri-Asl M, Shafeei M, Sheikhi M. 2012. Neuroprotective effects of vitexin, a flavonoid, on pentylenetetrazole-induced seizure in rats. *Chemical Biology & Drug Design* **80(2)**: 274-278.
2. Abuhamdah S, Chazot, PL. 2008. Lemon balm and lavender herbal essential oils; Old and new ways to treat emotional disorders. *Current Anaesthesia and Critical Care* **19**: 221-226.
3. Abuhamdah S, Abhuhamdah R, Howes MJ, Al-Olimat S, Ennaceur A, Chazot PL 2015. Pharmacological and neuroprotective profile of an essential oil derived from leaves of *Aloysia citrodora* Palau. *Journal of Pharmacy and Pharmacology* **67(9):** 1306-15.
4. Asamenew G, Bisrat D, Mazumder A, Asres K. 2011. *In vitro* antimicrobial and antioxidant activities of anthrone and chromone from the latex of *Aloe harlana* Reynolds. *Phytotherapy Research* 25**(12):** 1756-1760.
5. Baider C, Florens FBV, Baret S, Beaver K, Matatiken D, Strasberg D, Kueffer C. 2010. Status of plant conservation in oceanic islands of the Western Indian Ocean. *Proceedings of the 4th Global Botanic Gardens Congress*, 2 (http://www.bgci.org/files/Dublin2010/papers/Baider-Claudia.pdf) (accessed: 15 January 2016)
6. Bosser J, Cadet Th, Gueho J, Julier HR, 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 [in French]
7. Chandrashekara KT, Shakarad, MN. 2011. *Aloe vera* or resveratrol supplementation in larval diet delays adult aging in the fruit fly, *Drosophila melanogaster*. *The journals of gerontology. Series A, Biological sciences and medical sciences* **66(9):** 965-71.
8. CITES [online] 2015; https://cites.org/sites/default/files/eng/app/2015/E-Appendices-2015-02-05.pdf (accessed 20 December 2015)
9. El-Shemy HA. Aboul-Soud MA. Nassr-Allah AA. Aboul-Enein KM. Kabash, A. and Yagi, A. (2010). Antitumor properties and modulation of antioxidant enzymes' activity

by *Aloe vera* leaf active principles isolated via supercritical carbon dioxide extraction. *Current Medicinal Chemistry* **17(2):** 129-138.

10. Fawole OA, Amoo SO, Ndhlala AR, Light ME, Finnie JF, Van Standen J. 2010. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. *Journal of Ethnopharmacology* **127(2):** 235- 241.
11. Foster M, Hoster D, Saman N. 2011. Evaluation of the nutritional and metabolic effects of *Aloe vera*. Chapter 3. *Herbal Medicine: Biomolecular and Clinical Aspects*. 2nd edition. Benzie IFF, Wachtel-Galor S, editors. Boca Raton (FL): CRC Press/Taylor & Francis;
12. Freshney RI. 2001. Culture of animal cells: a manual of basic technique and specialized applications (6th Ed). Wiley-Blackwell.
13. Govinden-Soulange J , Lobine D, Frederich M , Kodja H , Coetzee MPA, Ranghoo-Sanmukhiya VD. 2017. Metabolomic and molecular signatures of Mascarene Aloes using a multidisciplinary approach. *South African Journal of Botany* **108:**137–143.
14. Grace OM. Simmonds MSJ, Smith GF, Van Wyk AE. 2008. Theraupeutic uses of *Aloe* L. (Asphodelaceae) in southern Africa. *Journal of Ethnopharmacolgy* **119:** 604-614.
15. Grace, OM. Dzajic, M. Jäger, AK. Nyberg, NT. Onder, A. and Ronsted, N. 2013. Monosaccharide analysis of succulent leaf tissue in Aloe. *Phytochemistry* **93**: 79-87.
16. Gurib-Fakim A. 2003. *An Illustrated Guide to the Flora of Mauritius and the Indian Ocean Islands.* Caractère Ltée: Baie du Tombeau, Mauritius, 2003
17. Howes MJ, Simmonds MSJ. 2015. Pharmacognosy in the United Kingdom: Past, present, and future. *Science* **350:** S58-S59.
18. Howes MJ. Simmonds, MSJ. 2014. The role of phytochemicals as micronutrients in health and disease. *Current Opinion in Clinical Nutrition and Metabolic Care* **17:** 558-566.
19. 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(26):** 7788-7791.
20. IUCN Red List of Threatened Species [Online] http://www.iucnredlist.org (accessed 20 December 2015]
21. Lee S, Do S.G, Kim SY, Kim J, Lee CH. 2012. Mass spectrometry-bases metabolite profiling and antioxidant activity of *Aloe vera* (*Aloe barbadensis* Miller) in different growth stages. *Journal Agricultural and Food Chemistry* **60(45):** 11222-11228.

22. Marais W, Coode MJE. 1978. [183. Liliacées. In Flore des Mascareignes: La Réunion, Maurice, Rodrigues. 177. Iridacées á 188. Joncacées,] eds. Bosser J, Cadet T, Guého J, Julien HR, Marais W. Mauritius Sugar Industry Research Institute, Port Louis, Mauritius, Office de la Recherche Scientifique et Technique Outremer, Paris and the Royal Botanic Gardens, Kew [in French]
23. Neergheen VS, Bahorun T, Pugo-Gunsam P, Lin D, Ramful D, Aruoma OI. 2010. Phenolic constituents and antioxidant efficacies of some Mauritian traditional preparations commonly used against cardiovascular disease. *International Journal of Pharmacognosy and Phytochemical Research* **2(3);** 44-52.
24. Ni, Y. Tizard, IR. 2004. Analytical methodology: the gel-analysis of *Aloe* pulp and its derivatives. In Aloe: The Genus Aloe, Reynolds T. (Ed). CRC Press, Boca Raton, USA, pp. 111–126.
25. Padmanabhan P, Jangle SN. 2012. *In-vitro* Antioxidant potential of a herbal preparation containing four selected medicinal plants. *Journal of Krishna Institute of Medical Science University* **1(2):** 52-53.
26. 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).] *Rev Écol (Terre et Vie)* **55:** 201-214 [in French]
27. Parihar MS, Chaudhary M, Shetty R, Hemnani T. 2004. Susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice: prevention by extracts of *Withania somnifera* and *Aloe vera*. *Journal Clinical Neuroscience* **11(4):** 397-402.
28. Perry E, Howes MJ. 2010. Medicinal Plants and Dementia Therapy: Herbal hopes for brain aging? *CNS Neuroscience & Therapeutics* **17:** 683-698.
29. Ranghoo-Sanmukhiya VM, 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.
30. Rathor N, Arora T, Manocha S, Patil AN, Mediratta PK, Sharma KK. 2014. Anticonvulsant activity of *Aloe vera* leaf extract in acute and chronic models of epilepsy in mice. *Journal of Pharmacy and Pharmacology* **66(3):** 477-485.
31. Reynolds T. (Ed.). 2004. *Aloes*: The genus *Aloe*. CRC Press, London

32. Reynolds, T. Dweck, AC. 1999. *Aloe vera* leaf gel: a review update. *Journal of Ethnopharmacology* **68**: 3–37.
33. Saada HN. Ussama ZS, Mahdy AM. 2003. Effectiveness of *Aloe vera* on the antioxidant status of different tissues in irradiated rats. Die Pharmazie. *An International Journal of Pharmaceutical Sciences* **58(12):** 929-931.
34. Saritha V, Anilakumar KR, Khanum F. 2010. Antioxidant and antibacterial activity of *Aloe vera* gel extracts. *International Journal of Pharmaceutical and Biological Archive*s **1(4):** 376-384.
35. Tian B, Hua Y. 2005. Concentration-dependence of prooxidant and antioxidant effects of aloin and aloe-emodin on DNA. *Food Chemistry* **91(3):** 413-418.
36. Uttara B, Singh AV, Zamboni P, Mahajan RT. 2009. Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. *Current Neuropharmacology* **7(1):** 65-74.
37. Wang Y, Cao L, Du G. 2010. [Protective effects of *Aloe vera* extract on mitochondria of neuronal cells and rat brain.] *Zhongguo Zhong Yao Za Zhi* **35(3):** 364-8. [in Chinese]
38. Williams P, Sorribas A, Howes MJ. 2011 Natural products as a source of Alzheimer's drug leads. *Nat. Prod. Rep* **28:** 48-77.
39. Wintola OA, Afolayan, AJ. 2011. Phytochemical constituents and antioxidant activities of the whole leaf extract of *Aloe ferox* Mill. *Pharmacognosy Magazine* **7(28):** 325-333.
40. Yang L, Yang Z, Zhang N, Tian Z, Liu S, Zhao M. 2014. Neuroprotective effects of vitexin by inhibition of NMDA receptors in primary cultures of mouse cerebral cortical neurons. *Molecular and Cellular Biochemistry* 386**:** 251-258.
41. Yoo EA. Kim SD, Lee WM, Park, HJ, Kim SK, Cho JY, Min W, Rhee MH. 2008. Evaluation of antioxidant, antinociceptive and anti- inflammatory activities of ethanol extracts from *Aloe saponaria* Haw. *Phytotherapy Research* **22(10):** 1389-1395.
42. Yu Z. Jin C. Xin M. and Jian Min H. 2009. Effect of *Aloe vera* polysaccharides on immunity and antioxidant activities in oral ulcer animal models. *Carbohydrate Polymers* **75**: 307-31.

Table 1: Aloin and Vitexin content of *Aloe* species from the Mascarene Islands

Species	Aloin (nmol/g)	Vitexin (nmol/g)

A. purpurea (Mauritius)	182.2	27.6
A. tormentorii	195.9	67.3
A. lomatophylloides	60.0	38.6
A. macra	nd	61.7
A. purpurea (Réunion)	546.6	54.0
A. vera	170.2	12.6

nd: not detected

Table 2: Antioxidant capacity of *Aloe* extracts measured Trolox and copper scavenging equivalents

Samples	**Trolox equivalent (mM)**	**Copper-reducing equivalents(µM)**
Standard	(Trolox)	(Vitamin A)
A. purpurea (Mauritius)	0.430 ± 0.049	0.295 ± 0.044
A. tormentorii	0.380 ± 0.030	0.311 ± 0.017
A. lomatophylloides	0.440 ± 0.027	0.255 ± 0.013
A. macra	0.360 ± 0.044	0.456 ± 0.033
A. purpurea (Réunion)	0.350 ± 0.033	0.643 ± 0.025
A. vera	0.550 ± 0.097	0.177 ± 0.021