Pluripharmacological potential of Mascarene endemic plant leaf extracts

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Abstract

Tropical forests of the islands of the West Indian Ocean region, have a rich and diverse collection of unique plants containing a multitude of chemical scaffolds that can potentially be developed into medicinal agents. Till date, endemic plants from the Mascarene Archipelago have been undervalued but they hold significant economic and biomedical potential. Hence, to better gauge the pharmacological potential of Mascarene Archipelago flora, the bioactivities of fourteen selected endemic species were investigated. Leaf extracts from the Ebeneceae, Myrtaceae, Sapotaceae and Combretaceae families were prepared via organic solvent extraction and evaluated for their polyphenolic content, antioxidant, antiproliferative, anti-tyrosinase, anti-elastase and antiplasmodial activities. The highest polyphenol content was observed in *Terminalia bentzoë* (385.0 ± 24.1 mg gallic acid equivalent (GAE)/ g) followed by *Sideroxylon sessiliflorum* (340.5 ± 13.4 mg GAE/ g) ($p < 0.05$). Each extract showed dose-dependent metal chelating and free radical scavenging activity, however, *T. bentzoë* demonstrated the most significant ($p < 0.05$) antioxidant activity in terms of ferric reducing antioxidant power, iron chelating activity, superoxide anion and nitric oxide free radical scavenging activity, followed by *Syzygium glomeratum.* *Eugenia orbiculata* and *E. pollicina* exhibited the highest anti-tyrosinase activity, while *Diospyros chrysophyllos* showed noteworthy anti-elastase activity. *Terminalia bentzoë* further showed potent dose-dependent antiplasmodial activity against Dd2^{luc} *Plasmodium falciparum* with an EC_{50} value of 6.7 µg/mL. Moreover, the extracts showed moderate to low toxicity against the non-tumorigenic Vero and HaCat cell lines. The findings highlight the potential of *T. bentzoë* and *S. glomeratum* as alternative sources of pharmaceutical compounds.

Keywords: *Terminalia bentzoë,* endemic, antiplasmodial, antioxidant, anti-elastase, anti-tyrosinase

1.0. INTRODUCTION

Throughout evolution, plants have developed valuable secondary metabolites (SMs) to colonise, grow and reproduce in order to protect against environmental challenges. The chemical structures of at least 100,000 SMs have been elucidated and identified from approximately 50,000 plant species (Parimelazhagan, 2016). The vast diversity in SMs showcases the extraordinary capacity of plants to synthesize diverse molecules with a plethora of biological and pharmacological effects that may be exploited to treat or mitigate broad spectrum human ailments (Atanasov et al., 2015; Pang et al., 2021; Singla et al., 2021). Secondary metabolites are multifaceted in terms of their molecular mechanisms of action, ranging from modulation of intracellular redox reactions to anti-inflammatory and anti-pathogenic reactions, amongst others (Jan et al., 2021). These metabolites also provide numerous health benefits and have been used for various medicinal purposes, both in preventive and curative care (Salmerón-Manzano et al., 2020).

It is estimated that at least 25% of pharmacopoeias around the world stem from SMs (Garcia, 2020). Moreover, approximately 80% of the developing world's population depends heavily on traditional and complementary medicine, including herbal medicine, to meet their primary healthcare needs (Shewamene et al., 2020). The use of herbal medicine is increasingly being recognised and acknowledged across the world. In the year 2019, 124 out of the 141 World Health Organisation (WHO) member states, compared to 65 member states in the year 1999, have incorporated herbal medicine regulation as part of their national policies (WHO, 2019).

Ten percent of the world's vascular plant species are known to have ethnomedicinal properties (Salmerón-Manzano et al., 2020). However, not more than 16% of the 28,187 documented medicinal plants, have been scientifically evaluated and validated for their pharmacological properties. Alarmingly, the Living Planet Report 2020, revealed that the survival of one-fifth of the global plant species is threatened in the wild (WWF, 2020). This extinction crisis is more pronounced in the tropics, with the Island of Mauritius being fourth in geographical regions with the highest total number of extinct endemic plant species (Humphreys et al., 2019). In less than four centuries, human settlements have caused mass deforestation, eradicating over 95% of the island`s pristine forest cover and causing the extinction of 11% of species, representing 30 out of 273 Mauritian endemic angiosperm species (Florens, 2013). The loss of plant species also means the permanent loss of potentially valuable and unique biomolecules that are yet to be uncovered.

Several drugs originating from the Madagascar rainforests have been successfully commercialized as clinically important drugs (Atanasov et al., 2015; Loh, 2008). However, plants from the Mascarene Islands have been underexplored in the search for novel therapeutic drugs. A literature survey from the year 1980 to 2016, revealed only 22 publications reporting the biological activities of 17% of the Mauritian endemic flora (Rummun et al., 2018). Although the *in-vitro* bioactivities of few selected Mauritian endemic plants have been described, reports regarding their safety and toxicological profile are considerably low. The toxicity profile of *T. bentzoe* leaf extract is restricted to one report concluding the extract being non-lethal, 15 days post oral administration, in male albino rate at a dose of 500 mg/kg body weight, (El-Rafie et al., 2016). The safety data for *Eugenia orbiculata* as well as for *E. tinifolia* is limited to their non-toxicity against non-malignant human cell lines, as assessed by anti-proliferative assays (Ramhit et al., 2018)

The scarcity of in-depth pharmacological data and therapeutic properties on the vast majority of flora unique to Mauritius, coupled with the lingering extinction crisis, prompted this study to investigate the biological activities of fourteen leaf extracts derived from endemic plants collected from the Island of Mauritius. The findings of this study demonstrate the pharmacological potential and economic value of the endemic species, therefore providing an incentive for its conservation. Furthermore, this research study provides the foundation to drive further investigation in the search for potential novel pharmaceutical compounds. Thus, the present study investigated the polyphenolic content, antioxidant, anti-elastase, antityrosinase, antiplasmodial and antiproliferative activities of fourteen selected Mauritian plants leaf extracts.

2.0. METHODOLOGY

2.1. SAMPLE COLLECTION AND EXTRACTION.

Permission for the collection of the Mauritian endemic plant samples was granted by the national park conservation service under the aegis of the Ministry of Agro-industry & Food Security. Fresh leaves of fourteen endemic plants were collected from the island of Mauritius from August 2014 to October 2015. The botanist at the Mauritius national herbarium assisted in the identification and collection of the samples. Voucher specimens were deposited at the Mauritius herbarium, where the species were authenticated. The family name and the herbarium accession barcode of the collected species are given in **Table 1**. The leaves were air-dried and exhaustively macerated with 80% methanol solution (v/v) for 24 hours per cycle, for three consecutive days, followed by freeze-drying as previously described (Rummun et al., 2013). The lyophilized powder was subsequently dissolved in water or methanol to yield aqueous or methanol extracts for use in the subsequent *in vitro* assays.

Table 1: Mauritian endemic plants under investigation

Species	Family	Mauritius herbarium accession code	% yield†
***Diospyros chrysophyllos* Poir.**[1]	Ebenaceae	MAU 0009431	14.7
***Diospyros egrettarum* I Richardson**[2]	Ebenaceae	MAU 0018728	9.8
***Diospyros leucomelas* Poir.**[3]	Ebenaceae	MAU 0016547	12.2
***Diospyros tessellaria* Poir.**[2]	Ebenaceae	MAU 0016639	16.8
***Eugenia lucida* Lam**[2]	Myrtaceae	MAU 0016552	7.6
***Eugenia orbiculata* Lam**[1]	Myrtaceae	MAU 0002703	14.7
***Eugenia pollicina* J. Guého & A. J. Scott**[4]	Myrtaceae	MAU 0017468	6.9
***Mimusops petiolaris* (A.DC.) Dubard**[2]	Sapotaceae	MAU 0016640	6.0
***Sideroxylon boutonianum* A.DC**[3]	Sapotaceae	MAU 0016546	9.1
***Sideroxylon cinereum* Lam**[1]	Sapotaceae	MAU 0016429	7.4
***Sideroxylon sessiliflorum* (Poir.) Capuron ex Aubrév.**[1]	Sapotaceae	MAU 0009459	8.7
***Syzygium glomeratum* (Lam.) DC**[7 Ψ]	Myrtaceae	MAU 0016432	2.3
***Syzygium guehoi* Bosser & Florens**[2]	Myrtaceae	MAU 0016549	9.8
Terminalia bentzoë* (L.) L.f. subsp. *bentzoë[2 Ψ]	Combretaceae	MAU 0016557	7.3

Superscripts on taxon name represent sample collection sites; (1) Gaulettes Serrées, (2) Réduit, (3) Black River, (4) Mare Longue, (5) Mondrain; Ψ represent species with documented ethnomedicinal uses (Rummun et al., 2018). † % yield is given relative to the fresh weight of leaves collected.

2.2. ESTIMATION OF POLYPHENOLIC CONTENTS

The total phenolic, flavonoid, and proanthocyanidin contents of the extracts were determined according to the Folin-Ciocalteau, the aluminium chloride, and the HCL/Butan-1-ol assays, respectively, as previously described (Rummun et al., 2013). The results were presented as mg of gallic acid equivalent (GAE)/g, quercetin equivalent (QE)/g, and cyanidin chloride equivalent (CCE)/g, respectively.

For the estimation of the total phenolic content, 0.25 mL of extract (dissolved in water), at concentrations ranging between 0.05 mg/mL and 1 mg/mL, was diluted with 3.5 mL distilled water followed by the addition of 0.25 mL of Folin-Ciocalteu reagent. After incubation at room temperature for 3 min, 0.75 mL of Na_2CO_3 (20% w/v) solution was added to the reaction mixture, followed by 40 min incubation at 40°C and thereafter cooled to room temperature. The absorbance of the formed chromogen was measured at 685 nm and the total phenolic content was calculated from a gallic acid standard curve ($Y= 0.117x$ and $R^2 = 0.9995$).

The total flavonoid content was estimated by mixing 100 µL of extract (dissolved in methanol), at concentrations ranging between 2 mg/mL and 100 mg/mL, with an equal volume of 2% $AlCl_3.6H_2O$. The absorbance after 10 min incubation, was measured at 440 nm. The total flavonoid content was calculated from a quercetin standard curve (Y= 0.0552x and R^2 = 0.9851).

For the determination of proanthocyanidin content, 3 mL of *n*-BuOH/HCl (95:5 v/v) was added to a test tube containing 0.25 mL extract, at concentrations ranging between 0.5 mg/mL and 15 mg/mL, followed by the addition of 0.1 mL of 2% $NH_4Fe(SO_4)_2.12\ H_2O$ in 2M HCl. The tubes were incubated for 40 min at 95°C and thereafter cooled to room temperature, where after the absorbance was measured at 550 nm. The total proanthocyanidin content was calculated from a cyanidin chloride standard curve (Y= 0.0525x and R^2 = 0.9757).

2.3. NON-CELL-BASED ANTIOXIDANT ASSAYS

The antioxidant potential of the extracts was determined using the ferric reducing antioxidant power (FRAP), iron-chelating, DPPH, superoxide anion and nitric oxide free radical scavenging assays, as previously established (Rummun et al., 2020a). Gallic acid served as the positive control, while the vehicle (distilled water or methanol) was used as the negative control. The metal chelating and free radical scavenging activity was expressed as a percentage of the negative control. The half-maximal activity (EC_{50}) values were determined using the GraphPad Prism 6 software (GraphPad Inc., USA).

2.3.1. Ferric reducing antioxidant potential (FRAP) assay

One hundred microlitres of extract, at final concentrations ranging between 10 µg/mL and 100 µg/mL, were diluted with 300 µL of distilled water followed by the addition of freshly prepared FRAP reagent (3 mL). The FRAP reagent contained 10 parts of 0.25 M acetate buffer (pH 3.6), 1 part of 20 mM ferric chloride and 1 part of 10 mM 2,4,6-tripyridyl-s-triazine. The reaction mixture was incubated at room temperature for 4 min and the absorbance was measured at 593 nm. The FRAP value was calculated relative to the ferrous sulphate calibration curve (Y= 0.0122x and R^2 = 0.9945).

2.3.2. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay was performed by mixing 1 part of extract (dissolved in methanol), at final concentrations ranging between 0.4 to 25 µg/mL, and 2 parts of methanolic DPPH (100 µM). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 492 nm.

2.3.3. Iron chelating assay

Forty microliters of extract, at final concentrations ranging between 0.3 to 10 mg/mL, was mixed with 10 µL of $FeCl_2{\cdot}4H_2O$ (0.5mM) and 150 µL of distilled

deionized water. After incubating the reaction mixture for 5 min at room temperature, 10 µL of ferrozine (2.5 mM) was added to the tube and the absorbance was measured at 562 nm.

2.3.4. **Superoxide scavenging assay**

Twenty-five microlitres of extract, at final concentrations ranging between 0.3 to 100 µg/mL, was mixed with 100 µL of nitroblue tetrazolium (156 µM) and 100 µL of beta-nicotinamide adenine dinucleotide reduced disodium salt hydrate (200 µM), followed by the addition of 30 µL of phenazine methosulphate. After 30 min of incubation at 25°C, the absorbance was measured at 560 nm.

2.3.5. **Nitric oxide scavenging assay**

Fifty microlitres of extract (dissolved in water), at final concentrations ranging between 0.3 to 100 µg/mL, was incubated with 100 µL of 5 mM of sodium nitroprusside (in phosphate saline buffer, pH 7.4) at 25°C for 150 minutes. Thereafter, 125 µL of the reaction mixture was transferred to a 96-well plate, to which 100 µL of 0.33% sulfanilic acid in 20% glacial acetic acid was added. After 5 min, 100 µL of 0.1% of *N*-1-napthyethylenediamine dihydrochloride was added and the pink colour formation was measured at 546 nm.

2.4. **ENZYME INHIBITORY POTENTIAL**

Extracts, which showed potential antioxidant activity in at least four of the five antioxidant assays, were further investigated for enzyme inhibitory activity, antiproliferative activity and antiplasmodial activity.

2.4.1. **Anti-tyrosinase assay**

The anti-tyrosinase activity of the extracts was determined as previously described (van Staden et al., 2017). Stock concentrations (20 mg/mL) of the extracts and the positive control, kojic acid, were prepared in dimethyl sulfoxide (DMSO). The extracts and positive control were added to a 96-well microtiter plate, to which the enzyme (333 Units/mL in phosphate buffer, pH 6.5) and the substrate L-tyrosine (637.50 µM) were added. The final concentration of the positive control and extracts ranged between 1.6 to 200.0 µg/mL. The tyrosinase activity was determined kinetically at 492 nm for 30 min (in 1 min intervals) using a BIO-TEK PowerWave XS multi-well plate reader set at 37 °C (van Staden et al. 2017). The 50% inhibitory concentration (IC_{50}) values were determined from the percentage inhibition values using GraphPad Prism 6 software.

2.4.2. **Anti-elastase assay**

The elastase inhibitory potential of the extracts was determined by measuring the release of p-nitroaniline from N-succinyl-ala-ala-ala-p-nitroanilide

spectrophotometrically at 405 nm (Fibrich et al., 2019). Stock concentrations (20 mg/mL) of the extracts and the positive control, ursolic acid, were prepared in DMSO and serially diluted to final concentrations ranging between 7.8 to 500 µg/mL. The reaction mixture consisted of the test sample, 100 mM Tris buffer (pH 8.0), and porcine pancreatic elastase type IV (5 mM) which was incubated at 37°C for 15 min. After incubation, N-succinyl-ala-ala-ala-p-nitroanilide (4 mM) was added to the reaction mixture and the change in the absorbance of the reaction mixture was kinetically measured for 15 min from which the rate was obtained. A vehicle control (2% DMSO, representing 100% activity) and negative controls (2% DMSO, 0% rate, no enzyme and no substrate respectively) were included. One unit of elastolytic activity is defined as the release of 1 µM of p-nitroaniline/min. The IC_{50} values were calculated from the percentage inhibition values using GraphPad Prism 4 software.

2.5 ANTIPROLIFERATIVE ASSAY

Two non-tumorigenic cell lines were used to determine the antiproliferative activity of the extracts using a previously described method (Lall et al., 2013). Human keratinocytes (HaCat) and African green monkey epithelial kidney cells (Vero) were donated by the University of Cape Town and the Department of Paraclinical Sciences (University of Pretoria), respectively. Cells were grown in a humidified incubator set at 5% CO_2 and 37 ºC, in medium supplemented with 10% fetal bovine serum (FBS), 1% amphotericin B (250 µg/ml) and 1% antibiotics (50 units/mL penicillin and 50 µg/mL streptomycin). The HaCat cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM), whereas the Vero cells were maintained in Minimum Essential Medium (MEM). Cells were sub-cultured once an 80% confluent monolayer formed using Trypsin-EDTA (0.25%). Cells were seeded at a concentration of 1×10^4 cells/well (1×10^5 cells/mL) in 96-well plates and incubated overnight for cells to adhere. Stock concentrations (20 mg/mL) of the extracts were prepared in DMSO. The final concentration at which the extracts were tested ranged from 12.5 to 400 µg/mL. Controls included a vehicle control (2% DMSO), untreated cells (100%), a 0% control (no cells) and the positive control, actinomycin D at final concentrations ranging from 1.56×10^{-3} to 0.05 µg/mL. Cells were incubated with the samples for an additional 72h after which 20 µL of the cell viability reagent (PrestoBlue®) was added and incubated for a further 2 h. Fluorescence was measured at an excitation/ emission of 560/590 nm using a VICTOR® Nivo™ microplate reader (Perkin Elmer Inc, Massachusetts, USA) to determine cell viability. The percentage cell viability was calculated for each sample where after an IC_{50} value was calculated using GraphPad Prism 4 software.

2.6 ANTIPLASMODIAL ACTIVITY

The *in vitro* evaluation of antiplasmodial activity was conducted using trophozoite stage (24–32 h post-infection) intraerythrocytic stages of the *Plasmodium falciparum* Dd2 strain using a 48 h Malaria Sybr Green I Fluorescence assay as previously described (Aldulaimi et al., 2017; Smilkstein et al., 2004). Stock

concentration of the extracts were prepared at 10 mg/mL (in DMSO). Initial evaluations were conducted using three-fold dilutions (100.0, 33.3 and 11.1 µg/mL) with technical duplicates and two biological repeats (n = 4). Determination of the 50% effective concentration (EC_{50}) was done by plotting the mean inhibition of a two-fold dilution series (three technical repeats of three biological repeats, n = 9) against the $\log_{10}$-transformed extract concentration. A non-linear regression (sigmoidal concentration-response/variable slope equation) in GraphPad Prism v 5.0 (GraphPad Software, Inc., San Diego, CA, United States) produced the $EC_{50}$and the 95% confidence intervals. In all experiments, the maximum final concentration of solvent was 0.6% (v/v).

2.7 STATISTICAL ANALYSIS

Each experiment was conducted in triplicate, with at least three (n = 3) independent experiments (unless otherwise stated). Results are reported as the mean ± standard error of the mean (unless otherwise stated). GraphPad Prism 4/5/6 software (GraphPad Inc., USA) was used for statistical analyses and to determine EC_{50} / IC_{50} values using non-linear regression analysis and sigmoidal dose-response curves. One-Way ANOVA followed by Tukey's or Dunnett's multiple comparisons Post Hoc tests were used to determine significance in mean phytochemical content, antioxidant activity, enzyme activities and antiproliferative activity (as indicated in each results section respectively). Pearson's correlation coefficient (r) were calculated from linear regression plots generated between polyphenolic contents and antioxidant activities using GraphPad Prism 6.0 software.

3 RESULTS

3.1 EXTRACTION AND PHYTOCHEMICAL ANALYSIS.

The yield of lyophilised extracts after extraction, ranged from 2.3 to 16.8% (**Table 2**). The maximum yield was obtained for *D. tessellaria*, followed by *D. chrysophyllos* and *E. orbiculata*. The estimated polyphenols content differed significantly ($p < 0.05$) among the evaluated species with concentrations ranging between 385.0 ± 24.1 and 123.6 ± 6.0 mg GAE/g. The highest level of total phenolic content was determined in *T. bentzoë* followed by *S. sessiliflorum*, while *S. boutonianum* had the lowest level of total phenolic content (**Table 2**). The amount of flavonoid content varied between 1.7 ± 0.0 and 12.9 ± 0.5 mg QE/g, with *T. bentzoë* showing the highest total flavonoid content, followed by *S. glomeratum.* The proanthocyanidin content, in terms of cyanidin chloride equivalents, ranged between 8.1 ± 0.67 and 151.5 ± 0.57 mg CCE/g, with the highest content determined in *S. sessiliflorum,* while negligible amounts were detected in other species, as detailed in **Table 2**.

3.2 *IN VITRO* ANTIOXIDANT ACTIVITIES OF ENDEMIC LEAF EXTRACTS

Five independent *in vitro* antioxidant assays allowed for the comparative evaluation of the antioxidant profile of the extracts. The antioxidant capacity among the extracts differed significantly ($p < 0.05$) in each assay model (**Table 2**). The FRAP value of *T. bentzoë* (24.8 ± 0.2 mmol Fe^{2+}) was comparable to that of the positive control, gallic acid (24.8 ± 0.3 mmol Fe^{2+}). Each extract showed dose-dependent metal chelating and free radical scavenging activity; the EC_{50} values are summarised in **Table 2**. The iron-chelating activity of *T. bentzoë* was the highest, which was 80-fold more potent than gallic acid. The known iron chelator, EDTA, showed an EC_{50} value of 6.9 ± 0.0 µg/mL (23.6 ± 0.2 µM), i.e., 14.5-fold more potent compared to that of *T. bentzoë*. *S. glomeratum* exhibited the highest DPPH free radical scavenging activity, while *T. bentzoë* showed superior superoxide and nitric oxide free radical scavenging activity. Taken together, *T. bentzoë* generally had the most significant ($p < 0.05$) antioxidant activity in terms of FRAP, iron chelating activity, superoxide anion and nitric oxide free radical scavenging activity, closely followed by *S. glomeratum* (**Table 2**).

Table 2: Phenolic content and antioxidant potential of investigated endemic leaf extracts.

Extract	Total phenolics[1]	Total flavonoids[2]	Total proanthocyanidins[3]	FRAP[4]	Iron chelating activity[5]	DPPH Scavenging activity[6]	Superoxide scavenging activity[6]	Nitric oxide scavenging activity[6]
D. chrysophyllos	217.6 ± 6.9 ^{f,g,h}	2.8 ± 0.1 ^{f,g}	91.3 ± 3.12 ^{e}	10.1 ± 0.7 ^{d,e,f}	1.6 ± 0.0 ^{b,c,d}	3.2 ± 0.1 $^{c,d,e,\delta}$	12.5 ± 0.4 ^{e}	14.8 ± 0.6 $^{a,b,\delta}$
D. egrettarum	219.1 ± 7.0 ^{f,g,h}	8.4 ± 0.1 ^{c}	38.4 ± 3.38 ^{h}	9.5 ± 0.4 ^{e,f}	2.8 ± 0.2 ^{e}	2.8 ± 0.2 $^{b,c,\delta}$	10.3 ± 0.3 $^{d,\delta}$	18.8 ± 0.6 $^{a,b,\delta}$
D. leucomelas	192.4 ± 5.9 ^{g,h}	1.7 ± 0.0 ^{i}	48.3 ± 1.39 ^{g}	6.8 ± 0.2 ^{g}	2.5 ± 0.0 ^{d,e}	5.2 ± 0.1 $^{h,\delta}$	13.6 ± 0.5 ^{e}	17.1 ± 0.5 $^{a,b,\delta}$
D. tessellaria	231.1 ± 8.4 ^{e,f,g}	3.1 ± 0.0 ^{e,f}	134.5 ± 1.60 ^{b}	8.2 ± 0.5 ^{f,g}	3.3 ± 0.2 ^{e,f}	4.6 ± 0.1 $^{g,\delta}$	9.8 ± 0.5 $^{c,d,\delta}$	14.7 ± 0.4 $^{a,b,\delta}$
E. lucida	300.6 ± 17.7 ^{b,c,d}	3.0 ± 0.0 ^{f}	8.1 ± 0.67 ^{j}	13.7 ± 0.4 ^{c}	0.8 ± 0.0 ^{a,b}	3.0 ± 0.2 $^{b,c,d,\delta}$	9.0 ± 0.2 $^{c,d,\delta}$	20.8 ± 0.5 $^{a,b,\delta}$
E. orbiculata	316.9 ± 6.9 ^{b,c}	2.2 ± 0.0 ^{h}	60.2 ± 0.46 ^{f}	19.7 ± 0.8 ^{a,b}	0.8 ± 0.0 ^{a,b}	3.0 ± 0.0 $^{b,c,d,\delta}$	8.6 ± 0.3 $^{c,d,\delta}$	18.0 ± 0.8 $^{a,b,\delta}$
E. pollicina	238.6 ± 10.1 ^{e,f,g}	2.9 ± 0.0 ^{f,g}	ND	2.6 ± 0.2 ^{i}	0.6 ± 0.0 ^{a}	3.7 ± 0.2 $^{e,f,\delta}$	10.5 ± 0.2 $^{d,\delta}$	47.1 ± 5.8 $^{c,\delta}$
M. petiolaris	265.0 ± 12.4 ^{d,e,f}	8.1 ± 0.2 ^{c}	104.8 ± 2.02 ^{c,d}	11.6 ± 0.3 ^{c,d,e}	1.9 ± 0.1 ^{c,d}	3.5 ± 0.1 $^{d,e,f,\delta}$	8.1 ± 0.3 $^{b,c,\delta}$	23.0 ± 0.8 $^{b,\delta}$
S. boutonianum	123.6 ± 6.0 ^{i}	2.6 ± 0.1 ^{f,g,h}	34.0 ± 1.58 ^{i}	6.1 ± 0.2 ^{h}	4.3 ± 0.4 ^{g}	7.6 ± 0.1 $^{i,\delta}$	13.2 ± 0.7 ^{e}	60.5 ± 6.1 $^{d,\delta}$

S. cinereum	179.3 ± 7.3 [h]	3.6 ± 0.1 [e]	99.4 ± 0.36 [d,e]	6.5 ± 0.2 [g]	4.2 ± 0.3 [f]	3.9 ± 0.1 [f,δ]	7.9 ± 0.4 [b,c,δ]	24.0 ± 1.3 [b,δ]
S. glomeratum	221.4 ± 7.0 [f,g,h]	11.6 ± [0.2] [b]	ND	21.4 ± 0.8 [a]	0.3 ± 0.0 [a]	1.8 ± 0.0 [a,δ]	8.5 ± 0.8 [c,d,δ]	15.6 ± 0.5 [a,b,δ]
S. guehoi	271.5 ± 6.7 [c,d,e]	2.4 ± 0.1 [g,h]	111.6 ± 2.92 [c]	12.1 ± 0.3 [c,d]	1.6 ± 0.0 [b,c]	2.9 ± 0.0 [b,c,δ]	6.4 ± 0.2 [a,δ]	17.5 ± 1.6 [a,b,δ]
S. sessiliflorum	340.5 ± 13.4 [a,b]	4.5 ± 0.1 [d]	151.5 ± 0.57 [a]	8.8 ± 0.2 [f,g]	4.6 ± 0.3 [g]	2.1 ± 0.0 [a,δ]	8.1 ± 0.2 [b,c,δ]	13.9 ± 1.0 [a,b,δ]
T. bentzoë [π]	385 ± 24.1 a	12.9 ± 0.5 [a]	ND	24.8 ± 0.2 [b]	0.1 ± 0.0 [a]	2.7 ± 0.1 [b]	5.2 ± 0.5 [a]	9.74± 3.1 [a]
Gallic acid	-	-	-	24.8 ± 0.3	8.0 ± 0.0	0.6 ± 0.0	5.5 ± 0.1	9.6 ± 0.9

[1]Values are expressed as mg of gallic acid equivalent (GAE)/g, [2]Values are expressed as mg of quercetin equivalent (QE)/g, [3]Values are expressed as mg of cyanidin chloride equivalent (CCE)/g; [4]Values are expressed in mmol Fe^{2+}; [5]EC_{50} values are expressed in mg /ml; [6]EC_{50} values are expressed in µg/mL; Data represent mean ± standard error of the mean (n=3). ND = Not detected. Different superscripts in between rows represent a significant difference between extracts, ($p < 0.05$). [δ] Represent no significant difference between extract and gallic acid (positive control), ($p > 0.05$). [π] Data adapted from (Rummun et al., 2020b)

3.3 CORRELATION BETWEEN PHYTOCHEMICAL CONTENT AND ANTIOXIDANT CAPACITY OF EXTRACTS

Pearson's correlation coefficient (r) calculated from bivariate correlation analysis was used to define the direction and strength of the linear correlation between different phytochemical contents and antioxidant potentials. The highest radical scavenging activity occurred in extracts with the highest phenolic content, as observed by a lower concentration at which 50% of free radicals were effectively scavenged. Thus, a significantly ($p \leq 0.01$) high negative association coefficient between the total phenolic content and the free radical scavenging activity of extracts, in terms of DPPH (r = -0.683) and superoxide anion radicals (r = -0.754), was noted. No significant relationship was observed between the polyphenolic content and the metal chelating activity as well as the ferric reducing potential of the extracts. The total flavonoid and proanthocyanidin contents do not appear to influence the free radical scavenging activity of the extracts.

3.4 *IN-VITRO* ANTI-TYROSINASE ACTIVITY

The *in vitro* anti-tyrosinase assay detected the formation of L-dopachrome using ultraviolet-visible spectrometry, which could also be observed visually through the colour change from clear to orange. The extracts exhibited moderate anti-tyrosinase activity (**Table 3**). The extracts of *E. orbiculata* and *E. pollicina* exhibited the highest anti-tyrosinase activity with IC_{50} values of 102.9 ± 1.2 and 94.63 ± 1.25 µg/mL, respectively. However, the IC_{50} values were much higher than the positive control, which had an IC_{50} value of 1.40 ± 1.2 µg/mL, indicating that the extracts did not show significant tyrosinase inhibition when compared to the control. This is to be expected as the positive control is a pure compound compared to the extracts.

3.5 *IN VITRO* ELASTASE INHIBITORY ACTIVITY

No inhibition was observed at the highest tested concentration (500 µg/mL) for *S. boutonianum*, *S. sessiliflorum,* S. *glomeratum, and T. bentzoë.* Moderate activity was observed for *E. lucida*, *E. orbiculata*, *M. petiolaris*, *S. cinereum and S. gueohi,* whereas low activity was noted for *D. tessellaria* and *E. pollicina*. The highest elastase inhibitory potential, which was similar to the activity reported for the positive control (9.54 ± 2.63 µg/mL), was observed for *D. chrysophyllos* which exhibited the lowest IC_{50}, (9.12 ± 0.96 µg/mL) followed by *D. egrettarum* (18.65 ± 2.88 µg/mL), *D. leucomelas* (16.70 ± 5.57 µg/mL), and *E. orbiculata* (26.02 ± 5.85/mL).

3.6 ANTIPROLIFERATIVE ACTIVITY

The antiproliferative activity of the extracts was evaluated against, HaCat and Vero, after 72h of exposure. According to guidelines suggested by Kuete and Efferth (2015), the extracts showed moderate toxicity (100 µg/mL < IC_{50} < 300 µg/mL) to low toxicity (300 µg/mL < IC_{50} < 1000 µg/mL) against the non-tumorigenic cell lines (**Table 3**). *Diospyros chrysophyllos, D. egretarrum, D. tessellaria, E. monticolum, E. lucida, E. pollicina, S. boutonianum* and *S. guehoi* showed low toxicity with IC_{50} values > 300 µg/mL against both HaCat and Vero cells. The highest antiproliferative activity was observed for *S. cinereum*, which showed moderate toxicity against Vero cells with an IC_{50} value of 144.05 ± 0.35 µg/mL, however, lower toxicity was observed against the HaCat cells with an IC_{50} value of 319.1 ± 1.56 µg/mL. Similarly, *D. chrysophyllos, D. egretarrum, E. lucida, E. orbiculata, E. pollicinia, S. sessiliflorum and S. glomeratum* showed lower toxicity towards Vero cells when compared to the toxicity towards HaCat cells. *Diospyros leucomelas, M. petiolaris, S. boutonianum, S. guehoi* and *T. bentzoë* showed lower toxicity towards HaCat cells when compared to Vero cells, suggesting that the extracts showed more targeted toxicity against Vero cells than keratinocytes.

Table 3: Antityrosinase, elastase inhibitory and antiproliferative activity of investigated endemic leaf extracts.

Extract	Antityrosinase	Anti-elastase	HaCat	Vero
	IC_{50} ± SD (µg/mL)			
D. chrysophyllos	260.9 ± 1.2	9.1 ± 1.0^{+}	304.9 ± 2.12	341.90 ± 17.96
D. egretarrum	181.4 ± 1.2	18.6 ± 2.9^{+}	386.27 ± 15.22	>400
D. leucomelas	319.0 ± 1.6	16.7 ± 5.6^{+}	301.3 ± 7.07	272.07 ± 9.62
D. tessellaria	> 500	101.8 ± 6.5	382.95 ± 12.52	388.65 ± 16.05

E. lucida	> 500	55.3 ± 3.7	390.45 ± 8.41	> 400
E. orbiculata	102.9 ± 1.2	26.0 ± 5.8	222.4 ± 10.47	301.4 ± 17.22
E. pollicina	94.6 ± 1.3	381.8 ± 16.5	372.95 ± 16.05	> 400
M. petiolaris	165.1 ± 1.3	46.7 ± 1.0	363.05 ± 4.03	239 ± 10.32
S. boutonianum	> 500	> 500	> 400	305.30 ± 9.19
S. cinereum	235.2 ± 1.2	57.3 ± 1.7	319.1 ± 1.56	144.05 ± 0.35
S. sessiliflorum	> 500	> 500	237.2 ± 3.39	323 ± 12.64
S. glomeratum	459.7 ± 1.5	> 500	258.15 ± 0.64	> 400
S. guehoi	> 500	58.3 ± 0.7	355.35 ± 11.24	315.30 ± 18.24
T. bentzoë	> 500	> 500	266.35 ± 6.86	207.30 ± 10.32
Positive control[a]	1.4 ± 1.2	9.54 ± 2.63	0.01 ± 7.3×10^{-3}	0.02 ± 9.9×10^{-3}

[a] Positive control for tyrosinase inhibition (kojic acid), anti-elastase activity (ursolic acid) and antiproliferative activity (actinomycin D). Values are expressed as mean ± SD (n=3). Extracts which showed significantly similar activity (+), when compared to the positive control, were determined using one-way ANOVA followed by Dunnett's multiple comparison test. Extracts which showed low activity were not statistically analysed.

3.7 ANTIPLASMODIAL ACTIVITY

Normalised percentage growth (%) of $Dd2^{luc}$ *P. falciparum* was used to determine the *in vitro* antiplasmodial activity of the extracts and calculated from a set of three concentrations tested for each extract (100, 33.3, and 11.1 µg/mL). For each of the tested extracts, there was a concentration-dependent decrease in antiplasmodial growth inhibition from 100 to 11.1 µg/mL (**Figure 1**). *Terminalia bentzoë* showed the most prominent antiplasmodial activity with almost complete growth inhibition of $Dd2^{luc}$ *P. falciparum,* with an EC_{50} value of 6.7 µg/mL. Both *Syzgium* extracts (*S. gueohii* and *S. glomeratum*) demonstrated conspicuous parasite growth inhibition, *S. gueohii* had 0% parasite growth at 100 and 33.3 µg/mL, and 17.13 ± 1.81% at 11.1 µg/mL, while *S. glomeratum* had 0% parasite growth for 100 and 33.3 µg/mL, and 2.69 ± 0.61% at 11.1 µg/mL.

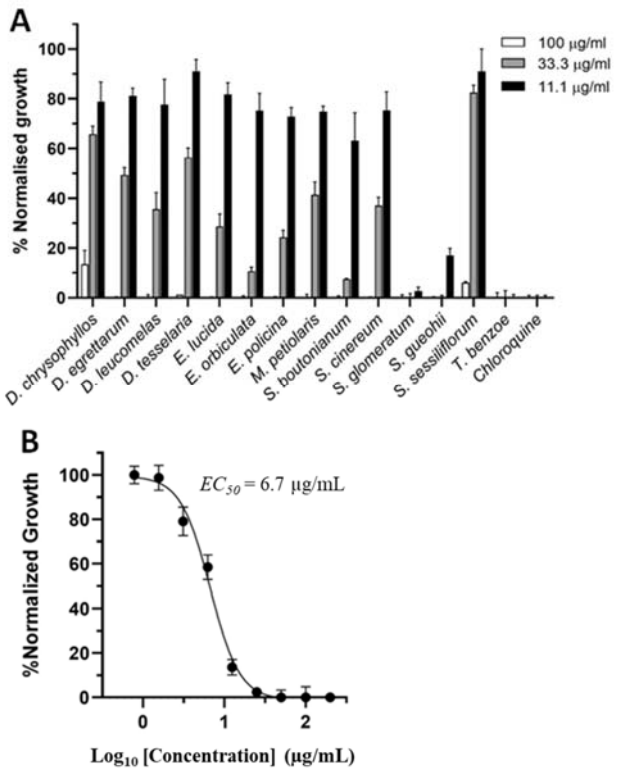

Figure 1: The effect of extracts on Dd2luc Plasmodium falciparum growth. (A) Screening fourteen extracts on *P. falciparum*. (B) Concentration-response growth inhibition of Dd2^{luc} *P. falciparum* by *T. bentzoë extract.* Trophozoite-stage Dd2^{luc} *Plasmodium falciparum* cultures (2% HCT, 1% parasitaemia) were incubated for 48 hours (37°C, 1% O_2, 3% CO_2, 96% N_2) in the presence of the indicated extracts, prior to the measurement of the absolute mean % normalized parasite growth (n = 4). A non-linear regression analysis was utilized to determine EC_{50} value (µg/mL), using GraphPad Prism 5.0.

4 DISCUSSION

Mauritius, being part of a biodiversity hotspot (Myers et al., 2000) harbours a high number of endemic taxa with the highest single island endemism (39%) among the Mascarene archipelago, exceeding those of the neighbouring Réunion (30%) and Rodrigues islands (31%) (Baider et al., 2010). Unfortunately, on a global scale, Mauritius is ranked as the third geographical area with the highest extinction rate of seed plants (Humphreys et al., 2019). In the IUCN red list criteria, 30 (10.9%) of the Mauritian endemic terrestrial plant species have been listed as extinct and 81.7% of the remaining endemic taxa are considered to be on the brink of extinction (Baider et al., 2010; BGCI, 2021). This represents an irreversible loss of molecular diversity. Approximately one-third of Mauritian endemic plant species have documented ethnomedicinal uses, however, only a limited number of the Mauritian endemic taxa have been evaluated for their biological activities (Rummun et al.,

2018). Therefore, the majority of endemic plants remain a valuable source for potential new pharmacological actives. With the aim to highlight the value of the native resources, this research study was designed to assess the pharmacological potential of leaf extracts from fourteen endemic taxa, focusing on the polyphenolic content, antioxidant, antiplasmodial, antiproliferative and anti-enzymatic activities.

The leaves from the plants have been investigated in this study, as the leaves are reported to generally represent an organ with high levels of plant SMs, the principal site of photosynthesis, most commonly used plant organ in traditional medicine, and causes minimum survival threat to the already endangered endemic plant species (Adhikari et al., 2018; Boadu and Asase, 2017). Plant polyphenols are ubiquitously occurring SMs that are highly valued for their therapeutic properties (Mutha et al., 2021). An exhaustive extraction using hydro-methanolic solvent (80% v/v) was carried out to ascertain maximum extraction of antioxidant-rich polyphenols from the leaf matrix (Altemimi et al., 2017). Extracts were further partitioned with dichloromethane to minimize the level of compounds such as waxes, oils, sterols, and chlorophylls, which can be possible sources of interference in different spectrophotometric assays (Stalikas, 2007). The yield of lyophilised leaf extracts ranged from 2.3 to 16.8%, with *D. tesselaria* having the highest yield (**Table 1**).

The highest phenolic content was estimated in *T. bentzoë* and *S. sessiliflorum* (**Table 2**). Several reports have delineated the phenolic content of selected Mauritian endemic plant leaf extracts (Neergheen et al., 2007, 2006; Soobrattee et al., 2008). Using a similar Folin-Ciocalteau method, the phenolic content in the hydro-ethanolic extract of *T. bentzoë* leaves cultivated in Cairo, Egypt, was reported to be 2-fold higher (765 mg GAE/g) compared to that estimated in the present study (385.1 ± 12.9 mg GAE/g) (El-Rafie et al., 2016). A possible explanation for the difference may be attributed to the accumulation of secondary metabolites, which is influenced by many environmental factors including light, drought, salinity and temperature stresses (Li et al., 2020). Similarly, high total phenolic content has been reported in other *Terminalia* species, in particular, leaf extracts of *T. catappa* (238 mg GAE/g extract), *T. chebula* (266 mg GAE/g extract) and *T. bellerica* (259 mg GAE/g extract) (Annegowda et al., 2010; Arya et al., 2012). The total phenolic content obtained for *E. policina* (238.6 mg GAE/g extract) in the present study, was higher compared to reported data (Ranghoo-Sanmukhiya et al., 2019), where the authors reported the total phenolic content to be as low as 6.4 mg GAE/g extracts, using a similar Folin-Ciocalteau method. A possible explanation for this discrepancy may be due to the difference in the extraction technique. The present study used air-dried leaves and exhaustive maceration with hydro-methanolic solvent for 72 hours, changing the extractant at 24-hour intervals, while Ranghoo-Sanmukhiya et al., (2019) used a combination of dichloromethane and methanol for 48 hours and used fresh leaves instead.

Among the polyphenolic compounds, flavonoids remain the most structurally diverse group present in plants (Ferreyra et al., 2012; Stalikas, 2007). The total flavonoid content of *T. bentzoë* and *S. glomeratum* were remarkably high compared to the other investigated extracts (> 10 mg QE/ g) ($p \leq 0.05$) (**Table 2).** The presence of polyphenolic compounds in the leaves of *T. bentzoë*, such as, gallic acid, ellagic acid, pyrogallol, catechin, chlorogenic acid, synergic acid, pyrocatechol, cinnamic acid, paraqumaric acid, caffeic acid, quercetin and rutin have been documented (El-Rafie et al., 2016). A recent study further reported the presence of methyl gallate, punicalagin, isoterchebulin, terflavin and orientin glycosides among the bioactive constituents present in the *T. bentzoë* leaf extract under study (Rummun et al., 2020b). Moreover, in the same study, the level of methyl gallate in *T. bentzoë* leaves was reportedly 1.7 fold higher than that of gallic acid (Rummun et al., 2020b). Plants from tropical regions are tolerant of environmental stress including a high level of ultraviolet radiation (UV) and pollutants rich in reactive radical species (Neergheen et al., 2007; Toyokuni et al., 2003). Exposure to high UV radiation is reported to upregulate the production of flavonoids, in particular, flavonols (Bornman et al., 2019; Ferreyra et al., 2012), which is consistent with their roles in protecting the plant tissue from UV induced damage (Stalikas, 2007). The accumulation of flavonoids in the leaves of Mauritian plants may be attributed to the high sunlight conditions and UV radiation (Bessafi et al., 2016) which are characteristic of tropical islands like Mauritius (Bahorun et al., 2012).

A study evaluating the health-promoting effects of herbal extracts have ascribed the wide-spectrum therapeutic potentials of the extracts, to their antioxidant-rich secondary metabolites (Yu et al., 2021). Polyphenols, in particular flavonoids, exhibit antioxidant capacity by directly scavenging free radicals, inhibiting the enzymatic activity of oxidases, stimulating activation of antioxidant enzymes, and chelating metal ions, amongst others (Procházková et al., 2011). Flavonoids are important plant antioxidants involved in conferring photoprotection to plants. The production of flavonoids in leaves was elevated in response to UV-B radiation (Bornman et al., 2019; Ferreyra et al., 2012). Mechanistically, antioxidants may neutralise free radicals either by single electron or by hydrogen atom transfer (Munteanu and Apetrei, 2021). Given the multifaceted nature via which phytochemicals exert their antioxidant activity, there is a growing consensus among scientific literature that a single assay method cannot predict the entire antioxidant efficacy of the test sample (Hernández-Rodríguez et al., 2019). Furthermore, since individual assay parameters vary considerably in function of pH, temperature, the presence of stable free radicals or *in situ* generation of free radicals; caution is needed when extrapolating data from a single assay model to predict efficacy within a biological setting. This prompted the use of five independent assays to evaluate the antioxidant potential of the extracts. The polyphenolic rich *T. bentzoë* and *S. glomeratum* were among the most effective antioxidants in nearly all of the antioxidant assays evaluated (**Table 2**).

The FRAP assay evaluated the potential of the leaf extracts to reduce Fe^{3+}/ferricyanide complex to the blue ferrous (Fe^{2+}) form (Benzie and Strain, 1996; Lewoyehu and Amare, 2019). Based on the FRAP assay, *T. bentzoë* and *S. glomeratum* had the highest reducing capacity. The FRAP value for Mauritian endemic plants reported in this study was comparable or even higher than the FRAP value for other plants cited in the literature. The FRAP value of methanolic extracts from 50 Chinese medicinal plants, used in alleviating symptoms of rheumatic disease, was reported to vary between 3.88 and 580 µmol Fe^{2+}/ g dry weight (Gan et al., 2010).

Given the catalytic role of Fe^{2+} ions to generate highly reactive hydroxyl radicals, the strong iron chelators from natural sources are highly desirable. In this study, the ability to chelate metal ions varied widely with *T. bentzoë* being the most potent chelator of Fe^{2+} ion followed by *S. glomeratum*.

Syzygium glomeratum was also a good scavenger of the DPPH• free radical. Although the DPPH assay is a widely used method (Munteanu and Apetrei, 2021; Nagarajan et al., 2017), the DPPH• radical lacks biological relevance. Therefore, the scavenging activity for biologically relevant free radicals in both physiology as well as in disease, namely superoxide anion and nitric oxide free radicals, was also tested (Alhayaza et al., 2020). *Terminalia bentzoë* showed potent dose-dependent free radical scavenging activity.

The antioxidant capacity of the endemic plants, in terms of DPPH• and superoxide anion free radical scavenging activity, correlated significantly with the total phenolic content ($p \leq 0.01$). The strong inverse association observed between the phenolic content and EC_{50} values indicated that the higher the abundance of phenolic content, the lower was the EC_{50} value and therefore, the more potent the extracts. The antioxidant activities of *T. bentzoë* and *S. glomeratum* extracts, reported in this study, might be attributed to the phenolic content (> 200 mg GAE/ g) of the extracts. The high antioxidant activity of Mauritian endemic leaf extracts was attributed to its high phenolic level (Ranghoo-Sanmukhiya et al., 2019). The positive association between phenolics and antioxidant activity is not restricted only to Mauritian endemic plants as a comparable relationship has also been reported for Mauritian citrus fruit (Ramful et al., 2010) and Mauritian tea (Luximon-Ramma et al., 2006) extracts. There was no clear correlation between the total polyphenolic contents and the anti-tyrosinase activity of the samples. To the authors' knowledge this is the first report of the anti-tyrosinase activity of the extracts of *E. pollicina* and *E. orbiculata*.

Terminalia bentzoë was identified to have potent antiplasmodial activity, with an EC_{50} value of 6.7 µg/mL (**Figure 1**). *Terminalia bentzoë* is utilised for its antimalarial activity as part of the traditional medicinal system in Reunion islands (Jonville et al., 2011, 2008; Rummun et al., 2018). In a study evaluating the antimalarial and cytotoxic activity of nine medicinal plant extracts from Reunion Island, including *T. bentzoë* leaf methanol extract, *T. bentzoë* had a half-maximal

inhibitory concentration value of 12.8 µg/mL against *P. falciparum*, which was considered as the second-best value from the said study (Jonville et al., 2008). The strong antiplasmodial activity of *T. bentzoë* leaf extract may be attributed to the high level of polyphenolic compounds such as methyl gallate and ellagic acid present in the extract (Rummun et al., 2020b). The potent antiplasmodial activity of methyl gallate (IC_{50} value of 0.03 mM) is reported to be superior to that of gallic acid (IC_{50} value of 194.84 mM) (Arsianti et al., 2018). Similarly, the potent antiplasmodial activity of ellagic acid was demonstrated, whereby ellagic acid was also observed to potentiate the activity of clinically used antimalarial agents (Soh et al., 2009).

Terminialia bentzoë, which showed potent antioxidant and antiplasmodial activity, was moderately toxic against non-tumorigenic cell lines (Vero and HaCat) (**Table 3**). However, SI values of 31 and 39.7 were calculated when comparing the antiplasmodial activity to the antiproliferative activity against Vero and HaCat cells respectively, indicating that the extract showed significantly higher antimalarial activity. No other reports on the antiproliferative activity of *T. bentzoë* against non-tumorigenic cells, were found, however *T. bentzoë* was shown to have promising activity against liposarcoma (SW872), lung (A549), hepatocellular (HepG2) and ovarian (OVCAR-4 and OVCAR-8) cancer cell lines (Rummun et al., 2020b). No other reports on the antiproliferative activity of *S. glomeratum* were found.

Members of the Diospyros genus showed the greatest anti-wrinkle potential as demonstrated by the elastase inhibitory results for *D. chrysophyllos*, *D. egrettarum* and *D. leucomelas* exhibiting the most noteworthy elastase inhibition having the lowest IC_{50} values and have not previously been investigated for their elastase inhibitory potential. However, *D. kaki*, commonly known as persimmon, has previously been investigated for a variety of anti-ageing properties. A study evaluating the elastase inhibitory potential of *D. kaki* leaves reported up to 78.1 % inhibition of the enzyme activity (An et al., 2006). A review which summarises the cosmetic potential of *D. kaki* suggested a range of additional investigations which may be conducted on these extracts to further expand on the potential cosmetic applications of these species (Kashif et al., 2017).

5.0 Conclusion

The lush green forest of the Mascarene islands is abundant in endemic plants with unique and untapped phytochemicals, having the potential to be exploited in the search for pharmaceutical lead compounds. The findings identified fourteen high-value Mascarene endemic plant species which can inspire the conservation of our cultural heritage, validate our traditional medicines and contribute to the development of new therapeutics. *Syzygium glomeratum* and *T. bentzoë* with their moderate to low antiproliferative activity were potent antioxidants and the latter showed substantial antiplasmodial activity, providing scientific rationale for its use in the mitigation of malarial infection as part of the ethnomedicinal system of the

Reunion island. The current data provided a basis for future in-depth mechanistic studies directed towards the molecular mechanism of action of the *T. bentzoë* and *S. glomeratum* leaf extracts against *P. falciparum*, as well as to investigate the bioactive compounds responsible for their antiplasmodial activity. The data suggests further *in vivo* and clinical studies to gauge its clinical efficacy and safety profile.

CRediT author statement:

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References

Adhikari, P.P., Talukdar, S., Borah, A., 2018. Ethnomedicobotanical study of indigenous knowledge on medicinal plants used for the treatment of reproductive problems in Nalbari district, Assam, India. J. Ethnopharmacol. 210, 386–407. doi:10.1016/j.jep.2017.07.024

Aldulaimi, O., Uche, F.I., Hameed, H., Mbye, H., Ullah, I., Drijfhout, F., Claridge, T.D.W., Horrocks, P., Li, W.W., 2017. A characterization of the antimalarial activity of the bark of Cylicodiscus gabunensis Harms. J. Ethnopharmacol. 198, 221–225. doi:10.1016/j.jep.2017.01.014

Alhayaza, R., Haque, E., Karbasiafshar, C., Sellke, F.W., Abid, M.R., 2020. The Relationship Between Reactive Oxygen Species and Endothelial Cell Metabolism. Front. Chem. 8, 1–21. doi:10.3389/fchem.2020.592688

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., Lightfoot, D.A., 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6. doi:10.3390/plants6040042

An, B.-J., Kwak, J.-H., Park, J.-M., Lee, J.-Y., Park, T.-S., Lee, J.-T., Son, J.-H.,

Jo, C., Byun, M.-W., 2006. Inhibition of Enzyme Activities and the Antiwrinkle Effect of Polyphenol Isolated from the Persimmon Leaf (Diospyros kaki folium) on Human Skin. Dermatologic Surg. 31, 848–855. doi:10.1111/j.1524-4725.2005.31730

Annegowda, H., Anwar, L., Mordi, M., Ramanathan, S., Mansor, S., 2010. Influence of sonication on the phenolic content and antioxidant activity of Terminalia catappa L. leaves. Pharmacognosy Res. 2, 368. doi:10.4103/0974-8490.75457

Arsianti, A., Astuty, H., Fadilah, Simadibrata, D.M., Adyasa, Z.M., Amartya, D., Bahtiar, A., Tanimoto, H., Kakiuchi, K., 2018. Synthesis and in vitro antimalarial activity of alkyl esters of gallate as a growth inhibitor of plasmodium falciparum. Orient. J. Chem. 34, 655–662. doi:10.13005/ojc/340207

Arya, A., Nyamathulla, S., Noordin, M.I., Mohd, M.A., 2012. Antioxidant and hypoglycemic activities of leaf extracts of three popular Terminalia species. E-Journal Chem. 9, 883–892. doi:10.1155/2012/859831

Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E.-M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H., Rollinger, J.M., Schuster, D., Breuss, J.M., Bochkov, V., Mihovilovic, M.D., Kopp, B., Bauer, R., Dirsch, V.M., Stuppner, H., 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol. Adv. 33, 1582–1614. doi:10.1016/j.biotechadv.2015.08.001

Bahorun, T., Ramful-Baboolall, D., Neergheen-Bhujun, V., Aruoma, O.I., Kumar, A., Verma, S., Tarnus, E., Da Silva, C.R., Rondeau, P., Bourdon, E., 2012. Phytophenolic Nutrients in Citrus: Biochemical and Molecular Evidence, in: Srivastava, A.K. (Ed.), Advances in Citrus Nutrition. Springer Netherlands, Dordrecht, pp. 25–40. doi:10.1007/978-94-007-4171-3_3

Baider, C., Florens, F.B.V., Baret, S., Beaver, K., Matatiken, D., Strasberg, D., Kueffer, C., 2010. Status of plant conservation in oceanic islands of the Western Indian Ocean, in: Proceedings of the 4th Global Botanic Gardens Congress. pp. 1–7.

Benzie, I.F.F., Strain, J.J., 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Anal. Biochem. 239, 70–76. doi:10.1006/abio.1996.0292

Bessafi, M., Oree, V., Khoodaruth, A., Chabriat, J.P., 2016. Mapping the incoming solar irradiance over Mauritius using time-series records of Satellite Application Facility on Climate Monitoring. 2015 IEEE Int. Conf. Smart Instrumentation, Meas. Appl. ICSIMA 2015 0–5. doi:10.1109/ICSIMA.2015.7559020

BGCI, 2021. State of the World`s trees, Botanic Gardens Conservation International. Island Press/Center for Resource Economics, Washington, DC.

Blom van Staden, A., De Canha, M., Nqephe, M., Rademan, S., Kumar, V., Lall, N., 2017. Potential medicinal plants for progressive macular hypomelanosis. South African J. Bot. 111, 346–357. doi:10.1016/j.sajb.2017.04.007

Boadu, A.A., Asase, A., 2017. Documentation of Herbal Medicines Used for the

Treatment and Management of Human Diseases by Some Communities in Southern Ghana. Evidence-Based Complement. Altern. Med. 2017, 1–12. doi:10.1155/2017/3043061

Bornman, J.F., Barnes, P.W., Robson, T.M., Robinson, S.A., Jansen, M.A.K., Ballaré, C.L., Flint, S.D., 2019. Linkages between stratospheric ozone, UV radiation and climate change and their implications for terrestrial ecosystems. Photochem. Photobiol. Sci. 18, 681–716. doi:10.1039/C8PP90061B

El-Rafie, H.M., Mohammed, R.S., Hamed, M.A., Ibrahim, G.E., Abou Zeid, A.H., 2016. Phytochemical and biological studies of total ethanol and petroleum ether extracts of Terminalia bentzoe (L.) leaves. Int. J. Pharmacogn. Phytochem. Res. 8, 592–603.

Ferreyra, F.M.L., Rius, S.P., Casati, P., 2012. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Front. Plant Sci. 3, 1–15. doi:10.3389/fpls.2012.00222

Fibrich, B., Gao, X., Puri, A., Banga, A.K., Lall, N., 2019. In vitro antioxidant, anti-inflammatory and skin permeation of myrsine africana and its isolated compound myrsinoside B. Front. Pharmacol. 10, 1–8. doi:10.3389/fphar.2019.01410

Florens, F.B.V., 2013. Conservation in Mauritius and Rodrigues: Challenges and Achievements from Two Ecologically Devastated Oceanic Islands, in: Raven, P.H., Sodhi, N.S., Gibson, L. (Eds.), Conservation Biology. John Wiley & Sons, Ltd, Oxford, UK, pp. 40–50. doi:10.1002/9781118679838.ch6

Gan, R.-Y., Kuang, L., Xu, X.-R., Zhang, Y., Xia, E.-Q., Song, F.-L., Li, H.-B., 2010. Screening of Natural Antioxidants from Traditional Chinese Medicinal Plants Associated with Treatment of Rheumatic Disease. Molecules 15, 5988–5997. doi:10.3390/molecules15095988

Garcia, S., 2020. Pandemics and Traditional Plant-Based Remedies. A Historical-Botanical Review in the Era of COVID19. Front. Plant Sci. 11, 1–9. doi:10.3389/fpls.2020.571042

Hernández-Rodríguez, P., Baquero, L.P., Larrota, H.R., 2019. Flavonoids, in: Bioactive Compounds. Elsevier, pp. 265–288. doi:10.1016/B978-0-12-814774-0.00014-1

Humphreys, A.M., Govaerts, R., Ficinski, S.Z., Nic Lughadha, E., Vorontsova, M.S., 2019. Global dataset shows geography and life form predict modern plant extinction and rediscovery. Nat. Ecol. Evol. 3, 1043–1047. doi:10.1038/s41559-019-0906-2

Jan, R., Asaf, S., Numan, M., Lubna, Kim, K.-M., 2021. Plant Secondary Metabolite Biosynthesis and Transcriptional Regulation in Response to Biotic and Abiotic Stress Conditions. Agronomy 11, 968. doi:10.3390/agronomy11050968

Jonville, M.C., Kodja, H., Humeau, L., Fournel, J., De Mol, P., Cao, M., Angenot, L., Frédérich, M., 2008. Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity. J. Ethnopharmacol. 120, 382–386. doi:10.1016/j.jep.2008.09.005

Jonville, M.C., Kodja, H., Strasberg, D., Pichette, A., Ollivier, E., Frédérich, M., Angenot, L., Legault, J., 2011. Antiplasmodial, anti-inflammatory and cytotoxic activities of various plant extracts from the Mascarene Archipelago. J. Ethnopharmacol. 136, 525–531. doi:10.1016/j.jep.2010.06.013

Kashif, M., Akhtar, N., Mustafa, R., 2017. An overview of dermatological and cosmeceutical benefits of Diospyros kaki and its phytoconstituents. Rev. Bras. Farmacogn. 27, 650–662. doi:10.1016/j.bjp.2017.06.004

Lall, N., Henley-Smith, C.J., De Canha, M.N., Oosthuizen, C.B., Berrington, D., 2013. Viability Reagent, PrestoBlue, in Comparison with Other Available Reagents, Utilized in Cytotoxicity and Antimicrobial Assays. Int. J. Microbiol. 2013, 1–5. doi:10.1155/2013/420601

Lewoyehu, M., Amare, M., 2019. Comparative evaluation of analytical methods for determining the antioxidant activities of honey: A review. Cogent Food Agric. 5. doi:10.1080/23311932.2019.1685059

Li, Y., Kong, D., Fu, Y., Sussman, M.R., Wu, H., 2020. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol. Biochem. 148, 80–89. doi:10.1016/j.plaphy.2020.01.006

Loh, K., 2008. Know the Medicinal Herb: Catharanthus roseus (Vinca rosea). Malaysian Fam. physician Off. J. Acad. Fam. Physicians Malaysia 3, 123.

Luximon-Ramma, A., Neergheen, V.S., Bahorun, T., Crozier, A., Zbarsky, V., Datla, K.P., Dexter, D.T., Aruoma, O.I., 2006. Assessment of the polyphenolic composition of the organic extracts of Mauritian black teas: A potential contributor to their antioxidant functions. Biofactors 27, 79–91.

Munteanu, I.G., Apetrei, C., 2021. Analytical Methods Used in Determining Antioxidant Activity: A Review. Int. J. Mol. Sci. 22, 3380. doi:10.3390/ijms22073380

Mutha, R.E., Tatiya, A.U., Surana, S.J., 2021. Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. Futur. J. Pharm. Sci. 7. doi:10.1186/s43094-020-00161-8

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature. doi:10.1038/35002501

Nagarajan, J., Ramanan, R.N., Raghunandan, M.E., Galanakis, C.M., Krishnamurthy, N.P., 2017. Carotenoids, in: Nutraceutical and Functional Food Components. Elsevier, pp. 259–296. doi:10.1016/B978-0-12-805257-0.00008-9

Neergheen, V.S., Bahorun, T., Jen, L.-S.S., Aruoma, O.I., 2007. Bioefficacy of mauritian endemic medicinal plants: Assessment of their phenolic contents and antioxidant potential. Pharm. Biol. 45, 9–17. doi:10.1080/13880200601026242

Neergheen, V.S., Soobrattee, M.A., Bahorun, T., Aruoma, O.I., 2006. Characterization of the phenolic constituents in Mauritian endemic plants as determinants of their antioxidant activities in vitro. J. Plant Physiol. 163, 787–799. doi:10.1016/j.jplph.2005.09.009

Pang, Z., Chen, J., Wang, T., Gao, C., Li, Z., Guo, L., Xu, J., Cheng, Y., 2021. Linking Plant Secondary Metabolites and Plant Microbiomes: A Review. Front. Plant Sci. 12. doi:10.3389/fpls.2021.621276

Parimelazhagan, T., 2016. Pharmacological Assays of Plant-Based Natural Products, Progress in drug research, Progress in Drug Research. Springer International Publishing, Cham. doi:10.1007/978-3-319-26811-8

Procházková, D., Boušová, I., Wilhelmová, N., 2011. Antioxidant and prooxidant properties of flavonoids. Fitoterapia 82, 513–523. doi:10.1016/j.fitote.2011.01.018

Ramful, D., Tarnus, E., Rondeau, P., Robert Da Silva, C., Bahorun, T., Bourdon, E., 2010. Citrus fruit extracts reduce advanced glycation end products (AGEs)-and H2O2 -induced oxidative stress in human adipocytes. J. Agric. Food Chem. 58, 11119–11129. doi:10.1021/jf102762s

Ramhit, P., Ragoo, L., Bahorun, T., Neergheen-Bhujun, V.S., 2018. Multi-targeted effects of untapped resources from the Mauritian endemic flora. South African J. Bot. 115, 208–216. doi:10.1016/j.sajb.2018.01.020

Ranghoo-Sanmukhiya, V.M., Chellan, Y., Soulange, J.G., Lambrechts, I.A., Stapelberg, J., Crampton, B., Lall, N., 2019. Biochemical and phylogenetic analysis of Eugenia and Syzygium species from Mauritius. J. Appl. Res. Med. Aromat. Plants 12, 21–29. doi:10.1016/j.jarmap.2018.10.004

Rummun, N., Neergheen-Bhujun, V.S., Pynee, K.B., Baider, C., Bahorun, T., 2018. The role of endemic plants in Mauritian traditional medicine – Potential therapeutic benefits or placebo effect? J. Ethnopharmacol. 213, 111–117. doi:10.1016/j.jep.2017.10.006

Rummun, N., Pires, E., McCullagh, J., Claridge, T.W.D., Bahorun, T., Li, W.W., Neergheen, V.S., 2020a. Methyl gallate – Rich fraction of Syzygium coriaceum leaf extract induced cancer cell cytotoxicity via oxidative stress. South African J. Bot. 137, 149–158. doi:10.1016/j.sajb.2020.10.014

Rummun, N., Rondeau, P., Bourdon, E., Pires, E., McCullagh, J., Claridge, T.D.W., Bahorun, T., Li, W., Neergheen, V.S., 2020b. Terminalia bentzoë, a Mascarene Endemic Plant, Inhibits Human Hepatocellular Carcinoma Cells Growth In Vitro via G0/G1 Phase Cell Cycle Arrest. Pharmaceuticals 13, 303. doi:10.3390/ph13100303

Rummun, N., Somanah, J., Ramsaha, S., Bahorun, T., Neergheen-Bhujun, V.S., 2013. Bioactivity of Nonedible Parts of Punica granatum L.: A Potential Source of Functional Ingredients. Int. J. Food Sci. 2013, 1–12. doi:10.1155/2013/602312

Salmerón-Manzano, E., Garrido-Cardenas, J.A., Manzano-Agugliaro, F., 2020. Worldwide Research Trends on Medicinal Plants. Int. J. Environ. Res. Public Health 17, 3376. doi:10.3390/ijerph17103376

Shewamene, Z., Dune, T., Smith, C.A., 2020. Use of traditional and complementary medicine for maternal health and wellbeing by African migrant women in Australia: a mixed method study. BMC Complement. Med. Ther. 20, 60. doi:10.1186/s12906-020-2852-6

Singla, R.K., Guimarães, A.G., Zengin, G., 2021. Editorial: Application of Plant Secondary Metabolites to Pain Neuromodulation. Front. Pharmacol. 11, 2020–2022. doi:10.3389/fphar.2020.623399

Smilkstein, M., Sriwilaijaroen, N., Kelly, J.X., Wilairat, P., Riscoe, M., 2004. Simple and Inexpensive Fluorescence-Based Technique for High-Throughput Antimalarial Drug Screening. Antimicrob. Agents Chemother. 48, 1803–1806. doi:10.1128/AAC.48.5.1803-1806.2004

Soh, P.N., Witkowski, B., Olagnier, D., Nicolau, M.L., Garcia-Alvarez, M.C., Berry, A., Benoit-Vical, F., 2009. In vitro and in vivo properties of ellagic acid in malaria treatment. Antimicrob. Agents Chemother. 53, 1100–1106. doi:10.1128/AAC.01175-08

Soobrattee, M.A., Bahorun, T., Neergheen, V.S., Googoolye, K., Aruoma, O.I., 2008. Assessment of the content of phenolics and antioxidant actions of the Rubiaceae, Ebenaceae, Celastraceae, Erythroxylaceae and Sterculaceae families of Mauritian endemic plants. Toxicol. Vitr. 22, 45–56. doi:10.1016/j.tiv.2007.07.012

Stalikas, C.D., 2007. Extraction, separation, and detection methods for phenolic acids and flavonoids. J. Sep. Sci. 30, 3268–3295. doi:10.1002/jssc.200700261

Toyokuni, S., Tanaka, T., Kawaguchi, W., Lai Fang, N.R., Ozeki, M., Akatsuka, S., Hiai, H., Aruoma, O.I., Bahorun, T., 2003. Effects of the phenolic contents of Mauritian endemic plant extracts on promoter activities of antioxidant enzymes. Free Radic. Res. 37, 1215–1224. doi:10.1080/10715760310001598150

WHO, 2019. WHO global report on traditional and complementary medicine 2019.

WWF, 2020. Living Planet Report 2020 - Bending the curve of biodiversity loss. WWF, Gland, Switzerland.

Yu, M., Gouvinhas, I., Rocha, J., Barros, A.I.R.N.A., 2021. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Sci. Rep. 11, 10041. doi:10.1038/s41598-021-89437-4