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# Multi-targeted effects of untapped resources from the Mauritian endemic flora

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ABSTRACT

The Mauritian endemic plants represent untapped resources, which must be probed into to establish therapeutic activities. This study aimed at determining a broad spectrum of *in vitro* bioactivities of twelve endemic plants. Total phenolics, flavonoids and proanthocyanidins were determined spectrophotometrically and by High Performance Liquid Chromatography (HPLC). Broth microdilution assay investigated the antibacterial effects of the extracts while their inhibitory effects on xanthine oxidase and acetylcholinesterase were evaluated. Cytotoxicity was studied against the human breast cancer cell line, Hs578T using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide. The highest level of total phenolics was measured in *Badula multiflora* while maximum total flavonoid and proanthocyanidin content were found in *Eugenia orbiculata* and *Diospyros tesselleria* respectively. A complex HPLC chromatogram indicating the presence of epigallocatechin gallate, kaempferol and quercetin in these extracts further emphasized the phenolic richness. *Diospyros* and *Eugenia* species had minimum inhibitory concentration values lower than 100 μg/ml against six Gram-negative and one Gram-positive bacteria. Eight extracts had better antimicrobial activity than at least one of the antibiotics used. *Badula multiflora* showed xanthine oxidase inhibition ($IC_{50}$ 0.08 ± 0.020 mg/ml) comparable to allopurinol (0.04 ± 0.001 mg/ml) ($p < 0.05$). *Diospyros tessellaria*, *Diopyros neraudii* and *Erythroxylum macrocarpum* were significantly better inhibitors of acetylcholinesterase than galantamine hydrobromide ($p < 0.05$). *Badula multiflora*, *Croton vaughanii*, *Diospyros neraudi*, *Eugenia elliptica* and *Psidia terebinthina* extracts exerted pronounced cytotoxicity against Hs578T cells in a dose-dependent manner. This study brings into the limelight the potential of these selected endemic plants and calls for further investigation of the bioactive components in relation to the activities.



## 1. Introduction

Healthcare and botany have evolved as inseparable domains of human activity over the past centuries. The use of natural products and their derivatives has been found to project up to 60% of all drugs in clinical use worldwide (Si-Yuan et al., 2013). Moreover, a number of developed countries are turning back to the use of traditional medicinal systems that encompass the use of plants as remedies and the WHO estimates that almost 65% of the world's population have incorporated medicinal plants for routine usage to manage and or cure different ailment conditions (Mukhopadhyay et al., 2012). Overwhelming epidemiologic data depicts that the phytochemicals present in a plant-based diet can reduce the risk of chronic diseases (Zhao et al., 2011; Das et al., 2016). Consequently, the important role that plant biodiversity can have in achieving the United Nation's Sustainable Development Goals (SDGs), which aim to ensure healthy lives and promote well-being for all at all ages is immense. In addition, the use of natural products for prophylactic and curative purposes is more than ever pressing because of the rise and spread of resistance to chemotherapy. The latter presents a real challenge to science and medicine and calls for revival of natural product discovery.

In addition, there is little doubt that medicinal plants play a fundamental role as a source of prescription drugs in the form of their active principles besides their role in traditional medicine (Anokwuru et al., 2011; Sasidharan et al., 2011; Tantiado, 2012). The secondary metabolites of plants through their multi-pharmacological effects account for health benefits (Leicach and Chludil, 2014). Specific classes of compounds including alkaloids and phenolic compounds produced by higher plants have been validated to exert positive health outcomes in humans and can potentially serve as antioxidants, anti-allergic, anti-inflammatory, anticancer, antihypertensive, and antimicrobial agents (Daglia, 2012; Ganesan and Xu, 2017).

Public acceptance coupled with the opportunity of obtaining complex compounds at an affordable rate (Koudous et al., 2014), and resistance to

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chemotherapy in cancer and microbial diseases has prompted the search for solutions from untapped natural resources. This is further encouraged by the ongoing biodiversity loss, which is altering ecosystem functions and their ability to provide goods and services for human health and well-being (Pecl et al., 2017). Therefore, bioprospecting from plant extracts remains a valid and timely strategy for the search of active extracts and bioactive compounds. Interestingly, Mauritius being a tropical island has a very rich and dense flora with about 315 endemic plants, a number of them having long-standing use as traditional medicine (Neergheen et al., 2006; Mauritius Wildlife Foundation, 2017). Previous studies highlighted the antioxidative richness of the plants under study (Neergheen et al., 2006, 2007; Soobrattee et al., 2008). As part of a systematic screening of Mauritian endemic plants with a view to identifying new sources of chemical compounds with biological studies, we pursue the investigation to determine additional biological targets of the endemic plant extracts in an attempt to show the promise of these untapped resources. Given the pluripharmacological effects of polyphenols, screening for major biological activities like antioxidant, antimicrobial, cytotoxicity and enzyme inhibition were envisaged. The screening will determine the plant extract and the most potent activity which subsequently will set the stage for more in depth mechanistic studies and phytochemical characterisation. In this endeavour, 12 Mauritian endemic plant extracts from Asteraceae, Ebenaceae, Euphorbiaceae, Erythroxylaceae, Meliaceae, Monimiaceae, Myrtaceae and Ochnaceae were probed for their qualitative and quantitative polyphenolic composition, further *in vitro* antioxidant activities, cytotoxic activity, anticholinesterase effects, anti-inflammatory and antimicrobial potential.

## 2. Methodology

### 2.1. Reagents used

The bacterial strains of Gram-negative *Escherichia coli* (ATCC 25922), *Klebsiella oxytoca* (ATCC 43086), *Salmonella enterica* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 27853), *Pseudomonas fluorescens* (ATCC 13525) and *Serratia marcescens* (*ATCC* 13880) and Gram-positive *Bacillus cereus* (ATCC 11778) were obtained from the American Type Culture Collection (ATCC). Breast cancer cell line, Hs578T (ATCC® HTB-126™) and normal breast cell line, Hs578BsT (ATCC® HTB-125™) were also obtained from American Type Culture Collection (ATCC). Nitroblue tetrazolium (NBT), β-nicotimamide-adenine dinucleotide (reduced disodium salt of NADH), phenazine methosulphate solution (PMS), sodium nitroprusside, phosphate saline buffer, sulphanilic acid (0.33% in 20% glacial acetic acid), naphthylethylenediamine dichloride, *p*-iodonitro-phenyltetrazolium (INT), xanthine oxidase, xanthine, 5,5′–dithio-bis-2-nitrobenzoic acid (DTNB), acetylcholinesterase from *Electrophorus electricus* (electric eel), acetylthiocholine iodide (ATCI), fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM),3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), epidermal growth factor (EGF), methanol (HPLC grade), ammonium iron(III)sulfate dodecahydrate, cyanidin chloride and acetonitrile were purchased from Sigma-Aldrich. All other organic solvents and chemicals used in this study were of analytical grade.

### 2.2. Plant materials

The leaves of *Badula multiflora* A. DC (E1), *Croton vaughanii* Croizat (E2), *Diospyros neraudii* A.DC (E3), *Diospyros tesselleria* Poir (E4), *Erythroxylum macrocarpum* O.E.Schultz. (E5), *Eugenia elliptica* Lam. (E6), *Eugenia orbiculata* Lam. (E7), *Eugenia tinifolia* Lam. (E), *Ochna mauritiana* Lam. (E9), *Psidia terebinthina* A.J. Scottt. (E10), *Tambourissa cordifolia* Lorence. (E11), and *Turraea rigida* Vent (E12) were obtained as detailed in Neergheen et al. (2006, 2007).Voucher specimens were deposited at the Mauritius Herbarium, Réduit and the Department of Biosciences, University of Mauritius (Table 1).

**Table 1**
Plant species under study.

| Plant species | Family name | Voucher number |
|---|---|---|
| *Badula multiflora* A. DC. | Myrsinaceae | BIO 2003001 |
| *Croton vaughanii* Croizat. | Euphorbiaceae | BIO 2003002 |
| *Diospyros neraudii* A.DC. | Ebeneceae | BIO 2002001 |
| *Diospyros tesselleria* Poir. | Ebeneceae | MAU 0019069 |
| *Erythroxylum macrocarpum* O.E.Schultz | Erythroxylaceae | BIO 2003003 |
| *Eugenia elliptica* Lam. | Myrtaceae | MAU 0013138 |
| *Eugenia orbiculata* Lam. | Myrtaceae | MAU 0013276. |
| *Eugenia tinifolia* Lam. | Myrtaceae | MAU 0013504 |
| *Ochna mauritiana* Lam. | Ochnaceae | MAU 0011605 |
| *Psidia terebinthina* A.J. Scottt. | Asteraceae | BIO 2003004 |
| *Tambourissa cordifolia* Lorence | Monimiaceae | MAU 0017470 |
| *Turraea rigida* Vent. | Meliaceae | BIO 2003005 |

### 2.3. Preparation of extracts

Leaves (50 g) were extracted first with acetone/water (70/30 v/v) (2 × 400 ml) and the residue derived after filtration was further macerated with pure methanol (3 × 400 ml). Filtrates were concentrated in vacuo and pooled, and the resulting aqueous extract was washed with dichloromethane to remove lipids before being freeze-dried. The extracts were prepared from the lyophilized powder as in Neergheen et al., 2006, 2007 for subsequent analyses.

### 2.4. Total phenolic content determination

The total phenolic content (TPC) was estimated as described in Neergheen et al. (2006). Briefly 0.25 ml of the extract was added followed by 3.75 ml of distilled water and 0.25 ml of Folin-Ciocalteu reagent. After 3 min, 20% sodium carbonate was added. The tubes were capped, mixed thoroughly, and heated at 40 °C for 40 min. The blue coloration was read at 685 nm against a blank standard. Results were expressed in mg of gallic acid equivalent (GAE)/g fresh weight (FW) of plant material.

### 2.5. Total flavonoid content determination

The total flavonoid content (TFC) of the extracts was determined as per Zhishen et al. (1999). A total of 150 μl of 5% aqueous $NaNO_2$ was added to 2.50 ml of extract. After 5 min, 150 μl of 10% aqueous $AlCl_3$ was added. A total of 1 ml of 1 M NaOH was added 1 min after the addition of aluminum chloride. The absorbance of the solution was measured at 510 nm. TFC was expressed in mg of quercetin equivalent (QE)/g FW of plant material.

### 2.6. Total proanthocyanidin content determination

The total proanthocyanidin content (TPrC) of the extracts was established using a modified hydrochloric acid (HCl)/butan-1-ol (BuOH) assay adapted from Porter et al. (1986). To each tube, 0.25 ml of the methanol extract was added, followed by 3 ml of butanol/HCl solution and 0.1 ml of $NH_4Fe(SO_4)_2 \cdot 12H2O$ in 2 M HCl. The tubes were incubated for 40 min at 95 °C. The absorbance of the red coloration developed was read at 550 nm and TPrC was expressed in mg of cyanidin chloride equivalent (CCE)/g FW of plant material.

### 2.7. Determination of the phenolic profiles of the extracts using HPLC

The methanolic extracts were centrifuged at 3500 rpm for 20 min and the supernatant was subjected to syringe filtration of pore size of 0.22 μm. Solvent A (10% acetonitrile *v*/*v*) and solvent B (50% acetonitrile *v*/*v*) were prepared and acidified to pH 2.6 using concentrated phosphoric acid.

HPLC analysis of extracts (12.5 mg FW/ml) and standards (stock concentration of 250 μg/ml) were carried out using a Hewlett Packard