# Molecular and bioactive profiling of selected *Eugenia* species from Mauritius Island

**R. V. Brunchault,[1]**
**Joyce Govinden Soulange,[1]**
**Vijayanti Mala Ranghoo Sanmukhiya,[1]**
**Jean Claude Sevathian[2]**

**[1]Faculty of Agriculture, University of Mauritius, Reduit; [2]Mauritian Wildlife Foundation, Vacoas, Mauritius**

## Abstract

The *Eugenia* genus is comprised of about 1011 species which share similar features resulting in the complexity of its taxonomy and nomenclature. *E. crassipetala*, *E. kanakana*, *E. tinifolia* and two undescribed *Eugenia species* all medicinal and endemic to Mauritius Islands were characterized using their phytochemical, bioactive and molecular profile. Biological activity was assessed using the broth microdilution assay and the DPPH assay. Significant minimal inhibitory concentration values of *E. crassipetala* against *E. coli* (1.56 mg/mL), *E. kanakana* against *P. mirabilis* (0.55 mg/mL) and *E. spp* (*small*) against *S. aureus* (0.43 mg/mL) validates the antibacterial ability of these plant extracts and could be attributed to their high content of antioxidants (flavonoids and phenols). Genetic diversity among these five species was assessed by amplification of genomic DNA using 60 RAPD and 25 ISSR markers. Hierarchical cluster analysis validates the uniqueness of each *Eugenia* species with *E. crassipetala* and *E. tinifolia* forming a separate cluster. Comparative analysis of phytochemical composition and bioactivity correlate with the branching pattern of the species in the dendogram.

## Introduction

The *Myrtaceae* is ranked eighth among the largest flowering plant families (Angiosperms) on earth, consisting of 5774 species within 145 plant genera.[1,2] The geographical origin of the *Myrtaceae* is Australia and its distribution is usually confined to warm regions: more concentrated in the tropics and subtropics. They are woody plants with generally entire leathery evergreen leaves with opposite exstipulates,[3] and constitute an important source of essential oils, flavonoids, tannins and other phenolics with biological activities including antibacterial, antifungal or anti-inflammatory properties.[4,5] Many members of this family share similar features and these result in the complexity of their taxonomy and nomenclature and may account for the lack of interest in the study of this plant family in the last decades. The level of specific diversity for the Myrtle family is estimated to have increased by 43% for the last two decades between publication of the first and latest editions of Mabberley.[6,7]

Molecular markers allow analysis of genetic diversity with solid evidence at the genetic level about relationships between species. They are more reliable compared to morphological or biochemical markers because they are stable irrespective of the organism's age, stage of development, remain unaffected by environment and allow detection of dominance or co-dominance.[8] The first molecular studies carried on species of the Myrtaceae family started in the mid-1990s and gave a new evolutionary structure to understand supraspecific relationships in the family.[2]

SPAR (Single Primer Amplification Reaction) methods are simple and economical techniques allowing assessment of genetic diversity. Many plant species of the Myrtaceae have been characterized by these methods, including *Psidium,*[9] *Metrosideros,*[10] and *Eucalyptus* among others.[11] ISSR (Inter Simple Sequence Repeat) analysis allows identification of closely related species, and the study of evolutionary relationships and phylogeny.[9] ISSRs produce numerous dominant *multilocus* markers from the nuclear genome which are highly reproducible, allowing a more accurate estimate of genetic variation compared to some other commonly used molecular markers.[10] RAPD (Random Amplified Polymorphic DNA) has been used in the molecular characterization of many plant species. The RAPD marker makes use of arbitrary primers and is a rapid and convenient molecular marker for the assessment of genetic diversity among various species including medicinal plants.[12] The use of ISSR and RAPD should complement each other in genetic identification and in mapping different regions of the *Eugenia* species genome.[13]

*Eugenia* species are members of the Myrtle family which bear berry fruits, with typically massive embryo and cotyledons which are not distinctly separate. Most studies done on species of the *Eugenia* genus were on *E. jambolana*, whose extract has antidiabetic properties.[14] The leaves of *E. jambolana* have been reported to contain β-sitosterol, betulinic acid, mycaminose, crategolic (maslinic) acid, n-hepatcosane, n-nonacosane, n-hentriacontane, noctacosanol, n-triacontanol, n-dotricontanol, quercetin myricetin, myricitrin, flavonol glycosides and acylated flavonol glycosides.[15,16] In the Mascarenes, 16 species are endemic to Mauritius (out of which five species have been recently transferred from the *Monimiastrum* genus to *Eugenia* in 2008 following ITS and ETS rDNA sequence studies,[2] 3 are endemic to Réunion island, 1 to Rodrigues and 2 have been introduced and naturalized. In Mauritius, *Eugenia* species including *E. tinifolia* (endemic) are traditionally used as purgative, diuretic and mixed with other green teas for the treatment of rheumatism.[17]

The increasing awareness of the use of traditional medicines to treat minor and major ailments justifies the need to further study endemic plant species with medicinal potential. The scientific validation of information on the antimicrobial and molecular attributes of such plant species constitutes a fundamental aspect of such investigations. Up to now most of the studies carried out on endemic *Eugenia* species from Mauritius were focused on the antioxidant potential of their extracts. The results obtained constitute the basis for the potential use of the *Eugenia* species leaf extract as a source of natural antioxidants.[18–20] The anti-proliferative and apoptotic activity of *E. pollicina* endemic to Mauritius, has also been reported.[21] In this study, we have used phytochemical and molecular tools in an attempt to characterize endemic *Eugenia* species from Mauritius islands. The use of phytochemical profiling coupled with molecular markers has previously been reported for the categorization of species, conservation

Correspondence: Joyce Govinden Soulange, Faculty of Agriculture, University of Mauritius, Le Reduit 80837, Moka, Mauritius.
Tel. +23.040.37400
E-mail: joyces@uom.ac.mu

Key words: *Eugenia*, antibacterial activity, DPPH, phenolics, RAPD, ISSR.

Acknowledgements: the authors of this work wish to acknowledge the commendable contribution of the Mauritian Wildlife Foundation during the course of the study which has led to the production of this document.

Contributions: the authors contributed equally.

Funding: this work was funded by the University of Mauritius.

Received for publication: 14 February 2013.
Revision received: 17 September 2013.
Accepted for publication: 17 September 2013.


*International Journal of Plant Biology 2014; 5:4728*
*doi:10.4081/pb.2014.4728*

practices and proper management of plant resources.[22]

## Materials and Methods

### Plant material

Plant specimens used in this study are described in Figure 1. Leaves of *E.* spp (big leaves) and *E.* spp (small leaves) were collected from mature plants at Choisy, Poste de Flacq (East of Mauritius) and those of *E. crassipetala*, *E. kanakana* and *E. tinifolia* were collected from Le Pouce Mountain (North of Mauritius). Voucher specimens of each were deposited at the Herbarium of the Mauritius Sugar Industry Research Institute (Réduit, Mauritius). Young leaves were kept for extraction of genomic DNA and mature leaves were kept for phytochemical analysis.

### Metabolite extraction

For the antioxidant assay, mature leaves (25 g) were cleaned and cut into small pieces (≈ 2×3 mm) and put to macerate in three different solvent systems (250 mL): dichloromethane only, dichloromethane/methanol (50/50 v/v) and methanol only for 48 hours. The crude extract was filtered and concentrated to dryness *in vacuo* at 45°C. Residues were resuspended in 100 mL 95% methanol and kept at 4°C. For the antimicrobial assay, residues from the methanol only solvent system were partitioned by solvent-solvent partitioning between hexane (200 mL) and methanol. The residues were resuspended in100 mL 95% methanol and kept at 4°C.

### Phytochemical screening

Phytochemical screening was based on qualitative methods described previously,[23,24] through a series of test tube tests for coumarins, tannins, anthraquinones, leucoanthocyanins and flavonoids. Phenols, alkaloids, steroids and terpenes were identified by thin layer chromatography (TLC) and UV techniques.

### Determination of total flavonoid content

The total flavonoid content (TFC) was determined using a spectrophotometric method. Initially, 2.5 mL of crude extract was treated with 5% $NaN0_2$ solution (150 µL) for 5 min followed by 10% $AlCl_3$ (150 µL) for 1 min. 1M NaOH (1 mL) was then added and the tubes were properly vortexed. Absorbance of the reaction mixture was read at 510 nm on Milton Roy Spectronic 1001 Plus UV-Visible. A quercetin standard curve was made (50-200 µg/mL). Total flavonoid content was determined as quercetin equivalents (µg/g of fresh weight) using the following formula:

$$TFC = \frac{R \times D.F \times V}{2.5\times W}$$

R = result obtained from the standard curve; D.F = dilution factor; V = volume of stock solution and W = weight of plant material used. Triplicates readings were taken for each sample and the result averaged.

### Determination of total phenolic content

The total phenolic content (TPC) was determined using a spectrophotometric method. Initially, 2.5 mL of crude extract was treated with Folin-Ciocalteu reagent (250 µL) and the mixture was properly vortexed. After 3 min 20% $Na_2CO_3$ (1 mL), the tubes were allowed to stand for 40 min in a 40°C water bath. The tubes were allowed to cool in the dark and the absorbance of the reaction mixture was read at 685 nm on Milton Roy Spectronic 1001 Plus UV-Visible. A gallic acid standard curve was made (50-300 µg/mL). Total phenolic content was determined as gallic acid equivalents (µg/g of fresh weight) using the following formula:

$$TPC = \frac{R \times D.F \times V}{2.5\times W}$$

R = result obtained from the standard curve; D.F = dilution factor; V = volume of stock solution and W = weight of plant material used. Triplicates reading were taken for each sample and the result averaged.

### Biological assays

#### Antioxidant assay

This was carried out according to the 2.2-diphenyl-2-picrylhydrazyl (DPPH) assay system. 3900 µL of a 0.3 mM DPPH methanol solution was added to 100 µL solution of each crude extract or standard (25-125 g/mL) and allowed to react in the dark at room temperature for 30 min. The absorbance of the resulting mixture was measured at 515 nm and converted to percentage antioxidant activity (AA%), using the formula:

$$AA\% = [(Abs_{blank} - Abs_{sample}) \times 100]/Abs_{blank}$$

Abs = Absorbance.

Methanol (3900 µL) plus extract solution (100 µL) was used as blank. 3900 µL of 0.3 mM DPPH plus methanol (100 µL) was used as a negative control. Solution of quercetin served as positive control. This assay was carried out in triplicates for each plant extract.

#### Antibacterial assay

The serial microdilution method was used to determine the minimum inhibitory concentration (MIC) for antibacterial activity of fractionated extracts.[25] Two millilitres of three bacterial cultures [one Gram-positive, *Staphylococcus aureus* (ATCC no. 29213)], two Gram-negative, *Escherichia coli* (ATCC no. 25922) and *Proteus mirabilis* (ATCC no. 12453) were prepared and incubated overnight at 37°C. The overnight cultures were standardized by dilution with sterile MH (Mueller-Hinton) broth (1 mL bacteria/100 mL MH) to an absorbance of 0.4-0.6 at 600 nm. 100 µL of compound solution tested for each bacterium was two-fold serially diluted with 100 mL sterile distilled water in a sterile 96-well microplate. A similar two-fold of chloramphenicol (10 mg/mL) was used as positive control against each bacterium. Methanol was used as negative control and 100 µL of bacterial suspension was added to each well. The plates were covered, sealed with parafilm and incubated overnight at 37°C. Bacterial growth was assayed with the addition of 40 µL of 0.2 mg/mL ρ-iodonitrotetrazolium violet (INT) to each well after incubation at 37°C for 30 mins. Bacterial growth in the wells was indicated by a red color and colorless wells indicated inhibition of tested extracts.

### Molecular study

#### Genomic DNA extraction

Genomic DNA was extracted from young leaves by a modified CTAB method.[26] About 80 mg of leaves were ground using liquid nitrogen, dispensed in 750 µL of cetyltrimethylammonium bromide (CTAB) extraction buffer made up 2.5% (w/v) CTAB, 1.25 M NaCl, 0.1M Tris-HCl (pH 8), 0.02M EDTA (pH 8), 4% (w/v) PVP and 4% (v/v) -mercaptoethanol; in a 15 mL corning tube and placed in a 65°C water bath with occasional swirling for 45 min. 500 µL equal volume chloroform:isoamyl alcohol (24:1, v/v) was added, the contents mixed to form an emulsion and tubes spun in MIKRO 220R microcentrifuge at 13,000 r.p.m., 4°C for 15 min (this was repeated until no interface was visible). The supernatant was transferred to a sterile microcentrifuge tube to which 5M NaCl (150 µL) followed by 500 µL ice-cold isopropanol were added. DNA was allowed to precipitate for 1 hour at -37°C and then the tubes were centrifuged at 14,000 r.p.m., 4°C for 30 min. The supernatant was discarded. The pellet was washed with 500 µL and air dried at room temperature for 15 min. The pellet was dissolved in 30 µL sterile distilled water. RNAse A/Phenol was then done.

#### DNA amplification

25 ng of genomic DNA were amplified in a volume of 25 µL of 1 × *Taq* polymerase buffer (BioRad), 2mM $MgCl_2$, 2.5 mM dNTPs, 0.5 µM primer, 1 U/µL *Taq* polymerase.

### RAPD analysis

60 Operon primers were screened. Amplifications products were obtained using primers OPA-08, OPA-19, OPA-02, OPA-10, OPA-11, OPA-12, OPA-04, OPL-05 and OPD-02. The thermal cycling program (Applied Biosystems 2720 Thermal Cycler) consisted of forty cycles. Each cycle consisted of denaturation at 92°C for 30 sec, annealing at 35°C for 1 min, an extension at 72°C for 3 min followed by a final extension at 72°C for 10 min.

### ISSR analysis

Out of 25 screened primers, amplification products were obtained using ISSR 2, ISSR 3 and ISSR 4. The thermal cycling program (My Cycler™ BIO RAD) consisted of thirty five cycles. Each cycle consisted of denaturation at 94°C for 30 sec, annealing at 53°C (ISSR 2 and ISSR3) and 50°C (ISSR 4) for 1 min, an extension at 72°C for 2 min followed by a final extension at 72°C for 10 min. All PCR products were resolved on 1.5% agarose gel at 115V for 2.5 hr. PCR products were visualized by UV-fluorescent staining with ethidium bromide (0.5 µg/mL).

## Statistical analysis

### Antioxidant assay analysis

Spearman's rank correlation coefficient is a bivariate evaluation of any relatedness which is employed with rank-order data. In this study, it was computed to find out which one, between TFC and TPC, was more related to the reported antioxidant activity of the *Eugenia* species plant extracts. The correlation coefficient was calculated using Excel (2010) and Minitab 16. The closer the $R^2$ or $r_s$ value obtained is to 1, stronger is the relationship between the two variables (*i.e.* here, TFC or TPC with antioxidant activity). The closer the $R^2$ or $r_s$ value obtained is to 0, weaker is the relationship between the two variables.

## Analysis of RAPD and ISSR profiling

Band positions obtained on amplification of genomic DNA were scored for each *Eugenia* species, from photographic prints of gels. Scores were defined as either by presence, corresponding to value 1, or absence of bands, corresponding to value 0. Data were processed using the statistical software NTSYS-PC and DARwin 5 software to construct a UPGMA dendrogram using hierarchical clustering.[27,28] Using NTSYS-PC, a dissimilarity matrix was calculated utilizing Jaccard's coefficient.[29]

# Results and Discussion

## Chemical Screening

Initial phytochemical screening of crude extracts and fractions (Figure 1) revealed the presence of coumarins, tannins, flavonoids, saponins, phenols, alkaloids and terpenes. However, anthraquinones were detected in *E. tinifolia* only and leucoanthocyanins appeared only in the methanol fractions of the extracts of all species. High concentrations of phenolics and flavonoids have also been recently reported in in *E. jambolana* polar seed extracts.[30] Total flavonoid content (TFC) of most *Eugenia* species studied seemed to be higher in extracts containing methanol than those with dichloromethane (Equation 1). TFC of *Eugenia* species ranged from 1115.91±93.15 µg QE $g^{-1}$ FW (*E. crassipetala* – DCM:MeOH) to 60.04±7.13 µg QE $g^{-1}$ FW [*E.*spp (small leaves) – DCM only]. A similar trend was observed in

**Figure 1. Morphological characters of endemic *Eugenia* species from Mauritius.**

| Leaf picture | Eugenia species | Brief description |
|---|---|---|
|  | *E.* spp.(big leaves) | Newly discovered species. Small plant 2m50 high.<br>The plant grows in rocky ground in a dry lowland native forest at Choisy, Poste de Flacq. |
|  | *E.crassipetala* | Small tree which can reach 4 m high, newly recorded on Le Pouce Mountain. |
|  | *E.kanakana* | Small, erect and slender tree, which can reach 5 m high, found in the Mauritian indigenous upland forests. Collected on Le Pouce Mountain.<br>Its extract has chemopreventive potential |
|  | *E.spp.* (small leaves) | Newly discovered species. Small plant 2.50 m high.<br>The plant grows in rocky ground in a dry lowland native forest at Choisy, Poste de Flacq. |
|  | *E.tinifolia* | Small plant which can reach 3 m high, found in a mixed vegetation of native and exotic species on sites.<br>Used as purgative |

Total Phenolic content (TPC) of *Eugenia* species ranging from 2162.91±6.67 (*E. tinifolia*) to 119.81±20.80 µg GAE $g^{-1}$ FW in *E. tinifolia* and *E. spp* (small leaves), respectively (Equation 2). It was also noted that *E. crassipelata* and *E. tinifolia* extracts had higher TFC and TPC than all other *Eugenia* species studied.

$$TPC = \frac{R \times D.F \times V}{2.5\times W} \quad \text{(Eq. 1)}$$

R = result obtained from the standard curve; D.F = dilution factor; V = volume of stock solution and W = weight of plant material used. Triplicates reading were taken for each sample and the result averaged. Triplicates readings were taken for each sample and the result averaged.

$$TFC = \frac{R \times D.F \times V}{2.5\times W} \quad \text{(Eq. 2)}$$

R = result obtained from the standard curve; D.F = dilution factor; V = volume of stock solution and W = weight of plant material used. Triplicates reading were taken for each sample and the result averaged. Triplicates readings were taken for each sample and the result averaged.

## Antioxidant assay

The DPPH free radical activity of the leaf extract of *Eugenia* species was maximum in those extracted by the two most polar solvent system: dichloromethane:methanol (1:1, v/v) and methanol only solvent systems (Supplementary Table S1). This high antioxidant activity ranged between 95.50±0.02 to 91.88±0.02% inhibition /100 µL of extract.

A correlation analysis was performed to identify whether only flavonoids or the synergistic effect of phenols (flavonoids and non-flavonoids) in the *Eugenia* extracts accounted directly for antioxidant activity. The Spearman's correlation coefficient was used since from the regression graph, it was seen that there was a non-linear relationship between either TFC or TPC and DPPH free radical activity. From Supplementary Table S1, it could be deduced that phenolics and antioxidant activity were more closely related compared to flavonoids and antioxidant activity. From the correlation coefficient (0.570) and the regression graph 67.5% of the data explain of the logarithmic relationship between total phenolic content and percentage DPPH scavenging activity. From the correlation coefficient of total flavonoid content (0.533) is not much far away from that of TPC and antioxidant activity. However, only 34.45% (<60%) of the data explain the logarithmic relationship between total flavonoid content and percentage DPPH scavenging activity ($P<0.05$). We therefore can deduce that a higher significant ($P<0.05$) positive correlation exists between phenolics content of the *Eugenia* extracts and antioxidant activity as compared to flavonoid level.

## Antibacterial assay

The antimicrobial properties of crude extracts and fractions of *E.* spp (big leaves), *E. crassipetala*, *E. kanakana*, *E.*spp (small leaves) and *E. tinifolia* were tested against two Gram negative and one Gram positive bacteria. It was noted that *E. crassipetala* and *E. tinifolia* extracts showed the most significant antibacterial activity when tested against *E. coli*, *P. mirabilis* and *S. aureus;* with minimum inhibitory concentrations (MIC) lower than the positive control, chloramphenicol. Moreover, it was noted that antimicrobial activity of the *Eugenia* species leaf extracts seemed to be more important in crude dichloromethane fractions than in the methanol or hexane fractions (Supplementary Table S1). The antibacterial activity of *E. jambolana* seed extracts against *S. aureus*, *P. pneumoniae* and *P. aeruginosa* was reported to be more significant in polar fractions of same,[30] indicating that leaf and seed extracts of *Eugenia* differ with respect to their antimicrobial properties.

## Molecular study

Assessment of genetic diversity among the five *Eugenia* species was carried out using the 13 RAPD and 3 ISSR markers which gave the maximum polymorphic bands. Banding patterns for RAPD and ISSR amplification are represented in Equation 3 and Supplementary Figures. A higher percentage of polymorphic markers were observed as compared to the number of monomorphic markers (Supplementary Table S3). Unique bands have also been observed for *E. tinifolia.*156 RAPD markers and 48 ISSR markers were used to construct a dendogram. The five *Eugenia* were grouped in two distinct clades (X and Y) in the dendogram (Supplementary Figures).

$$AA\% = [(Absblank - Abssample) \times 100]/Abs\ blank \quad \text{(Eq. 3)}$$

$$AA\% = [(Absblank - Abssample) \times 100]/Abs\ blank$$

Abs = Absorbance.

In this study, the presence of monomorphic bands obtained with both RAPD markers OPA-19 (Supplementary Figures), OPA-4, OPA-02 and OPA-12 (Supplementary Figures), (at 850bp, 1000bp, 1200bp, 1200bp and 1750bp respectively); and ISSR markers - ISSR 2, ISSR 3 and ISSR 4 (Supplementary Figures),(at 1200bp and 1000bp; 3000bp and 475bp respectively) corroborates with the taxonomical classification of these five species in the same genus and the similarities in their phytochemical profiles on average. Leucoanthocyanins were detected in all the five species only upon fractionation probably due to impurities or non-polar compounds forming complexes with leucoanthocyanins and preventing free anthocyanin or anthocyanidin to be completely hydrolyzed (on heating with acid) to anthocyan (glucosidal) pigments giving a red solution as observed from the magnesium-acid reduction test.

Furthermore, since anthraquinones were identified only in *E. tinifolia*, therefore it may be hypothesised that presence of unique bands [OPA-10 (650bp), OPP-20 (1400bp, 1000bp and 550bp), OPL-05 (700bp), OPA-12 (4000bp, 1200bp and 1000bp), OPA-11 (800bp and 750bp), OPA-02 (1000bp), OPA-19 (700bp and 750bp) and OPA-08 (1000bp)] and ISSR markers [ISSR-2 (1200bp), ISSR-3 (700bp), and ISSR-4 (450bp)] in lanes containing *E. tinifolia* could be associated with presence of anthraquinones in the methanolic crude extract and fractions. These unique bands obtained with *E. tinifolia* were indicated by arrows on Supplementary Figures.

The UPGMA dendogram generated using the banding pattern obtained with RAPD and ISSR markers clearly showed that *E.spp (big)*, *E. kanakana* and *E.spp (small)* are clustered in the same clade; and *E. crassipetala* and *E. tinifolia* are clustered together in another clade as shown by clade X and Y in Supplementary Figures. Within this cluster analysis, based on the dissimilarity matrix, it was found that *E. tinifolia* was the most different from the rest of the species of the group. *E. tinifolia* and *E. kanakana* had genotypes which were most distantly related (0.999) and in contrast, *E.*spp (big leaves) and *E.*spp (small leaves) were the two closest species (0.4863) as shown by the dissimilarity matrix.

Clustering of the *Eugenia* species within the dendogram corroborates with results obtained from phytochemical screening and biological assays. Phytoprofiling clearly revealed that *E.*spp (big leaves), *E. kanakana* and *E.* spp (small leaves) (members of clade X) were the species to contain saponins, while only traces of saponins were detected in the two species of clade labelled Y (Table 1). Total flavonoid and phenolics content evaluation showed that *E. crassipetala* and *E. tinifolia* extracts contained far more flavonoid and phenolics compared to the three species of clade labelled X. This may account for the high antibacterial properties of these two extracts as shown by the MIC values (Supplementary Table S2). The following mechanism most probably occurred: hydroxyl group present in the phenol molecule ionizes in the presence of a base to give $-O^-$ ion.[31] These interact with electronegative atoms in peptide bonds by forming hydrogen bonds or positively charged side chains of basic amino acids (lysine, histidine, arginine respectively)

**Table 1. Phytochemical composition of crude extracts and fractions of *Eugenia* species of Mauritius.**

| *Eugenia* species | *E.* spp (big leaves) | *E. crassipetala* | *E. kanakana* | *E.* spp (small leaves) | *E. tinifolia* |
|---|---|---|---|---|---|
| Crude extract | | | | | |
| Coumarins | ++ | + | + | + | - |
| Tannins | ++ | ++ | ++ | ++ | ++ |
| Anthraquinones | - | - | - | - | ++ |
| Leucoanthocyanins | - | - | - | - | - |
| Flavonoids | ++ | ++ | ++ | ++ | ++ |
| Saponins | ++ | + | ++ | ++ | + |
| Alkaloids | ++ | ++ | ++ | ++ | ++ |
| Phenols | ++ | ++ | ++ | ++ | ++ |
| Terpenes | ++ | ++ | ++ | ++ | ++ |
| Hexane fraction | | | | | |
| Coumarins | - | - | - | - | - |
| Tannins | ++ | ++ | ++ | ++ | ++ |
| Anthraquinones | - | - | - | - | ++ |
| Leucoanthocyanins | - | - | - | - | - |
| Flavonoids | - | - | - | - | - |
| Alkaloids | ++ | ++ | ++ | ++ | ++ |
| Phenols | ++ | ++ | ++ | ++ | ++ |
| Terpenes | ++ | ++ | ++ | ++ | ++ |
| Methanol fraction | | | | | |
| Coumarins | - | - | - | - | - |
| Tannins | ++ | ++ | ++ | ++ | ++ |
| Anthraquinones | - | - | - | - | ++ |
| Leucoanthocyanins | ++ | ++ | ++ | ++ | ++ |
| Flavonoids | ++ | ++ | ++ | ++ | ++ |
| Alkaloids | - | + | - | - | - |
| Phenols | ++ | ++ | ++ | ++ | ++ |
| Terpenes | - | - | - | - | - |

+ Trace, ++ Metabolite Present. - Metabolite Absent



of proteins by stronger ionic bonds. This eventually affects microorganisms' membrane proteins permeability, causing water to enter into the cells by osmosis resulting in cell death when these burst. As noted by the MIC values obtained with *E. tinifolia* crude extract and fractions, anthraquinones, another phenolic compound, add up to the antimicrobial activity of the plant extract. Moreover, correlation of TPC with antioxidant activity may be explained by the synergistic antioxidant effects of flavonoids and non-flavonoids possibly in terms of hydrogen-ion donors: higher amounts of phenolics components results in rapid and increased antioxidant activity, since there are more hydrogen ions dissociations from the hydroxyl groups of phenol at a given time. At the morphological level *E. tinifolia* and *E. crassipetala* share certain morphological features namely shape of the leaves which are obvate as compared to circular in species of clade X. In fact *E. tinifolia* and *E. crassipetala* were previously classified in the genus *Monimiastrum* and have been recently transferred to Eugenia.[32]

According to the dendogram, clade X clusters the two undescribed *Eugenia* species together. This classification corroborates with the similarities in their morphological features and in their phytochemical profiles. These species have significant similarities in their total phenolics content (Equation 2) and leaf morphology (Table 1) – both are slightly crinkled and orbicular in shape, except that *E.* spp (small leaves) leaves are smaller. Taking into account climatic conditions prevailing in the area of the sample collection of both species, it is to be noted that it is relatively dry. *E.* spp (small leaves) may be hypothesized to have evolved after *E.* spp (big leaves) with more adaptive features: The smaller the leaves are, the lower the surface area to volume ratio and rate of transpiration. Since less water is lost by plants with smaller leaves, the plant can fix more carbon dioxide by photosynthesis. This is the first study making use of phytochemical and molecular tools for the classification of *Eugenia* species endemic to Mauritius. Phytoprofiling of these endemic species clearly showed that these species are rich in a diverse range of compounds and also that phytochemical data strongly correlates with morphological and molecular features. The presence of anthraquinones in *E. tinifolia* validates its use as a purgative. Biological assays clearly demonstrated that *E. crassipetala* and *E. tinifolia* could be coined as having powerful antimicrobial and antioxidant properties. These are of paramount importance since it paves the way to more in-depth studies for the classification of the species, and unveiling medicinal virtues for those, medicinally-speaking, unknown species through the isolation and characterization of bioactive molecules.

## References

1. The plant list. Eugenia. Available from: http://www.theplantlist.org/browse/A/Myrtaceae/Eugenia/.
2. Snow N, Mc Fadden J, Evans TM, et al. Morphological and molecular evidence of polyphyly in Rhodomyrtus (Myrtaceae: Myrteae). Systemc Botany 2011;36:390-404.
3. Rendle AB. The classification of flowering plants II dicotyledons. 1st Ed. London: Cambridge University Press; 1975. pp 381-385.
4. Stefanello ME, Pascoal AC, Salvador MJ. Essential oils from neotropical Myrtaceae: chemical diversity and biological properties. Chem Biodivers 2011;8:73-94.
5. Lis-Balchin M, Hart SL, Deans SG. Pharmacological and antimicrobial studies on different tea-tree oils (Melaleuca-alternifolia, Leptospermum scoparium or Manuka and Kunzeaericoides or Kanuka),

originating in Australia and New Zealand. Phytother Res 2000;14:623-9.
6. Mabberley DJ. The plant book. A portable dictionary of the higher plants. Cambridge: Cambridge University Press; 1989.
7. Mabberley DJ. Mabberley's plant book. A portable dictionary of the higher plants. 3rd ed. Cambridge: Cambridge University Press; 2008.
8. Meloni M, Perini D, Filigheddu R, Binnelli G. Genetic variation in five Mediterranean populations of Juniperus phoenicea as revealed by Inter-Simple Sequence Repeat (ISSR) Markers. Ann Botany 2006;7:299-304.
9. Mani A, Mishra R, Thomas G. Elucidation of diversity among Psidium species using morphological and SPAR methods. J Phytol 2011;3:53-61.
10. Wright ME, Ranker TA. Dispersal and habitat fidelity of bog and forest growth forms of Hawaiian Metrosideros (Myrtaceae). Biol J Linn Soc 2010;162:558-71.
11. Keil M, Griffin AR. Use of random amplified polymorphic DNA (RAPD) markers in the discrimination and verification of genotypes in Eucalyptus. Theor Appl Genet 1994;89:442-50.
12. Nejatzadeh-Barandozi F, Naghavi MR, Enferadi ST, et al. Genetic diversity of accessions of Iranian Aloe vera based on horticultural traits and RAPD markers. Ind Crop Prod 2012;37:347-51.
13. Levi A, Thomas CE, Newman M, et al. ISSR and AFLP markers differ among American watermelon cultivars with limited genetic diversity. J Am Soc Horticult Sci 2004;129: 553-8.
14. Tripathi AK, Bhoyar PK, Baheti JR, et al. Herbal antidiabetics: a review. Int J Res Pharmaceutic Sci 2011;2:30-7.
15. Mahmoud II, Marzouk MS, Moharram FA, et al. Acylated flavonol glycosides from Eugenia jambolana leaves. Phytochemistry 2001;58:1239-44.
16. Sagrawat H, Mann AS, Kharya MD. Pharmacological potential of Eugenia jambolana: a review. Pharmacogn Mag 2006;2;96-105.
17. Gurib-Fakim A, Gueho J. Plantes médicinales de Maurice. Vol 2. Rose Hill: Edition de l'Ocean Indien; 1996
18. Ramful D, Aumjaud B, Neergheen VS, et al. Polyphenolic content and antioxidant activity of Eugenia pollicina leaf extract in vitro and in model emulsion systems. Food Res Int 2011;44:1190-6.
19. Neergheen VS, Soobrattee MA, Bahorun T, Aruoma OI. Characterization of the phenolic constituents in Mauritian endemic plants as determinants of their antioxidant activities in vitro. J Plant Physiol 2006;163:787-99.
20. Neergheen VS, Bahorun T, Pugo-Gunsam P Ng Foong Lin D Ramful D Aruoma O I. Phenolic constituents and antioxidant efficacies of some Mauritian traditional preparations commonly used against cardiovascular disease. Int J Pharmacogn Phytocheml Res 2010;2:44-52.
21. Neergheen VS, Bahorun T, Aruoma OI. In vitro anti proliferative and apoptotic screening of Mauritian endemic plants. Univ Mauritius Res J 2011;17:240-55.
22. Messaoud C, Bejaoui A Boussaid M. Fruit color, chemical and genetic diversity and structure of Myrtus communis L. var. italica Mill. morph populations. Biochem Syst Ecol 2011;39:570-80.
23. Harborne JB, Mabry TJ, Mabry H. The flavonoids. New York : Academic Press, 1975.
24. Trease GE, Evans EC. Pharmacology. 11th ed. London: Balliere Tindall; 1989. pp 60-75.
25. Eloff JN. A sensitive and quick plate microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 1998;64:711-3.
26. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 1987;19:11-5.
27. Rohlf FJ. 2005. NTSYSpc (Numerical taxonomy and multivariate analysis system). Version 2.2. 2005. Applied Biostatistics Inc., New York.
28. Perrier X, Jacquemoud-Collet JP. DARwin Software. 2006. Available from: http://darwin.cirad.fr/Download.php
29. Jaccard P. New research on the floral distribution. Bull Soc Vaudoise Sci Nat 1908;44:223-70.
30. Bag A, Bhattacharya SK, Pal NK, Chattopadhyay RR. In vitro antibacterial potential of Eugenia jambolana seed extracts agaist multidrug-resistant human pathogens. Microbiol Res 2012;167:352-7.
31. Walton NJ, Brown DE. Chemicals from plants: perspectives on plant secondary products. London: Imperial College Press; 1999.
32. Mauritius Sugar Industry Research Institute. Flore des Mascareignes. Rhizophoracees to Araliacees. Réduit 1990.