Biochemical and phylogenetic analysis of *Eugenia* and *Syzygium* species from Mauritius

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Abstract

The Myrtaceae represents a plant family with several species having important medicinal virtues. In view of these properties four *Syzygium* species namely *S. latifolium*, *S .commersonii*, *S. coriaceum*, *S. petrinense* and two *Eugenia* species namely *E. pollicina* and *E. pyxidata* were studied. Phytochemical analysis showed that the methanol extracts had higher content of total phenolics and total flavonoids than the hexane extracts. The *in vitro* antioxidant activity evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant potential (FRAP) assays was significant. The antibacterial activity of the extracts against *Staphylococcus epidermidis*, *Staphylococcus aureus, Propionibacterium acnes* and *Escherichia coli* was also promising. Species studied displayed significant tyrosinase inhibitory activity with *S. petrinense* having the highest activity. Interestingly, when the expression analysis of the tyrosinase cDNA in mouse melanocyte cells was studied it was observed that the methanolic *Syzygium* extracts inhibited melanogenesis by upregulating or downregulating the tyrosinase gene. Phylogenetic analysis of the ribosomal DNA sequences showed the genetic uniqueness of each plant species reflecting in their unique biological activity. Bioprospecting of endemic Mauritian *Eugenia* and *Syzygium* could be envisaged against acne vulgaris, skin hyperpigmentation and possible exploitation as phytotherapeutics.

Keywords: Antioxidant, antimicrobial, expression analysis, phylogenetics, Myrtaceae

Graphical abstract

Endemic *Syzygium latifolium, Syzygium coriaceum, Syzygium commersoni, Syzygium petrinense, Eugenia pollicina, Eugenia pyxidata* from Mauritius

Terpenes, Tannins, Phenols, Alkaloids, Saponins, Anthraquinones, Leucoanthocyanins, Flavonols

Antimicrobial activity

Antioxidant activity

Antityrosinase activity (except for *Syzygium coriaceum* and *Eugenia pyxidata*)

1. Introduction

The Myrtaceae is regarded as the eighth largest family of angiosperms comprising about 140 genera and 3800-5800 species including the *Syzygium* Gaertn. and *Eugenia* L. genera. The Myrtaceae family has been reported to have therapeutic uses such as treating dysentery, gastroenteritis, stomach ache and diabetes (Azevedo et al., 2012). The threat of increasing antibiotic resistant bacteria and increase in the price of synthetic drugs is leading mankind towards the development of novel antimicrobials. More than 600 commonly used plants form part of the Mauritian pharmacopoeia out of which 15% have been identified as endemic to the island (Gurib-Fakim, 2007) ;

This study reports the bioprospecting of endemic species of the genera *Syzygium* and *Eugenia* species from Mauritius Island for the detection of novel phytotherapeutic leads.

Syzygium is the largest genus in Myrtaceae, consisting of about 1200 species and is distributed over the tropics and subtropics of the Old World (Biffin et al., 2010). This genus has the highest degree of diversity that extends from Malaysia to north eastern Australia (Ayyanar and Subash-Babu, 2012). *Syzygium* was considered as a section of *Eugenia* until Schmid (1972) revealed that these two genera are independent lineages by analysing anatomical data and this was confirmed by molecular studies (Byng et al., 2015). Scott (1990) provided a description of fourteen *Syzygium* species endemic to Mauritius and recently Bosser and Florens (2000) discovered another species, *S. guehoi*, native to the island. A new species, *S. pyneei*, has been discovered in Mondrain Reserve in Mauritius (Byng et al., 2015). Species of this genus usually have economic importance and they have been utilized in food and medicine. *S. glomeratum*, native to Mauritius, is used in traditional medicine for the treatment of headaches and coughs while *S. aromaticum* is used as a spice (Cheong and Ranghoo-Sanmukhiya, 2013). The four *Syzygium* species reported in this study are *S. petrinense*, *S. coriaceum*, *S. commersonii* and *S. latifolium*.

The genus *Eugenia* consists of about 1009 species and is distributed from Mexico and the Caribbean to northern Argentina (Moresco et al., 2016). Cultivation of some species can be seen in the tropics and subtropics (Azevedo et al., 2012). Sixteen *Eugenia* species, vernacular name “Bois de clous”, are native to Mauritius. *E. tinifolia* is used as a laxative and diuretic while *E. pollicina* has anti-proliferative and apoptotic activities (Neergheen et al., 2011). A very rare

species occurring on the island is *E. bojeri* while *E. longuensis* is regarded as extinct. New species have also been discovered e.g. *E. alletiana* was found in 2006 in the Brise Fer forest located in the Black River Gorges National Park (Baider and Florens, 2013). The two *Eugenia* species used in this research are: *E. pyxidata* and *E. pollicina*.

The phytochemical profiles found in several *Eugenia* and *Syzygium* species have been investigated and are responsible for their different biological activities. A study carried out by Joshi et al., (2011) on *Eugenia caryophyllata* (clove), revealed the presence of tannins and glycosides in its ethanol extracts. The leaves of *Syzygium cumini*, commonly called Jambolan, were shown to contain alkaloids, flavonoids, saponins, tannins, phenols and steroids (Gowri and Vasantha, 2010). Research performed on extracts of *Eugenia polyantha*, demonstrated that saponins, flavonoids, tannins, phenol, steroid and terpenoids were present (Hassan et al., 2015). Phytochemical screening conducted on *Eugenia brejoensis* showed high flavonoid content in the leaves while in *Eugenia floccosa*, almost all of the main phytoconstituents were detected (Azevedo et al., 2012; Tresina and Mohan, 2014).

The antioxidant virtues of *Syzygium* and *Eugenia* species have been reported (Eshwarappa et al., 2014, Bagetti et al., 2011). Eshwarappa et al., (2014) showed that the methanolic extract of *S. cumini* leaves possessed higher antioxidant potential than the standard ascorbic acid. The extracts of the purple-fleshed *E. uniflora* fruit had the highest antioxidant capacity compared to the extracts of the red and orange-fleshed fruits (Bagetti et al., 2011). Ethanolic extracts of leaves of *E. catharinae* were shown to have antioxidant activity (Moresco et al., 2016).

The antimicrobial virtues of both *Syzygium* and *Eugenia* have been previously explored. Djipa et al., (2000) evaluated the antimicrobial activity of the acetone and aqueous extracts of *S. jambos* against *Staphylococcus aureus*, *Yersinia enterocolitica*, and coagulase negative *Staphylococci.* Gowri and Vasantha (2010) revealed that the methanol extract of the leaves of *S. cumini* were more effective than the aqueous extract against seven Gram-negative bacteria and two Gram-positive bacteria. Brunchault et al., (2014) investigated the antimicrobial activities of the crude extracts and fractions of some Mauritian *Eugenia* species against *S. aureus*, *Escherichia coli* and *Proteus mirabilis* and the extracts of *E. crassipetala* and *E. tinifolia* revealed to be the most active against the tested microorganisms. Tresina and Mohan (2014) demonstrated that the methanol extract of *E. floccosa* leaf was effective against fifteen pathogenic bacteria out of the sixteen that were tested.

Antibacterial activity of *Syzygium* and *Eugenia* species against *S. aureus* and *E. coli* has not been reported yet.

There are no previous studies on the anti-acne properties of the endemic *Eugenia* and *Syzygium* species from Mauritius. Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit. Inflamed lesions develop due to the presence of the bacterium, *Propionibacterium acnes*. *P. acnes* plays a central role in acne vulgaris and releases a number of enzymes that metabolizes sebum triglycerides into free fatty acids. These free fatty acids act as chemokines generating an inflammatory response (Burkhart and Burkhart, 2007).

Melanin is a pigment responsible for hair, skin and eye colour. The tyrosinase enzyme plays a pivotal role in the production of melanin. This copper containing enzyme catalyses the hydroxylation of tyrosinase to 3,4-dihydroxyphenylalanine (DOPA) and the oxidation reaction of DOPA to DOPAquinone is the second step of melanogenesis. Skin hyperpigmentation is a term used to describe the over-production of melanin either in the epidermis or dermis layers of the skin. Various factors can result in stimulating tyrosinase, resulting in an overproduction of melanin and ultimately hyperpigmented lesions are visible on the surface of the skin (Ando et al., 1998; Momtaz et al., 2008; Rigopoulos et al., 2007). Prevention of hyperpigmentation would require suppressing the melanogenesis pathway and although many synthetic compounds exist in the cosmeceutical industry, most have adverse damaging effects (Solano et al., 2006). Natural ingredients, such as plant extracts, could offer safer alternatives for depigmentation than chemical medical cosmetics. No previous studies have investigated the tyrosinase inhibitory activity or down-regulation of tyrosinase as well as the anti-acne properties of the selected *Eugenia* and *Syzygium* species.

Molecular markers have been used to study the endemic *Eugenia* and *Syzygium* species of Mauritius (Brunchault et al., 2014, Cheong and Ranghoo-Sanmukhiya., 2013). The chloroplast and nuclear markers were used to review the phylogenetic relationships of South African *Eugenia* species and some of the Mauritian species (Van der Merwe (2005); Murillo et al., 2012). Van der Merwe (2005) and Mazine et al., (2006) focused on neotropical *Eugenia* species using chloroplast DNA sequences. The molecular systematics of *Syzygium* species was carried out by Biffin et al., (2010) while the whole chloroplast genome of *Syzygium cumini* was unveiled by Asif et al*.,* (2013).

Molecular phylogenetics of the endemic *Eugenia* and *Syzygium* species under study in this paper has not been previously reported.

This study therefore aimed at unveiling the biological properties of *Syzygium* and *Eugenia* species including their antioxidant, antimicrobial, anti-acne, antityrosinase activities and down-regulation of tyrosinase gene expression in mouse melanocyte cells. Moreover the phylogenetics of *S. latifolium*, *S. commersoni*, *S. coriaceum*, *S. petrinense*, *E. pollicina* and *E. pyxidata* was unveiled using nuclear Internal Transcribed Spacer (ITS) sequences to authenticate the plant material being used.

2. Material and Methods

2.1. Plant material collection

The four *Syzygium* and two *Eugenia* species were collected from the National Parks and Conservation Services (NPCS), GPS 20.4264° S, 57.4509° E. Voucher specimens of each species were deposited at the Mauritius Herbarium (MAU) and accession numbers were provided (Table 1).

Table 1
Syzygium and *Eugenia* species used in phylogenetic analysis (Species sequenced in this study are in bold).

Species used	Genebank Accession Number	Herbarium Accession Number
Syzygium nervosum	KP093040	
Syzygium caryophyllatum	KU301787	
Syzygium samarangense	KC815990	
Syzygium australe	KM064931	
Syzygium paniculatum	KM064993	
Syzygium cordatum	EF026625	
Eugenia orbiculata	KJ187631	
Eugenia brongniartiana	KJ187615	
Eugenia neoglomerata	KJ187626	
Eugenia bimarginata	KJ187611	
Eugenia pisiformis	KJ187634	
Eucalyptus tetragona	AF190364	
Syzygium petrinense	**581203**	**MAU 0018402**
Syzygium commersonii	**KY58120**	**MAU 0018404**
Eugenia pyxidata	**KY581205**	**MAU 0018405**
Eugenia pollicina	**KY581206**	**MAU 0018975**
Syzygium latifolium	**KY581207**	**MAU 0018974**
Syzygium coriaceum	**KY581208**	**MAU 0018403**

2.2. Extract preparation

Methanol and hexane extracts were prepared from the leaves as reported previously (Brunchault et al., 2014).. Mature leaves (25g) were cut into 2 x 3mm pieces) and macerated in three different solvent systems (250ml): dichloromethane only, dichloromethane/methanol (50/50 v/v) and methanol for 48 hours. The crude extract was filtered and concentrated to dryness *in vacuo* at 45°C. Residues were re-suspended in 100ml 95% methanol and kept at 4°C. For the antimicrobial assay, residues from the methanol system were extracted by solvent-solvent partitioning between hexane (200ml) and methanol. The residues were re-suspended in100ml 95% methanol and kept at 4°C for antioxidant, antibacterial, antityrosinase and tyrosinase down- regulation investigation

2.3. DNA Isolation

Genomic DNA extraction was done using a protocol described previously by Govinden-Soulange et al., (2007) and Cheong and Ranghoo-Sanmukhiya (2013). Essentially 0.075 g of fresh leaf tissue was ground in liquid Nitrogen and was then transferred into a tube containing 0.75 ml of CTAB buffer with 0.2%) β- mercaptoethanol and 2% (v/v) PVP. The tube was incubated in a 60˚C water-bath for 25-30 minutes followed by extraction with 2/3 volume of chloroform:isoamyl alcohol. The mixture was then micro-centrifuged at 10,000 rpm for 10 minutes and this purification step was repeated at least two times. Genomic DNA was precipitated with 2/3 volume of ice-cold isopropanol and the tube was left overnight at 4˚C to allow further precipitation of DNA and then spun in a microcentrifuge for 30 minutes at 13,000 rpm. The supernatant was discarded and washed with 70% alcohol. The pellet was air dried under the hood and re-dissolved in 50 µl sterile distilled water.

2.4. PCR amplification

The ITS region was amplified using primer pairs AB101 (5'-ACGAATTCATGGTCCGGTGAAGTGTTCG-3') and AB102 (5-GAATTCCCCGGTTCGCTCGCCGTTAC-3') (Murillo *et al.,* 2012). Each 100 µl of reaction consisted of 4 U of DreamTaq™ DNA Polymerase, 100 mM KCl, 20 mM Tris-HCl pH 8.8, 20mM MgCL2, 80 mM dNTP, 0.4 mM primer and approximately 20 ng of template DNA. Thirty amplification cycles were performed on a Biorad minicycler. An initial denaturation step was performed at 95 °C for 5 min followed by a denaturation step at 94 °C for 1 min, annealing at 55 °C for 1 min and primer extension at 72 °C for 10 mins. The final primer elongation segment of

the run was extended to 10 min at 72 °C. PCR products were separated on a 1.5% (w/v) ethidium bromide-stained agarose gel and the bands were visualized under UV illumination

2.5. DNA Sequencing

PCR amplicons were purified using Fermantas PCR purification kits following the manufacturers' instructions. DNA sequencing reactions were done using a Big Dye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) following the protocol outlined by the manufacturers. Sequencing reaction products were purified by ExoSAP method. All DNA sequences were determined with an ABI 3500 DNA sequencer (Applied Biosystems) at Inqaba Biotechnical Industries (Pty) Ltd, South Africa.

2.6. Phylogenetic analysis

2.6.1. Sequence editing and alignment.

Forward and reverse sequences were assembled and edited using CLC Main Workbench Version 7.6 (https://www.qiagenbioinformatics.com/). All sequences were deposited in GenBank (Table 1). DNA sequences were aligned using the online version of MAFFT 5.66 (Katoh et al., 2005) using the iterative refinement method and the following settings: Needleman–Wunsch algorithm active, 2 tree rebuilding steps, 1000 iterations and the program's default values for gap opening and gap extension penalties. No further manual manipulation of the alignment was performed. A number of *Syzygium* and *Eugenia* species were retrieved from the GenBank and used in the phylogenetic analysis (Table 1). *Eucalyptus tetragona* was used as the outgroup.

2.6.2. Phylogenetic analyses and tree generation.

Maximum parsimony searches were performed with the software PAUP* (Swofford, 2002). Gaps were treated as missing characters and cladograms were generated using heuristic searches based on 1000 random sequence additions, tree branch swapping (TBR) and MULPARS effective. Bootstrap analyses were done using the same settings with a 1000 replicates. Bayesian inference was performed using MrBayes v.3.1.1 (Huelsenbeck and Ronquist, 2001). The nucleotide substitution model that best fit each sequence matrix was determined with JModeltest (Darriba et al., 2012) with model selection based on the Akaike information criterion (AIC). The Monte Carlo Markov Chains (MCMC) run in MrBayes was set to 5×10^6 generations with tree sampling every

100th generations. The log-likelihood scores for the sampled tree were analysed to determine the number of generations that could be excluded before stationary has been reached (i.e. the "burn-in" value). The trees generated were viewed in FigTree v.1.4.0 (http:// tree.bio.ed.ac.uk/software/figtree/) to obtain tree topologies and the posterior probability values for their nodes.

2.7. Phytochemical Screening

Phytochemical screening was based on qualitative methods through a series of test tube tests. Phenols, alkaloids, steroids and terpenes were identified by thin layer chromatography (TLC) and UV techniques (Harborne et al., 1975; Trease and Evans, 1989).

2.7.1. Determination of total phenolic content (TPC)

The Folin-Ciocalteu procedure (Singleton et al., 1965) was employed to estimate the TPC of the extracts. Absorbance at 760 nm was recorded, against a methanol (MeOH) blank, using a spectrophotometer (Milton Roy Spectronic 1001 Plus UV-visible). The results were expressed as means of triplicate analyses in milligram per gram extract (mg GAE/g). Gallic acid served as the positive control.

2.7.2. Determination of total flavonoid content (TFC)

TFC was determined using the method of Brunchault et al., (2014). Absorbance of the reaction mixture was read at 510 nm on Milton Roy Spectronic 1001 Plus UV-Visible. Total flavonoid content was determined as quercetin equivalents (µg/g of fresh weight). Triplicates readings were taken for each sample.

2.8. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Radical scavenging activity of plant extracts against stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined using a modified method of Wong et al*.,* 2006. The control was composed of methanol and DPPH solution while blanks contained methanol instead of DPPH solution.

Quercetin was used as the positive control. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula, (Mensor et al., 2001)

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

Where: A_0= absorption of control

A_1 = absorption of test samples

2.9. Ferric reducing antioxidant potential (FRAP)

The adapted method of Benzie and Strain (1996) was used to assess the ferric reducing antioxidant power of each plant extract. Absorbance was read at 593 nm on Milton Roy Spectronic 1001 Plus UV-Visible spectrophotometer. A calibration curve of ferrous sulfate (0.1 to 1.0 mM) was used to express the results in terms of mM Fe^{2+} per milligram extract (mM Fe^{2+}/mg). All determinations were carried out in triplicates. Ascorbic acid served as the positive control.

2.10. Antibacterial Assay

Minimum inhibitory concentrations (MIC's) of the crude extracts against *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 12228 and *Staphylococcus aureus* ATCC 29213 was determined using the broth microdilution assay (Eloff, 1998). Overnight bacterial cultures were standardised with sterile Mueller-Hinton broth to achieve an absorbance of 0.4-0.6 at 600 nm. Chloramphenicol (Sigma) (0.01 mg/mL) was used as a positive control while MeOH and (Dichloromethane) DCM were used as a negative control. Following overnight incubation at 37 °C, the MIC which is the lowest concentration at which bacterial growth inhibition occurred, was determined by an indicator dye (p-iodonitrotetrazolium violet (INT) with a 0.2 mg/mL) clear wells indicated growth inhibition. All measurements of MIC values were repeated in triplicates.

2.11. Propionibacterium acnes antibacterial assay

The two extracts of all six species (methanol and hexane) were tested against *Propionibacterium acnes* (ATCC 6919) by determining the (MIC) values obtained by a microdilution method as described by Sharma et al., (2014) with slight modifications. *P. acnes* was cultured from a Kwik-Stick on mouse brain and heart agar and incubated under anaerobic conditions at 37 °C for 72 h.

The 72 h bacterial culture was suspended in nutrient broth and adjusted to an optical density (OD_{600}) of 0.132. In a sterile 96-well plate, 100 µl of the plant extracts (2 mg/mL in 10% dimethyl sulphoxide (DMSO) and the positive control tetracycline were diluted with nutrient broth. Twofold serial dilutions were made in the nutrient broth with a concentration ranging from 2000-15.6 µg/mL for the plant extracts and 100-0.78 µg/mL for the positive control, tetracycline. The bacterial suspension (100 µl) was added to all the wells of the microtiter plate. To the control wells, 2.5% DMSO and bacterial suspension without samples served as the negative and bacterial controls respectively. The plates were incubated for 72 h at 37 °C under anaerobic conditions. The MIC was determined visually after the addition of PrestoBlue reagent (20 µl) as described by Lall et al., (2013)

2.12. Anti-tyrosinase assay

The two plant extracts of all six species (methanol and hexane) were dissolved in dimethyl sulfoxide (DMSO) to a final stock concentration of 20 mg/mL. The stock solutions were two-fold serially diluted in in 50 mM potassium phosphate buffer (pH 6.5) to a final concentration of 600 µg/mL. Kojic acid was the positive control with a final concentration ranging between 400-3.1 µg/mL. In a 96-well plate, the sample solutions (70 µl) were combined with 30 µl of mushroom tyrosinase (48 Units/mL) in triplicate. The plate was incubated for 5 minutes at room temperature before the addition of 2 mM L-tyrosine (110 µl) to each well. The final concentration of the extracts ranged between 1000-7.8 µg/mL. The optical densities of the wells were analysed at 492 nm for 30 minutes at room temperature with the BIO-TEK PowerWave XS multi-well plate reader. The fifty percent inhibitory concentration (IC_{50}) value was determined by the use of Graph Pad Prism 4 software (Momtaz et al., 2008).

2.13. Mouse melanocyte cell treatments with plant extracts

In a 24-well plate with mouse melanocyte cell treatments with plant extracts (MEM), B16-F10 mouse melanocyte cells were cultured to 1 x 10^6 cells per well and respective wells were then treated with 70 µg/mL *S. petrinense,* or 130 µg/mL *S. commersonii* or 70 µg/mL *S. latifolium* in triplicate and incubated for 72 hours at 37 °C at a 5% CO_2 level. To extract RNA from treated B16-F10 mouse melanocyte cells, the Qiagen RNeasy minikit (Qiagen, Hilden, Germany) was used with few modifications. The additional on-column DNAse enzyme digestion using the RNase

free DNase purification kit (Qiagen, Hilden, Germany) was included to eliminate genomic DNA contamination. The purity of the samples were then assessed on the NanodropTM 2000 spectrophotometer (Thermo Scientific, Minnesosta, USA). Further evaluation of the mRNA quality included a Seakem® LE Agarose (Lonza Rockland, USA) gel electrophoresis of all the RNA samples.

2.14. Tyrosinase gene amplification and Real Time quantitative PCR: Relative Expression Analysis of Tyrosinase cDNA in Mouse Melanocyte Cells

mRNA isolated from mouse melanocytes treated with each of *S. petrinense, S. commersonii* and *S. latifolium* extracts, was used to synthesize cDNA by means of designed oligo (dT) primers and random hexamers. These primers were manufactured by Integrated DNA Technologies (IDT, USA). The cDNA synthesis kit (Thermo Scientific, Minnesosta, USA) was used to convert the mRNA into cDNA.

For all subsequent PCR reactions, the primers for the tyrosinase gene and the housekeeping gene were selected by stability tests obtained from the Genevestigator V3 database (Hruz et al., 2008). The primer sequences used for *M. musculus* tyrosinase (P11344) and *M. musculus* carnitine palmitoyltransferase 2 (CPT2) (NC_000070) were designed with Primer3 Software (version 0.4.0).

Real-time quantitative PCR (RT-qPCR) analysis was conducted with the Biorad CFX96 TouchTM Real-Time PCR detection system (Bio-rad Laboratories, Johannesburg, South Africa) and the SYBR® Green Master Mix (Life Technologies, Johannesburg, South Africa). The gene expression of tyrosinase across all samples was quantified by assessing its stability against the housekeeping gene, CPT2, for reference.

The cDNA of each mouse melanocyte treated sample was placed in triplicate into the 96-well plate using the maximization strategy (Vandesompele et al., 2002). Each plate consisted out of one gene, either TYR or (Carnitine palmitoyltransferase) CPT2, for analysis. A ten-fold cDNA serial dilution series from a 1:5 standard dilution (5 ng/µl), to a 1:10 000 000 000 standard dilution (2.56 x 10-6) was also incorporated in triplicate for each plate. For RT-qPCR amplification, reaction volumes of 10 µl consisted of 5 µl 1x SYBR® Green Master Mix, 0.2 µM forward and reverse primer, 4 µl ddH$_2$0 and 1 µl cDNA template (1.25 ng/µl) or water in the case of non-template

controls. The samples were run for 10 min/95 °C then forty cycles of amplification were carried out with the following thermal profile: 10 sec/95 °C, 30 sec/ 59 °C, 10 sec/72 °C. To verify the primer specificity, a melting point analysis was included where the samples were heated from 65 °C to 95 °C with 5 second increments.

The Biorad CFX ManagerTM software was used to generate Crossing Point values (CP) as well as the melting curves, standard curves and amplification plots for the TYR and CPT2 genes. The TYR gene relative expression values were normalized to the reference gene CPT2 expression values (Vandesompele et al., 2002). Statistical analysis of the relative expression data was done with GraphPad Prism 5.04 software using log-transformed normal distributed relative quantification data. A one-way ANOVA analysis, measured at a $p \leq 0.05$ level of statistical significance, was used to detect significant differences between the DMSO and *S. petrinense, S. commersonii* and *S. latifolium* plant extract mouse melanocyte treated samples.

2.15. Statistical Analysis

All readings were taken in triplicates and the experimental results were expressed as means ± standard error (SE). One way analysis of variance (ANOVA) and Tukey's test were carried out to test any significant differences among the different plant extracts. Correlations between TPC, TFC, total antioxidant capacity, and antibacterial activities were carried out. All analyses were done in Minitab statistical software, version 17.0 and all charts and graphs were done using Microsoft Excel, version 2007. Results were considered significantly different with $p < 0.05$

3. Results and Discussion

3.1. PCR amplification of the ITS regions

Amplification of the ITS region yielded single amplicons of around 621bp for all taxa studied, 550 characters were not informative and 71 parsimony-informative.

3.2. Phylogenetic Reconstruction

Parsimony analysis of the ITS sequences yielded three most parsimonious trees with tree length = 113 steps, consistency index (CI) = 0.7788 and retention index (RI) = 0.9164 (Fig. 1). Phylogenetic trees generated from parsimony and Bayesian analysis clustered *Eugenia* species separate from

the *Syzygium* species. The Mauritian *Eugenia* and *Syzygium* species clustered in separate monophyletic group (Bootstrap =72, posterior probability (PP) =1). *Eugenia pixidata* and *E. polliciana* clustered in a separate monophyletic group along with *E. orbiculata* (Bootstrap =67, posterior probability (PP) =1). The Mauritian *Syzygium* species clustered in a separate clade (Bootstrap =58, posterior probability (PP)=0.9).

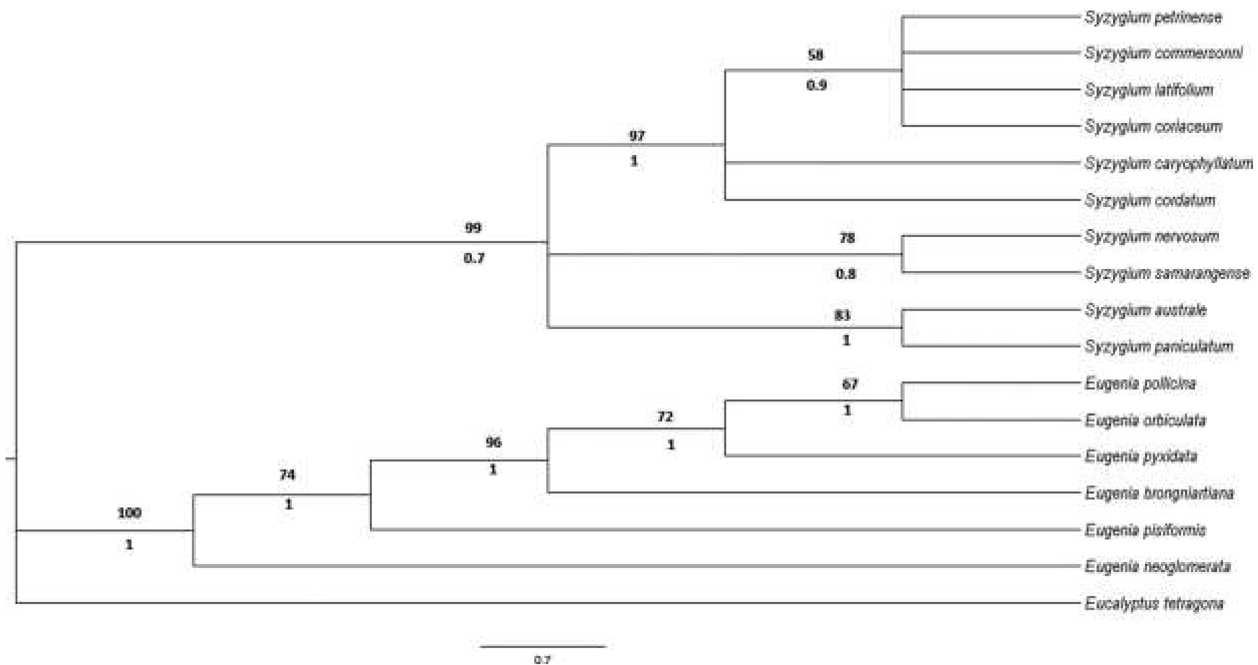

Fig. 1. Phylogenetic relationships of *Syzygium* and *Eugenia* species.

The ITS region of the ribosomal genes proved to be very efficient in determining the variation among the *Syzygium* and *Eugenia* as reported previously (Murillo et al., 2012). DNA sequence alignment revealed significant differences in the ITS sequences of Mauritian *Syzygium* and *Eugenia* species allowing clustering of these species in separate clades with other species studied (Murillo et al., 2012). Significant differences were observed between the Mauritian *Syzygium* and other *Syzygium* studied and these data can further contribute to the broader phylogeny of the Myrtaceae family. Genome sequencing may reveal significant differences which can be further used to explain unique transcriptomic profiles to account for their biological activities.

Table 2
Phytochemicals detected in methanol and hexane extracts of *Syzygium* and *Eugenia* species.

Species	*Syzygium latifolium*		*Syzygium coriaceum*		*Syzygium commersonii*		*Syzygium petrinense*		*Eugenia pollicina*		*Eugenia pyxidata*	
Solvent used	M	He	M	He	M	He	M	He	M	He	M	He
Coumarins	–	–	–	–	–	–	–	–	–	–	–	–
Terpenes	+ +	+	+ +	+ +	+	+ +	+ +	+ +	–	+	–	+
Hydrolysable Tannins	+ +	+	+ +	+	+ +	+	+ +	+	+ +	+	+ +	+
Phenol	+ +	+	+ +	+	+ +	–	+ +	+	+ +	+	+ +	+
Alkaloids	–	+	–	+	–	–	–	+	–	+	–	+
Saponins	+	–	+ +	–	+ +	–	+ +	–	+ +	–	+	–
Anthraquinones	+	–	+ +	–	+ +	–	–	–	–	–	+	–
Leuco-anthocyanins	–	+	+	+	–	–	–	–	–	–	–	+
Flavonols	+ +	+	+ +	+	+ +	–	+	–	+ +	–	+ +	+

Key: '+'= present in trace amounts, '+ +'= present, '-'= absent, 'H'= hydrolysable tannins, 'M'= methanol, 'He'= hexane.

3.3. Phytochemical screening

Hydrolysable tannins were present in all the methanol and hexane extracts. Terpenes and phenols were detected in all the six species through TLC analysis. Saponins and flavonols were detected in all the six species. The analysis also revealed the presence of anthraquinones and leucoanthocyanins in only 4 of the species under study (*Syzygium latifolium*, *S.coriaceum*, *S. commersonii* , *Eugenia pyxidata*) (Table 2).

3.4. Total Phenolic Content

The highest level of phenolics was recorded in the methanol extract of *S. petrinense* (8.2 ± 0.04 mg Gallic Acid Equivalent (GAE) g^{-1} extract) followed by the methanol extract of *E. pyxidata* (7.2 ± 0.17 mg GAE g^{-1} extract). All of the hexane extracts of contained significantly lower levels of phenolics than the methanol extracts ($p<0.05$) (Table 3).

3.5. Total Flavonoid Content

Hexane extracts contained lowest level of flavonoids. The highest level of total flavonoid content was detected in *E. pyxidata* with methanol (0.78 ± 0.00 mg QE g^{-1} extract) ($p<0.05$) (Table 3).
The phytochemical profile of the studied Myrtaceae revealed the presence of bioactive metabolites such as terpenes, tannins, phenols, alkaloids, saponins, anthraquinones, leucoanthocyanins and flavonols in the extracts as observed by Brunchault et al., (2014) in other *Eugenia* species, namely *E. crassipetala*, *E. kanakana* and *E. tinifolia*. The total phenolics in the methanol extracts of the six species under scrutiny were considerably higher than their hexane extracts. In general, methanol has been shown to extract low molecular weight polyphenols more efficiently (Dai and Mumper, 2010). Similarly, the total flavonoid content of the methanol extracts was generally higher than that of the hexane extracts. The bioactive compounds may have had greater solubility in methanol than in hexane. Comparable results were obtained previously with *S. caryophyllatum* and *S. densiflorum* and endemic *Eugenia* species. (Subramanian et al., 2014; Eshwarappa et al., 2014; Brunchault et al., 2014).

Table 3
TPC, TFC, DPPH and FRAP values of the methanol and hexane extracts of *Eugenia* and *Syzygium* species from Mauritius.

Species	TPC content (mg GAE/g extract)		TFC content (mg QE/g extract		DPPH IC_{50} (µg/mL)	FRAP value (mM Fe2+/mg extract)	
	M	He	M	He	M	M	He
S. latifolium	4.29 ± 0.31^{e}	0.29 ± 0.21^{f}	0.31 ± 0.55^{f}	0.18 ± 0.02^{g}	40.91 ± 1.94^{c}	23.34 ± 0.921 e	2.04 ± 0.103 g
S. commersonii	4.80 ± 0.24^{d}	0.22 ± 0.23^{f}	0.11 ± 0.00^{e}	0.34 ± 0.00^{h}	18.73 ± 1.96^{d}	43.58 ± 0.492 c	2.55 ± 0.0444^{fg}
S. coriaceum	6.88 ± 0.38^{bc}	0.65 ± 0.85^{f}	0.44 ± 0.00^{c}	0.11 ± 0.01^{h}	71.72 ± 10.48^{a}	81.07 ± 0.75^{a}	3.28 ± 0.02^{fg}
S. petrinense	8.18 ± 0.39^{a}	0.57 ± 0.12^{f}	0.44 ± 0.00^{c}	0.12 ± 0.00^{h}	18.50 ± 2.39^{d}	81.11 ± 0.51^{a}	3.39 ± 0.01^{f}
E. pyxidata	7.18 ± 0.17^{b}	0.3 ± 0.12^{f}	0.78 ± 0.02^{a}	0.38 ± 0.00^{d}	54.19 ± 3.98^{bc}	33.91 ± 0.19^{d}	3.74 ± 0.00^{f}
E. pollicina	6.4 ± 0.15^{c}	0.34 ± 0.36^{f}	0.55 ± 0.01^{b}	0.08 ± 0.01^{i}	63.86 ± 2.41^{ab}	47.65 ± 0.65^{b}	3.18 ± 0.01^{fg}
Positive Control (Quercetin)					21.19 ± 1.45^{d}		
Positive control (Ascorbic Acid)						31.07 ± 42.31^{d}	0.045 ± 0.02^{h}

Each value in the table is expressed as mean ± SE (n = 3).
Data was analysed using one-way ANOVA and Tukey's multiple comparison test.
Different letters between rows and columns indicate significant differences between extracts ($p < 0.05$).

3.6. Bioactivity

3.6.1. The DPPH scavenging assay

S. coriaceum and *S. commersonii* had significant ($p>0.05$) lowest IC_{50} values indicating highest free radical scavenging activities significantly similar to the positive control (Table 3). No DPPH free radical scavenging activity was observed in hexane extracts.

The DPPH IC_{50} values of the hexane extracts were not reported since at 1 mg/mL, the percentage inhibition was less than 50%. Both *Eugenia* and *Syzygium* methanol extracts had potent free radical scavenging activities, Interestingly, the extracts of *S. coriaceum* and *S. commersonii* had significantly similar scavenging activity as the positive control quercetin (IC_{50} : 21.19 µg/mL). Previous studies have reported a high radical scavenging activity in *S. cumini* and *S. fruticosum* with an IC_{50} value of the order of 9.90 µg/mL *S. cumini* and *S. fruticosum* (Eshwarappa et al., 2014; Islam et al., 2013).

3.6.2. The FRAP assay

Hexane extracts showed significantly ($p<0.05$) lowest Ferric reducing ability than methanol extracts although most extracts had higher FRAP values than the positive controls.

The highest FRAP values were obtained with the methanol extract of *S. petrinense* and *S.coriaceum*The methanol extracts of *E. pollicina*, *S. commersonii*, *E. pyxidata* and *S. latifolium*. The decreasing order of the antioxidant activity was as follows:

S. petrinense (MeOH)> *S. coriaceum* (MeOH)> *E. pollicina* (MeOH)> *S. commersonii* (MeOH)> *E. pyxidata* (MeOH)> *S. latifolium* (MeOH)> *E. pyxidata* (Hex)> *S. petrinense* (Hex)> *S. coriaceum* (Hex)> *E. pollicina* (Hex)> *S. commersonii* (Hex)> *S. latifolium* (Hex) (Table 3).

All the methanol extracts of *Eugenia* and *Syzygium* species were found to have higher ferric reducing power than the hexane extracts. The FRAP values of the methanol extracts of *S. petrinense* and *S. coriaceum* were not significantly different ($p>0.05$) whereas there was a significant difference between the FRAP values of the other four methanol extracts ($p<0.05$). Ruan et al*.*, (2008) reported that the ferric reducing power of *S. cumini* leaves was higher in the methanol extract than in the hexane fraction. The high antioxidant power of the methanol extracts may be attributed to the high phenolic and flavonoid contents in these extracts.

Correlation studies indicated that both TPC and TFC of the extracts had a strong positive linear relationship with the antioxidant activity. The high antioxidant activity of the extracts could be attributed to the high content of total phenolics and total flavonoids. Wong et al., (2006) reported

that polyphenols are usually responsible for the antioxidant activity of plant extracts and that a high antioxidant activity is attributed to the high amount of polyphenols. Sobeh et al ., (2018) attributed the significant antioxidant activity of *S. jambos* to its high phenolic content 466 mg gallic acid equivalents (GAE)/g extract.

Both DPPH and FRAP assay indicated a positive correlation between total phenolic content(r = 0.948) and the total flavonoid content (r =0.935) of the extracts.

3.6.3. Antibacterial activity

The methanol extract of *S. coriaceum* had the lowest MIC value of 0.39 mg/mL when it was tested against *E. coli* which was significantly ($p<0.05$) lower than that of chloramphenicol. The methanol extract of *E. pyxidata* and the hexane extract of *E. pollicina* were equally active against all the three bacteria tested with MIC values of 1.56 mg/mL and 3.13 mg/mL respectively. All methanol extracts had higher antimicrobial activity that the reference antibiotic (chloramphenicol) against *E.coli*. Hexane extracts of *S. coriaceum*, *S. commersonii* and *S. latifolium* were not active.

The methanol extract of *E. pollicina* had the lowest MIC value of 0.031 mg/mL against *P. acnes*. *S. petrinense* and *E. pyxidata* methanol and hexane extracts, moderately inhibited *P. acnes* at MIC values of 0.063 mg/mL and 0.5 mg/mL respectively. The methanol extracts of all the samples tested had a higher antibacterial activity than the hexane extracts (Table 4). The antibacterial activity against *S. epidermidis* and *E. coli* was positively correlated with the total phenolic content (r = 0.530 and r = 0.672 respectively), implying that a higher TPC was associated with higher antibacterial activities of the methanol extracts. The antibacterial activity against *S. epidermidis* and *E. coli* was positively correlated with the total flavonoid content (r = 0.784 and r = 0.477 respectively). This implies that a high TFC value was associated with higher antibacterial activities of the methanol extracts.

Table 4
Antimicrobial activity expressed as MIC (Minimum Inhibitory Concentration) of the methanol and hexane extracts of *Syzygium* and *Eugenia* species.

Sample	Solvent	MIC in mg/mL			
		S. epidermidis ATCC[*] 12228	*S. aureus* ATCC[*] 29213	*E. coli* ATCC[*] 25922	*P. acnes* ATCC[*] 6919
S. petrinense	Methanol	6.25	0.78	1.56	0.063
	Hexane	3.13	6.25	3.13	0.5
E. pyxidata	Methanol	1.56	1.56	1.56	0.063
	Hexane	6.25	3.13	6.25	0.5
E. pollicina	Methanol	1.56	3.13	1.56	0.031
	Hexane	3.13	3.13	3.13	NI[a]
S. latifolium[d]	Methanol	1.56	3.13	1.56	0.063
S. commersonii[d]	Methanol	3.13	3.13	1.56	0.125
S. coriaceum[d]	Methanol	0.78	1.56	0.39	0.125
Chloramphenicol[b] Tetracycline[c]	–	0.31[b]	0.63[b]	2.50[b]	0.00078[c]

a: No inhibition at the highest concentration tested (500 µg/mL).
b: Positive control tested against: *S. epidermidis, S. aureus, E. coli.*
c: Positive control tested against: *P. acnes*d : The yield from hexane extracts was too low to carry out MIC's.

The leaf extracts of all the 6 species under scrutiny were shown to inhibit both Gram-positive and Gram-negative bacterial growth. *S. cumini, S. travancoricum, S. aromaticum* and *S. jambos* have been reported to have broad antimicrobial activity against both Gram-positive and Gram-negative bacteria (Shafi et al., 2002; Arora and Kaur, 2007; Mohanty and Cock, 2009). Strong antimicrobial activity was observed against *E. coli* for most of the extracts which is noteworthy as the MIC values were lower than the control. Studies carried out with other *E. brejoensis* also showed that the latter were effective against Gram-positive and Gram-negative bacteria (Azevedo *et al*., 2012). *E. crassipetala* and *E. tinifolia*, endemic to Mauritius demonstrated significant antibacterial activity against *E. coli, S. aureus* and *P. mirabilis* (Brunchault *et al.,* 2014). *E. uniflora* essential oil extracts have also been reported to exhibit antimicrobial activity against *Bacillus licheniformis* (MIC: 0.63 mg/ml) (Sobeh et al., 2016).

The methanolic extract of *E. pollicina* displayed a notable antibacterial activity against *P. acnes* with an MIC of 0.031 mg/mL. *S. petrinense*, *E. pyxidata* and *S. latifolium* all moderately inhibited

P. acnes bacterial growth with MIC values of 0.063 mg/mL. No previous studies have evaluated the antibacterial activity of the selected plant species on *P. acnes*.

3.6.4. Tyrosinase inhibition assay

The methanol and hexane extracts of *Syzygium* and *Eugenia* species were tested for their tyrosinase inhibitory activity. The concentration of *S. petrinense* that inhibited half of the tyrosinase enzyme activity (IC_{50}) was 25.5±4.2 µg/mL. *E. pollicina* and *S. latifolium* displayed inhibition of the tyrosinase enzyme at IC_{50} values of 30.0±6.4 µg/mL and 32.2±7.1 µg/mL respectively (Table 5). Although *E. pollicina* and *S. latifolium* had moderate enzyme inhibitory activity, their IC_{50} values were significantly different ($p<0.001$) from the positive control, Kojic acid.

The use of tyrosinase inhibitors has become increasingly important in the cosmeceutical industry to treat skin hyperpigmentation. However, these agents have been found to have chronic, cytotoxic and mutagenic effects on the patient. There is a need for safer alternative herbal agents to treat skin hyperpigmentation. *Eugenia* and *Syzygium* species have been evaluated for their ability to inhibit the tyrosinase enzyme compared to a commercially available compound used to treat skin hyperpigmentation, Kojic acid. *S. petrinense* displayed the highest inhibition of the enzyme at an IC_{50} of 25.5±4.2 µg/mL with a p-value that was significantly different ($p<0.01$) from the positive control, Kojic acid. *S. latifolium* also displayed a noteworthy tyrosinase inhibitory activity with an IC_{50} of 32.2±7.1 µg/mL ($p<0.001$). Previous studies done by Palanisamy *et al*., (2011) found a propylene glycol extract from the leaves of *S. aqueum* to have a noteworthy tyrosinase inhibitory activity with an IC_{50} value of about 60 µg/mL. The tyrosinase activity of *S. aqueum* was suggested to be due to the presence of myricetin. Previous studies have isolated myricetin, a flavonoid from *Syzygium* species which displayed tyrosinase inhibitory activity *in vitro*. It was suggested that myricetin together with other phenolic compounds could contribute to the tyrosinase activity of certain *Syzygium* species. Research conducted by Huang *et al*., (2010) demonstrated the ability of myricetin to protect keratinocytes when exposed to UVB radiation. Flavonoids have previously been identified to inhibit the tyrosinase enzyme due to their ability to chelate the copper active site of the enzyme (Parvez et al., 2007). The results found in the current study for *S. petrinense* and *S. latifolium* could be due to the presence of the flavonoid, myricetin and could not only be used to treat skin hyperpigmentation but can also serve as a preventative measure for skin pigmentation. No previous studies have evaluated the tyrosinase inhibitory activity of the selected plant species.

Table 5
Anti-tyrosinase activity expressed as fifty percent inhibitory concentration (IC_{50}) ± Standard Deviation (SD) of the methanol and hexane extracts of *Syzygium* and *Eugenia* species.

Sample	Solvent	IC_{50} ± SD (μg/mL)	One-way ANOVA[b]
S. petrinense	Methanol	25.5 ± 4.2	$p < 0.01$
	Hexane	NI[a]	–
E. pyxidata	Methanol	NI[a]	–
	Hexane	NI[a]	–
E. pollicina	Methanol	30.0 ± 6.4	$p < 0.001$
	Hexane	NI[a]	–
S. latifolium	Methanol	32.2 ± 7.1	Pp < 0.001
S. commersonii	Methanol	50.9 ± 1.4	Pp < 0.001
S. coriaceum	Methanol	NI[a]	–
Kojic acid	–	2.94 ± 0.02	–

a: No inhibition at the highest concentration tested (200 μg/mL).
b: Data was analysed using one-way ANOVA and Tukey's multiple comparison test, n = 3.

3.6.5. Real Time quantitative PCR Relative Expression Analysis of Tyrosinase cDNA in Mouse Melanocyte Cells

Relative quantification provides a means to compare the potential changes in gene expression with the fold difference indicating whether the change in treatment is significant or not (Fraga et al., 2008). RT-qPCR analysis of the treated and untreated mouse melanocyte samples for both the TYR and CPT2 genes were performed with three technical replicates each. The CPT2 gene is expressed in mouse melanocyte cells at levels similar to the expression of tyrosinase (Fraga et al., 2008). Whether the tyrosinase expression was increased or decreased for each sample was revealed by comparison with CPT2 level expression. Besides normalising the tyrosinase gene expression levels by relative quantification, the reference gene CPT2, also ensured that the comparison made was between equivalent amounts of starting sample.

The relative expression of tyrosinase, normalised against the CPT2 reference gene, can be seen in Figure 2. The untreated mouse melanocyte cells indicated the highest expression of the tyrosinase gene, whilst all the other treatments down-regulated the tyrosinase mRNA compared to this control. Since the plant extracts were dissolved in DMSO prior to treatment, and DMSO clearly

had an effect on tyrosinase expression, it was used as a measure of comparison. The relative expression of the plant extract treated samples was compared against the DMSO control and a fold-change was calculated.

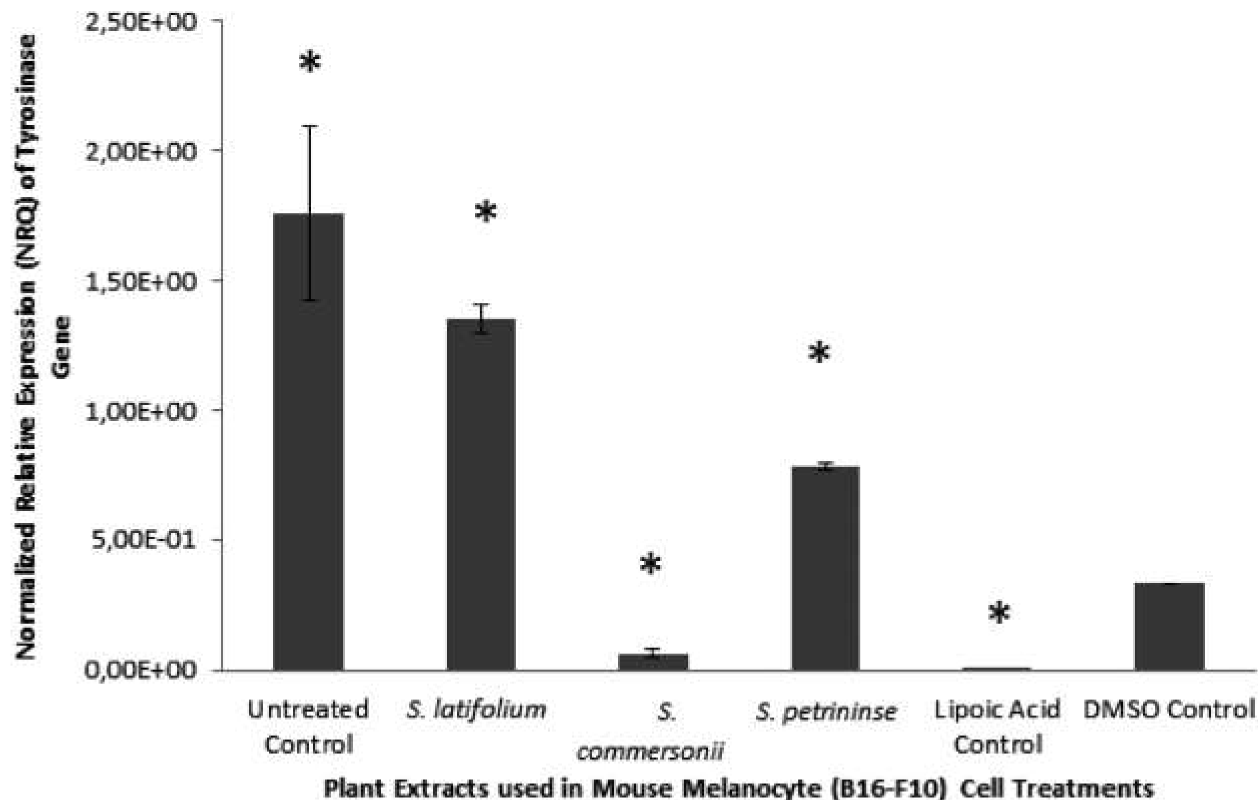

Fig. 2. Normalised RT-qPCR expression profiles of the tyrosinase mRNA in B16-F10 mouse melanocyte cells. The melanocyte cells were treated *with S. latifolium, S. commersonii, S. petrinense,* with lipoic acid, DMSO and untreated controls. RT-qPCR reactions were performed in triplicate and the average tyrosinase gene relative expression values were normalized relative to the expression of the CPT2 gene expression. Standard error of the average relative expression is indicated by the error bars in the figure. Statistical analysis of the relative expression data was done by one-way ANOVA analysis. * Indicates a statistical significant change in mean expression value when compared to the DMSO control. The extract *S.commersonii* inhibited tyrosinase gene expression (p-value < 0.05).

The one way ANOVA test indicated that all the samples were significantly different to the DMSO control at a calculated probability equal to or smaller than 0.05. Only the plant extract of *S. commersonii* down-regulated the tyrosinase gene expression by 5.2 fold respectively when

compared to the DMSO control, whilst extracts of *S. latifolium* and *S. petrinense* exhibited tyrosinase expression values 4.1 and 2.4 fold greater than the DMSO control, respectively. Tyrosinase gene expression in mouse melanocytes treated with these latter two extracts was similar to the untreated control. The positive control, lipoic acid is known to inhibit tyrosinase gene expression with the lowest 0.004 relative expression to the tyrosinase gene expression. Lipoic acid down-regulated the expression of the tyrosinase gene by 81.3 fold compared to the DMSO control. The melanogenesis pathway starts with the transcription of proteins required for melanin synthesis (Tang and Chen, 2015). True tyrosinase inhibitors would be different from melanin inhibitors, and would inhibit the transcription of proteins required in melanogenesis, regardless of direct enzyme interaction. Tyrosinase transcription may be inhibited by down-regulating either MC1R activity or MITF expression (Tang and Chen, 2015). In this study, the amount of tyrosinase mRNA, after the mouse melanocyte cell treatments with methanol extracts of *S. latifolium, S. commersonii* and *S. petrinense*, would indicate the potential genetic inhibiting activity of the methanolic *Syzigium* extracts. Down-regulating the expression of tyrosinase could include influencing the transcription factors, promoters or other molecular mechanisms of action within the melanogenesis pathway. The key regulating pathway of melanogenesis includes MITF and the MC1R/ cAMP pathways which induce it (Park et al., 2009). The natural plant extract *S. commersonii*, resulted in down-regulation of the tyrosinase gene expression in mouse melanocytes by 5.2 fold when compared to the DMSO control (p-value < 0.05). The plant extracts most likely down-regulated tyrosinase expression through the suppression of MITF expression.

Many effective inhibitors of the cAMP pathway of melanogenesis are flavonoid, anti-inflammatory, or antioxidant compounds (Nanda et al., 2006; Chan et al., 2014; Choi et al., 2008). In the phytochemical analysis*, S. commersonii* contained many flavonoids and anti-oxidant compounds, with the greatest scavenging activity noted for *S. latifolium, S. commersonii* or *S. petrinense*. The *S. commersonii* extract might therefore have down regulated the tyrosinase gene through reduction of the intracellular cAMP levels. If the melanin reducing effects of *S. commersonii* want to be considered further, a microarray analysis could indicate which genes, and thereby which pathways, the extracts have an effect on melanocyte and keratinocyte cells. Such a study would help understand their mechanism of action whilst detecting any potential off-target effects (Lall et al*.,* 2016).

Although the *S. petrinense* and *S. latifolium* extracts up-regulated the expression of the tyrosinase gene, they illustrated inhibition of melanogenesis by inhibiting the tyrosinase activity directly. It is clear therefore that different chemical constituents, present in the different plants, may act through various mechanisms to inhibit melanogenesis before melanin synthesis, during melanin synthesis or after melanin synthesis (Briganti et al., 2003).

4. Conclusion

Using the ribosomal DNA sequences the genetic uniqueness of each plant species used in this study was unveiled. Both *Eugenia* and *Syzygium* species had significant biological activities. The methanol extracts of two *Syzygium* species displayed noteworthy antioxidant, antibacterial and tyrosinase inhibitory activity. The results obtained for the methanol extracts of *S. petrinense* and *S. latifolium* suggest their potential to be used against acne vulgaris, skin hyperpigmentation and possible exploitation as phytotherapeutics. In-vivo studies revealed that *S. commersonii* down regulated the gene expression of the tyrosinase enzyme and as a result the melanogenesis pathway was suppressed and less melanin was formed.

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