



## Original article

# Antiglycation, comparative antioxidant potential, phenolic content and yield variation of essential oils from 19 exotic and endemic medicinal plants

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## ARTICLE INFO

## Article history:

Received 6 October 2017

Revised 27 April 2018

Accepted 1 May 2018

Available online 3 May 2018

## Keywords:

Antiglycation

Antioxidant

Phenolic

Essential oil

Yield variation

Nutraceutical

Pharmaceutical

Mauritius

## ABSTRACT

The antioxidant potential, antiglycation, and total phenolic content of essential oils (EOs) extracted from 19 medicinal plants were assessed. The variation in yield of the EOs with respect to altitude and season was also studied. The antioxidant potential of *Pimenta dioica* (L.) Merr., *Psiadia terebinthina* A.J. Scott, *Laurus nobilis* L., *Piper betle* L., and *Citrus hystrix* DC. showed IC<sub>50</sub> values less/equivalent to the positive controls. Weak correlations were observed between the 1,1-diphenyl-2-picryl hydrazyl (DPPH) and xanthine oxidase (XO) assays as well as between the DPPH and nitric oxide radical scavenging (NO) assay and between the XO and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay. *Cupressus macrocarpa* Hartw., *L. nobilis*, *Cinnamomum zeylanicum* Nees, and *Psidium guajava* L. successfully inhibited *in vitro* glycated end-products (IC<sub>50</sub>: 451.53 ± 3.00, 387.04 ± 1.53, 348.59 ± 3.34 and 401.48 ± 2.86 µg/mL respectively) compared to aminoguanidine (IC<sub>50</sub>: 546.69 ± 3.57 µg/mL). Some of the EOs had a high content of phenolic compounds. EOs such as *P. dioica*, *P. terebinthina*, *L. nobilis*, *P. guajava*, and *C. hystrix* were found to be rich in eugenol and other phenolic compounds. The EOs evaluated in the present study may have applications in the nutraceutical and pharmaceutical industries.

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## 1. Introduction

The biological properties of essential oils (EOs) derived from natural products have been recognized and used for centuries, although the scientific basis of this activity is still being investigated (El-Soud et al., 2012; Raut and Karuppaiyil, 2014). More than 300 EOs out of the approximately 3000 known EOs worldwide have gained importance for their wide range of biological activities (El-Soud et al., 2012; Raut and Karuppaiyil, 2014). For instance, EOs have been purported to be potent sources of new compounds which can be used in both the food industry and for medical purposes, as anti-mutagenic, anticancer, antioxidant, anti-inflammatory, immunomodulatory,

antiprotozoal agents (Ribeiro-Santos et al., 2017; Pandey et al., 2016; Perricone et al., 2015; Bakkali et al., 2008), as well as in the treatment of neurodegenerative diseases (Öztürk, 2012), diabetes, and hyper pigmentation. Additionally, EOs have been widely appraised as antimicrobial agents having the ability to overcome the resistant phenotype of multiple drug-resistant bacteria and to act against food-borne pathogens (Pandey et al., 2016; Shaaban et al., 2012; Burt, 2004).

Tropical islands like Mauritius are endowed with a rich floral biodiversity comprising of an interesting microcosm with diverse species including aromatic medicinal plants offering interesting biological activities (Aumeeruddy-Elalfi et al., 2015, 2016). Abiotic environmental factors (altitude, temperature, moisture, and other climatic conditions) have also been reported to affect chemical composition and yield of EOs (Moghaddam et al., 2015; Santos-Gomes and Fernandes-Ferreira, 2001). To the best of our knowledge, no study has attempted to study the biological properties of EOs isolated from common medicinal plants adapted to the tropical climate of Mauritius.

This report is therefore the first study designed to evaluate the antioxidant, antiglycation, and total phenolic content of EOs from

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Peer review under responsibility of King Saud University.



common medicinal plants of Mauritius – a tropical island that belongs to the Mascarene archipelago. For this purpose, a panel of *in vitro* assays was selected to assess the EOs. For the isolation of the EOs, 19 plants have been included among which two are endemic plants (*Psiadia arguta* Pers. (Voigt) and *Psiadia terebinthina* A.J. Scott), and the remaining 17 plants (*Citrus grandis* L., *Citrus hystrix* D.C., *Citrus reticulata* (Blanco), *Psidium guajava* L., *Pimenta dioica* L., *Lavandula x intermedia* var. Grosso L., *Salvia officinalis* L., *Cupressus macrocarpa* H., *Laurus nobilis* L., *Piper betle* L., *Rosmarinus officinalis* L., *Cymbopogon citratus* D.C. (Stapf), *Melaleuca quinquenervia* S.T. Blake (Cav.), *Cinnamomum zeylanicum* Nees, *Schinus terebinthifolius* R. and *Triphasia trifolia* (Burm. f.) P. Wilson are exotic to Mauritius.

## 2. Experimental

### 2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH), methanol, ascorbic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), potassium persulfate, xanthine, dimethylsulfoxide (DMSO), xanthine oxidase (XO), allopurinol, 2,4,6-tripyridyl-s-triazine (TPTZ), sodium acetate, glacial acetic acid, dilute hydrochloric acid (HCl), concentrated HCl, anhydrous iron (III) chloride (FeCl<sub>3</sub>·6H<sub>2</sub>O), trolox, sodium carbonate, Folin-Ciocalteu reagent, gallic acid, hydrogen peroxide, iron chloride, ethylenediaminetetraacetic acid (EDTA), ascorbic acid, 2 deoxy-D-ribose, phosphate buffer, sodium phosphate, sodium dihydrogen phosphate, sodium chloride, trichloroacetic acid, thiobarbituric acid, sodium nitroprusside, Griess Reagent, AAPH (2,2'-Azobis (2-amidinopropane) dihydrochloride), and fluorescein were purchased from Sigma-Aldrich (St. Louis, USA).

### 2.2. Collection of plant materials

The plants included in the present study are used traditionally by the local people as medicinal herbs and food plants (Gurib-Fakim et al., 1996; Nunkoo and Mahomoodally, 2012). Plants were collected from the central region of Mauritius which is 151 m above sea level and benefits from a mild tropical maritime climate throughout the year. The leaves of *Pimenta dioica* Linn. Merr (PD), *Lavandula x intermedia* var. Grosso Linn. (LI), *Salvia officinalis* Linn. (SO), *Cupressus macrocarpa* Hartw. (CM), *Citrus grandis* Linn. (CGI), *Laurus nobilis* Linn. (LN), *Piper betle* Linn. (PB), *Rosmarinus officinalis* Linn. (RO), *Cymbopogon citratus* D.C. (Stapf.) (CC), *Melaleuca quinquenervia* (Carv.) S.T. Blake (MQ), *Cinnamomum zeylanicum* Nees (CZ), *Schinus terebinthifolius* Raddi. (ST), *Psidium guajava* Linn. (PG), *Psiadia arguta* Pers. (Voigt.) (PA), *Psiadia terebinthina* A.J. Scott. (PT), *Triphasia trifolia* (Burm. f.) P. Wilson (TT) and fully ripened fruits of three citrus species namely *Citrus grandis* Linn. Osbeck (CGp), *Citrus hystrix* D.C. (CH), and *Citrus reticulata* Blanco (CR) were collected at the University farm. Each plant was identified by a local botanist. A voucher specimen (2014-AE2) has been deposited at the Local Herbarium, Mauritius. A local repository database was constructed whereby plant samples were assigned a collection number for future data mining and sharing.

### 2.3. Extraction of the EOs

The leaves of the plants were gently plucked, washed, and finely cut into pieces, while the fruits were peeled off carefully with the use of a sharp knife to avoid any damage of the oil glands and finely reduced to uniform size. The plant materials were then subjected to the hydrodistillation process for a period of 3 h using a Clevenger apparatus (Soković and van Griensven, 2006). The

distillates of the EOs thus yielded were then dried over anhydrous sodium sulfate, filtered and stored at –4 °C until further analysis (Hussain et al., 2008).

### 2.4. Yield assessment of *P. betle* and *C. citratus*

The yield of the EOs were investigated with respect to various environmental and geographical parameters. In this endeavor, two plants having exhibited the best yield and which are also easily available, were collected from the three regions mentioned below. The following variables were considered; altitude, humidity, amount of rainfall and temperature. To study the possible variation of the EOs yield and composition, the plants were selected monthly and the EO extracted immediately after collection.

Fresh whole plant samples were collected from the different regions of Mauritius:

- (i) Location 1: North (latitude 20° 0' 49S, longitude 57° 34' 26E and altitude 107 m above sea level),
- (ii) Location 2: South (latitude 20° 29' 7S, longitude 57° 33' 3E and altitude 151 m above sea level),
- (iii) Location 3: Central region (at the University of Mauritius farm in Réduit: latitude 20° 13' 39S, longitude 57° 29' 33E and altitude 283 m above sea level).

Mauritius benefits from a mild tropical maritime climate throughout the year. The country has two seasons: warm humid summer from November to April and a cooler dry winter from June to September. October and May are the transition period between each season. The mean summer temperature is 24.7 °C and the mean winter temperature is 20.4 °C. The temperature difference between the seasons is only 4.3 °C. The warmest months are January and February with average daytime maximum temperature reaching 29.2 °C and the coolest months are July and August when average night minimum temperatures drops down to 16.4 °C. Long term mean annual rainfall over the Island is 2010 mm with the wettest months being February and March and the driest month, October. Most of the rainfall occurs in the summer months. The island receives 6.5 h to above 8.0 h of bright sunshine daily. In summer months around 6.0 h of bright sunshine are received at location 3, whereas location 1 are exposed to 7.5 to over 8.0 h of bright sunshine. In winter months, location 3 receives around 5.0 h of bright sunshine whereas region 1 above 7.5 h of bright sunshine.

### 2.5. Antioxidant assays

#### 2.5.1. 1,1-Diphenyl-2-picryl hydrazyl (DPPH)

The free radical scavenging capacity was assessed as described by Muanda et al. (Muanda et al., 2010), with slight modification, whereby 200 µL of 100 µM DPPH (2,2-diphenyl-1-picrylhydrazyl) (in a solution of 50–50% (v/v) methanol-distilled water) was mixed with the 100 µL EO of different concentrations, (diluted in methanol). For the positive control, 200 µL of DPPH solution 100 µM were added to 100 µL of ascorbic acid at different concentrations. For the blank wells (reaction mixture without EO), 200 µL of DPPH solution 100 µM were added to 100 µL of methanol. The reaction mixture was incubated in the dark for 30 min, and the optical density was recorded at a wavelength of 517 nm against the blank. The optical density of DPPH in samples with regard to the control system was used to calculate the antioxidant activity as inhibition percentage (I%) of DPPH radical, with  $I\% = [(A_b - A_s)/A_b] * 100$ ; where  $A_b$ : absorbance of the blank sample after  $T_{30min}$ ;  $A_s$ : (absorbance of the sample at  $T_{30min}$ ) – (absorbance of the sample at  $T_{0min}$ ). The assay was carried out in triplicate of each sample and control, at different concentrations.

### 2.5.2. 2,2'-Azinobis(3-ethylbenzothiazoline-6 sulphonic acid) (ABTS) assay

The technique for the generation of the 2,2'-azino-bis (3-ethylbenzothiazoline-6 sulphonic acid) radical, ABTS<sup>•+</sup>, was evaluated according to the method described by Re et al. (1999), with slight modification. The assay involves the direct formation of the compound ABTS<sup>•+</sup>, a bluish green chromophore through the reaction between ABTS salt and potassium persulfate which has a maximum absorption at wavelengths 645 nm, 734 nm and 815 nm. ABTS<sup>•+</sup> stock solution was prepared by mixing 50 mL of two millimolar ABTS salt to 300  $\mu$ L 17 mM potassium persulfate, 16 h prior to the experiment in order to have a stable maximal absorbance. The reaction was initiated by adding 190  $\mu$ L of ABTS<sup>•+</sup> (diluted from ABTS<sup>•+</sup> stock solution prepared, to a final absorbance of 0.70 ( $\pm$ 0.02) at 734 nm) to 10  $\mu$ L of EOs at different concentrations, diluted in ethanol. The spectrophotometric analysis of ABTS<sup>•+</sup> scavenging activity was determined and for comparison of the activity, a positive control, ascorbic acid and a blank (ethanol was used instead of EOs) were also evaluated. The optical density of ABTS<sup>•+</sup> in samples with regard to control system was used to calculate the antioxidant activity as inhibition percentage (%) of ABTS radical;  $\% = [(A_b - A_s)/A_b] * 100$ ; where  $A_b$ : absorbance of the blank sample after six mins ( $T_{6min}$ ) at 734 nm,  $A_s$ : (absorbance of the sample at  $T_{6min}$  at 734 nm) – (absorbance of the sample at  $T_{0min}$  at 734 nm).

### 2.5.3. Xanthine oxidase (XO) assay

The inhibitory effect on XO was determined spectrophotometrically by monitoring the increase in the absorbance at 295 nm (Kong et al., 2000). The reaction mixture was prepared for this assay, consisting of 400  $\mu$ L of 200 mM sodium pyrophosphate buffer (pH 7.5), 200  $\mu$ L of 0.6 mM xanthine, 20  $\mu$ L of EOs at different concentrations (or positive control, allopurinol) dissolved in dimethylsulfoxide (DMSO), and 200  $\mu$ L XO (0.1 U). The formation of uric acid was observed by an increase in absorption at absorbance of 295 nm. All EO evaluations were performed in triplicate. The dosages for XOD inhibitory activity were assayed at 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781  $\mu$ g/mL and expressed as, inhibitory (%) =  $(1 - b/a) * 100$ , where,  $a$  = change in absorbance per min without the sample and  $b$  = change in absorbance per min with the sample.

### 2.5.4. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging capacity of each EO was estimated according Bozin et al. (2006) with slight modification. Phosphate buffer, pH 7 was prepared by diluting 0.24 g of sodium phosphate and 0.21 g sodium dihydrogen phosphate in 500 mL distilled water. The pH of the buffer was measured using a pH meter and 4.36 g sodium chloride was added to adjust the pH to 7. The reaction mixture consisted of 100  $\mu$ L hydrogen peroxide (150  $\mu$ mol/L), 100  $\mu$ L iron chloride (30 M), 100  $\mu$ L EDTA (30 M), 100  $\mu$ L (0.22%) ascorbic acid, 100  $\mu$ L 2 deoxy-D-ribose (25.8 M) and 100  $\mu$ L EO at different concentrations and completed to 1 mL with phosphate buffer pH 7. The reaction mixture was incubated for 30 min at 37 °C, after which, 1 mL trichloroacetic acid (60 g/L) and 0.5 mL thiobarbituric acid (1 g in 100 mL of 0.05 mol/L NaOH) were added and boiled for 20 min for the development of the light pink chromogen. The reaction mixture was allowed to cool and absorbance was read at 532 nm against a blank containing buffer and 2-deoxyribose. Ascorbic acid was used instead of EOs for the evaluation of the positive control. The inhibition percentage (%) of deoxyribose degradation was evaluated as  $\% = [(A_b - A_s)/A_b] * 100$ ; where  $A_b$ : absorbance of the blank sample,  $A_s$ : absorbance of the EOs (or positive control).

### 2.5.5. Nitric oxide radical scavenging assay

The nitric oxide radical scavenging assay was performed as per Yen et al. (2001), with slight modifications. Graded concentrations of the EOs diluted in DMSO were assayed in the test tubes with sodium nitroprusside solution (25 mM) to comprise a reaction mixture of 1 mL. The tubes containing the reaction mixture were incubated at 25 °C for 1.5 h. An aliquot (0.25 mL) of the solution was withdrawn and diluted with 0.15 mL Griess Reagent (1% sulfanilamide in 5% H<sub>3</sub>PO<sub>4</sub> and 0.1% naphthylethylene-diamine-dihydrochloride). This reaction generated instantly a chromophore which was identified at 570 nm using a spectrophotometer. Nitric oxide generated from the decomposition of sodium nitroprusside was measured (15). All tests were performed in triplicate and Trolox (at different concentrations) was used as positive standard while DMSO instead of EOs was used as blank. Percentage inhibition was calculated as follows: % inhibition =  $[(A_{blank} - A_{sample})/A_{blank}] * 100$ , where A is absorbance measured by spectrophotometer.

### 2.5.6. Ferric reducing antioxidant power (FRAP)

The antioxidant capacity of each EO was estimated according to Pulido et al. (2000) with slight modification. Acetate buffer (100 mL at 300 mM, pH 3.6) was prepared from sodium acetate (3.10 g) and glacial acetic acid (16 mL). FRAP solution was prepared using 25 mL acetate buffer to 2.5 mL of 2-4-6 tripyridyl-s-triazine (TPTZ) (10 mM in 40 mM HCl) and warmed at 37 °C. The reaction mixture was then constituted by adding 90  $\mu$ L of distilled water and 30  $\mu$ L of the test sample (EOs of varying concentrations) to 900  $\mu$ L of the warmed FRAP solution. Readings at a maximum absorption of 593 nm were monitored at 30 s interval, up to 30 min. In this method, we are using the properties of EOs to reduce Fe<sup>3+</sup> ions to Fe<sup>2+</sup>, causing a change in color from colorless complex of TPTZ-Fe<sup>3+</sup> to the blue Fe<sup>2+</sup> TPTZ-Fe complex, measured at 593 nm. Solutions of known ferrous sulfate concentrations in the range of 100–2000  $\mu$ mol/L (FeSO<sub>4</sub>·7H<sub>2</sub>O) were used for calibration. The results of the reducing capacity of the EOs were expressed in  $\mu$ M Fe<sup>2+</sup>/mg EO.

### 2.5.7. Oxygen radical absorbing capacity (ORAC) assay

The ORAC assay performed as reported by Dávalos et al. (2004), consists of a kinetic study based on the measurement of radical scavenging activities of the 19 selected EOs against peroxy radicals triggered by the addition of the AAPH (2,2'-Azobis(2-amidino propane) dihydrochloride) radical. Different dilutions of EOs and positive control, Trolox, were prepared in phosphate buffer, and incubated with fluorescein (70 nM final concentration) for 10 min. The pro-oxidant agent AAPH (12 mM final concentration) was then added as peroxy radical generator, simulating the oxidative stress. Fluorescence was then measured every minute for 8 min (excitation 485 nm and emission 520 nm) at 37 °C. Antioxidant activity was determined using area under curve (AUC) and results were compared to a standard curve of Trolox and expressed in g TE (Trolox equivalent)/g EO that is the antioxidant capacity of the EO as compared to the standard Trolox.

## 2.6. Antiglycation assay

The antiglycation end products assay was performed according to the method reported by Chen et al. (2011) and Ramkissoon et al. (2012). Briefly, the reaction solution which included 2.5 mL of total volume of glycation reaction solution, diluted EOs (0.5 mL), 20 mg/mL BSA, 500 mM glucose, 0.02% (w/v) sodium azide and phosphate buffer saline (100 mM, pH 7.4). This assay was conducted during a 3 week interval. At the end of each week, the reaction was stopped by adding 10  $\mu$ L of 100% (w/v) trichloroacetic acid (TCA). The TCA-added mixture was kept at 4 °C for 10 min before centrifugation at

15,000 rpm. The precipitate obtained was re-dissolved with 0.8 mL PBS (pH 7.4), and the products formed were monitored by measuring the fluorescence intensity (excitation 360 nm and emission 450 nm). The percentage inhibition of each EO was calculated as follows:  $\%I = [1 - (F_{\text{BSA} + \text{glucose} + \text{sample}} - F_{\text{BSA} + \text{sample}}) / (F_{\text{BSA} + \text{glucose}} - F_{\text{BSA}})] \times 100$ , where F is the fluorescence intensity. Aminoguanidine, which is a common glycation inhibitor, was used as positive control in this assay.

### 2.7. Evaluation of the total phenolic content (TPC)

The TPC in the EOs were quantified using the Folin-Ciocalteu colorimetric reaction as described by [Chen et al. \(2011\)](#). Different concentrations of EO samples were mixed with NaCO<sub>3</sub> and Folin-Ciocalteu reagent. Samples were then incubated in darkness at room temperature. Absorbance was read at 750 nm by use of a spectrophotometer. Gallic acid was used as a reference standard, and the total polyphenol content was expressed as gallic acid equivalents (GAE, µg/µg EO). The total phenolic content in the EOs was calculated as follow:  $T = C * V/M$ ; where, T is the total phenolic content in µg of gallic acid per µg of EO, C is the concentration of gallic acid established from the calibration curve in mg/mL, V is the volume of the extract solution in mL and M is the weight of the extract in g.

### 2.8. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) analysis of the 19 EOs diluted in hexane were carried out using a Perkin Elmer Clarus 500 GC (Shelton, CT06484, USA), coupled with Perkin Elmer Clarus 500 mass spectrometer equipped with RTX-5 (60 m × 0.32 mm, 0.25 µm film thickness) capillary column. The EOs were filtered using syringe filters of 0.45 µm and passed over anhydrous magnesium sulfate to remove any trace of humidity during sample preparation. The carrier gas used for the GC–MS apparatus was Helium, launched at a flow rate of 1 mL/min. The ionization of the sample components was performed in EI mode of 70 eV. Injector temperature was set to 210 °C. A solvent delay of five mins was applied. The oven temperature was programmed as follows: 60–220 °C at the rate of 2 °C/min and finally held isothermally for 15 min. The constituents were identified by matching of the GC relative retention times obtained for the EOs with those of pure compounds. Retention indices (RI) were determined using retention times of the C8–C20 alkane standards injected as reference and the retention times of the unknown EO components for which the RI is being determined.

The retention indices of the individual components and MS fragmentation patterns were also compared and matched with those of the Wiley Mass Finder 2.1 Library, Nist 98 and with those available in the literature ([Joulain et al., 2001](#)).

### 2.9. Statistical analysis

Results were presented as mean ± SEM of experiments. In order to determine the reproducibility of the measurements, each experimental procedure was carried out three times. Difference between groups and percentage inhibition of the assays were compared using unpaired t-test with one-tailed test. These differences were considered significant at  $p < 0.05$ . Pearson's correlation was used to determine the correlation of the several antioxidant activities. The yields of the EOs were analyzed using ANOVA (Analysis of Variance) procedure with plant, month of collection and sea-level as main factors. All statistical analysis were performed with SPSS version 14.0 statistical package.

## 3. Results and discussion

### 3.1. Yield of EOs

This preliminary experiment was geared to study the monthly yield of EOs from two common medicinal plants, CC and PB in 3 different areas and with respect to different environmental parameters. A monthly yield evaluation at different altitudes and across the season revealed that CC gave a better yield during the summer period. The plants were at their full vegetative state in summer which can account for the rich EO content during this period of the year. Among the three locations, the yield of CC sampled at location 1 was less abundant for the summer period, compared to locations 2 and 3. Location 1 received around 7.5–8.0 h of bright sunshine compared to location 3, which received only 6.0 h of bright sunshine which may account for the difference in yield observed. The partial evaporation of some constituents of the EOs due to the high temperatures observed in summer at location 1 could also account for this difference. [Fig. 1](#) illustrates the variation of the yield of the EOs with respect to the month of collection and altitude. On the overall, the yield of CC is generally higher across the months except in May and August, where the EO PB has a much higher yield. Along with altitude and climate, other parameters from previous studies have shown that higher plant population density also affects EO production due to decreasing nutrient absorption ([Khorshidi et al., 2009](#)). This might be one reason to justify the decreasing EO yield recorded in the present study at higher density areas. For instance, PB, which had the highest density at location 3 showed a decrease during the monthly record from September 2013 to April 2014 while CC showed considerable decrease from November 2013 to May 2014 at location 1 where the plant population density was highest.

### 3.2. Antioxidants assays

EOs have been reported in the literature as having significant antioxidant properties ([Bozin et al., 2006](#); [Chen et al., 2011](#)). There has been a growing interest in the use of EOs as natural antioxidants as synthetic antioxidants are nowadays challenged to be potentially harmful to human health. Additionally, the use of EOs in edible products, either by direct mixing or in active packaging and edible coatings, may represent a legitimate alternative to prevent autoxidation and extend shelf life. In the present study, we have assessed the EOs through a panoply of antioxidant assays. The evaluation of the antioxidant performance of EOs in different assays is a crucial issue, since many commonly used assays do not give results which tally with one another and are often marked inappropriate due to the contradictory results.

Antioxidant assay results were summarized by the respective IC<sub>50</sub> of all EOs and presented in [Table 1](#). Several antioxidant assays have been used to assess the potency of the EOs in decreasing oxidative stress through the inhibition of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (NO, HO, OX, and ORAC assays) as well as for their ability to act as reducing agent (FRAP assay) and to scavenge free radicals (DPPH, ABTS, OH, NO assays).

Correlation results obtained for the different antioxidant assays are detailed in [Table 2](#). In relation to DPPH activity, out of the 19 EOs tested, PD, CGp, LN, PB, CH, PT and TT were found to be the most active and statistically significant ( $p < 0.05$ ) compared to ascorbic acid. The most potent EO was PB with an IC<sub>50</sub> of  $0.425 \pm 0.045$  µg/mL compared to ascorbic acid having an IC<sub>50</sub> of  $1.703 \pm 0.022$  µg/mL. The percentage inhibition of the free radicals increased with the dose of EO in the reaction mixture and inhibited up to 89, 83, 84, 80 and 79% for LN, PB, CH, TT and PT respectively and 87% for both PD and CGp. The DPPH scavenging activity of

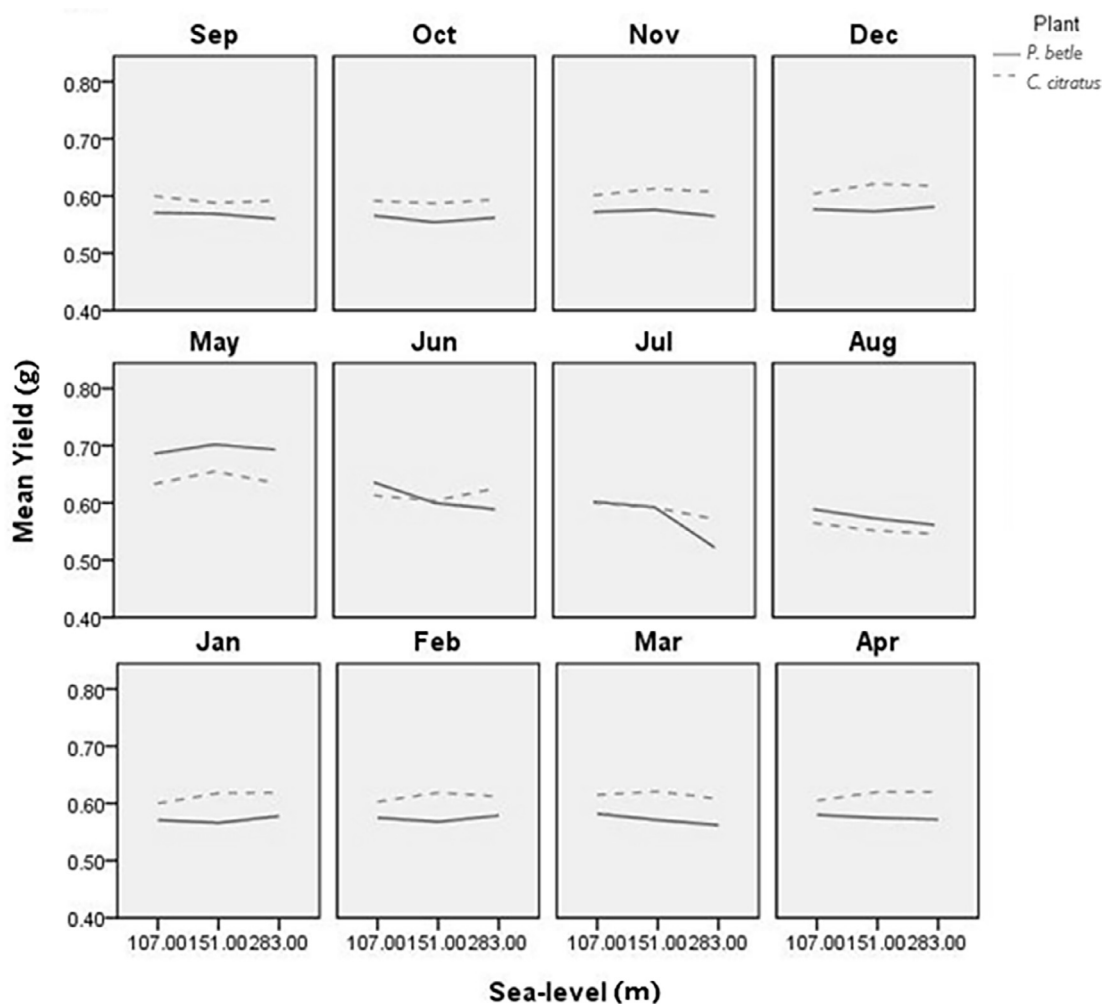


Fig. 1. Monthly variation in EO yield with respect to altitude and season.

phenolic compounds from EOs is well documented in the literature (Zheng and Wang, 2001).

The ABTS radical scavenging assay was used to confirm the results obtained for the DPPH scavenging assay. Among the 19 EOs, only PD, SO, CM, LN, PB, PT and PA were found to be the most active as summarized in Table 1. The most potent EO is *P. dioica* ( $IC_{50}$  of  $0.686 \pm 0.032 \mu\text{g/mL}$ ) compared to ascorbic acid ( $IC_{50}$  of  $1.111 \pm 0.071 \mu\text{g/mL}$ ). The  $IC_{50}$  of PD, SO, CM, PA and PT in the ABTS assay were relatively lower than that of the DPPH assay ( $0.988 \pm 0.014$ ,  $5.264 \pm 0.182$ ,  $3.667 \pm 0.090$ ,  $1.294 \pm 0.021$  and  $0.931 \pm 0.065 \mu\text{g/mL}$  respectively). Our observation is in accordance to previous studies whereby it has been purported that EOs contains pigmented and hydrophilic antioxidants compounds which are better reflected by the ABTS radicals than the DPPH radicals (Kim et al., 2002; Floegel et al., 2011). However, statistical analysis of the DPPH assay compared to the ABTS assay showed that there exists a positive linear correlation (Table 2) between these two assays for the 19 EOs tested, with correlation coefficient,  $r$ , of 0.457 and  $p$  value of 0.030 at  $\alpha = 0.05$ . These results also indicate that more than 50% of EOs tested simultaneously possess high ABTS scavenging activity and high DPPH radical activity.

Xanthine oxidase (XO) has a major function in the oxidation of xanthine or hypoxanthine to uric acid (Pacher et al., 2006). The mean  $IC_{50}$  value of allopurinol, a clinically used XO inhibitory drug against hyperuricemia, was found to be  $0.723 \pm 0.022 \mu\text{g/mL}$ . CZ, PD, SO, and PB inhibited XO ( $IC_{50}$ :  $0.518 \pm 0.051$ ,  $0.582 \pm 0.042$ ,  $0.677 \pm 0.069$  and  $0.579 \pm 0.092 \mu\text{g/mL}$  respectively) while the

activity of MQ ( $IC_{50}$ :  $0.795 \pm 0.039 \mu\text{g/mL}$ ) was comparable to allopurinol (Table 1). Jirovetz et al. (2007) also reported the capacity of PD to inhibit the XO activity. However, the aqueous extract of CZ has been described by Roohbakhsh et al. (2009) as having no significant effect on the activity of XO. The hydroxyl and nitric oxide radical scavenging assay showed the existence of a positive correlation (Table 2) with the XO assay, with Pearson correlation coefficients,  $r$  equivalent to 0.729 and 0.562 respectively ( $p$ -value 0.001 and 0.019 respectively).

The hydroxyl radical is known to be the most reactive one, inducing severe damage to adjacent molecules. There are several ways to determine the capacity of a compound to form hydroxyl radicals and the most common being the deoxyribose test. Most active EOs for this assay were PD, LI, SO, LN and PB with  $IC_{50}$  of  $0.684 \pm 0.042$ ,  $0.909 \pm 0.514$ ,  $0.922 \pm 0.042$ ,  $0.909 \pm 0.014$  and  $0.562 \pm 0.021 \mu\text{g/mL}$  respectively compared to ascorbic acid (mean  $IC_{50}$ :  $1.006 \pm 0.069 \mu\text{g/mL}$ ). The hydroxyl radical scavenging capacity of EOs of CGp, CGI, CH, MQ and PT were considered as equivalent to ascorbic acid in this assay with  $IC_{50}$  values of  $1.145 \pm 0.374$ ,  $1.069 \pm 0.099$ ,  $1.151 \pm 0.124$ ,  $1.156 \pm 0.127$  and  $1.103 \pm 0.087 \mu\text{g/mL}$  respectively. Our results also showed a significantly high positive correlation (with  $p = 0.007$  and 0.001 respectively at  $\alpha = 0.01$  as shown in Table 2) when the data obtained for the OH assay was compared to that obtained for the NO and ABTS assays, with Pearson correlation coefficients of 0.721 and 0.628 respectively.

Excess production of NO in the body has major consequences and has been associated with several diseases (Ialenti et al.,

**Table 1**  
Summary of biological activities of 19 EOs.

EOs	DPPH IC <sub>50</sub> (µg/mL)	ABTS IC <sub>50</sub> (µg/mL)	XO IC <sub>50</sub> (µg/mL)	OH IC <sub>50</sub> (µg/mL)	NO IC <sub>50</sub> (µg/mL)	ORAC (gTE/gEO)	TPC (GAE (µg/µg EO))	FRAP (µM Fe <sup>2+</sup> /mg EO)
PD	0.988 ± 0.014 <sup>†</sup>	0.686 ± 0.032 <sup>†</sup>	0.582 ± 0.042 <sup>†</sup>	0.684 ± 0.042 <sup>†</sup>	0.928 ± 0.031 <sup>**</sup>	1.756 ± 0.052	867.20 ± 1.72	120.02 ± 3.10
LI	1.629 ± 0.093 <sup>**</sup>	1.199 ± 0.420 <sup>**</sup>	1.289 ± 0.514	0.909 ± 0.514 <sup>†</sup>	1.502 ± 0.451	0.329 ± 0.210	188.25 ± 6.33	106.57 ± 2.10
SO	5.264 ± 0.182	0.848 ± 0.054 <sup>†</sup>	0.677 ± 0.069 <sup>**</sup>	0.922 ± 0.042 <sup>†</sup>	0.971 ± 0.072	1.471 ± 0.256	121.11 ± 3.25	114.24 ± 6.52
CM	3.667 ± 0.09	1.068 ± 0.015 <sup>**</sup>	1.546 ± 0.522	1.258 ± 0.358	1.325 ± 0.915	0.925 ± 0.315	1223.17 ± 8.67	106.87 ± 8.55
CGp	0.991 ± 0.011 <sup>†</sup>	1.221 ± 0.053 <sup>**</sup>	1.622 ± 0.418	1.145 ± 0.374 <sup>**</sup>	1.441 ± 0.532	0.683 ± 0.251	322.64 ± 7.40	131.27 ± 1.09
CGI	1.722 ± 0.004 <sup>**</sup>	1.951 ± 0.078	1.054 ± 0.098	1.069 ± 0.099 <sup>**</sup>	1.687 ± 0.782	0.227 ± 0.750	349.50 ± 6.65	88.62 ± 1.15
LN	0.522 ± 0.023 <sup>†</sup>	0.712 ± 0.087 <sup>†</sup>	1.052 ± 0.041	0.909 ± 0.014 <sup>†</sup>	0.982 ± 0.097	1.602 ± 0.540	806.59 ± 8.20	97.49 ± 2.71
PB	0.425 ± 0.045 <sup>†</sup>	0.808 ± 0.051 <sup>†</sup>	0.579 ± 0.092 <sup>†</sup>	0.562 ± 0.021 <sup>†</sup>	0.956 ± 0.061	1.715 ± 0.216	551.12 ± 6.52	113.36 ± 8.02
CH	0.761 ± 0.049 <sup>†</sup>	1.170 ± 0.223 <sup>**</sup>	1.342 ± 0.245	1.151 ± 0.124 <sup>**</sup>	1.249 ± 0.352	0.647 ± 0.336	228.54 ± 1.31	123.45 ± 5.66
RO	1.283 ± 0.077 <sup>†</sup>	1.294 ± 0.048	1.341 ± 0.066	1.535 ± 0.047	1.342 ± 0.482	0.958 ± 0.422	524.29 ± 2.22	121.08 ± 1.94
CC	1.245 ± 0.049 <sup>†</sup>	1.345 ± 0.059	1.270 ± 0.085	1.582 ± 0.064	1.572 ± 0.519	0.612 ± 0.117	1203.63 ± 6.11	96.63 ± 3.52
MQ	2.825 ± 0.048	1.904 ± 0.040	0.795 ± 0.039 <sup>**</sup>	1.156 ± 0.127 <sup>**</sup>	1.733 ± 0.402	1.104 ± 0.200	231.57 ± 2.06	127.14 ± 2.37
CZ	1.809 ± 0.037 <sup>**</sup>	3.115 ± 0.158	0.518 ± 0.051 <sup>†</sup>	1.285 ± 0.182	2.056 ± 0.595	2.015 ± 0.169	595.22 ± 1.08	116.02 ± 7.10
ST	6.553 ± 0.092	2.645 ± 0.474	1.450 ± 0.078	1.326 ± 0.114	2.229 ± 0.714	0.312 ± 0.524	499.45 ± 5.64	76.05 ± 3.41
PG	5.194 ± 0.088	3.091 ± 0.942	2.515 ± 0.968	1.904 ± 0.341	2.714 ± 0.932	0.275 ± 0.921	209.16 ± 6.15	44.41 ± 1.82
PA	1.294 ± 0.021 <sup>†</sup>	1.105 ± 0.094 <sup>**</sup>	1.200 ± 0.092	1.225 ± 0.489	1.265 ± 0.074	1.113 ± 0.614	426.51 ± 2.30	98.45 ± 1.48
PT	0.931 ± 0.065 <sup>†</sup>	0.994 ± 0.34 <sup>†</sup>	1.092 ± 0.053	1.103 ± 0.087 <sup>**</sup>	0.902 ± 0.052 <sup>**</sup>	1.294 ± 0.406	368.18 ± 6.35	111.85 ± 8.63
TT	1.050 ± 0.062 <sup>†</sup>	2.502 ± 0.024	1.612 ± 0.125	1.412 ± 0.197	2.482 ± 0.817	0.854 ± 0.124	659.52 ± 3.19	82.64 ± 3.30
CR	1.641 ± 0.026 <sup>**</sup>	1.667 ± 0.035	1.580 ± 0.096	1.549 ± 0.062	1.963 ± 0.525	1.307 ± 0.641	306.50 ± 1.51	100.03 ± 8.10
PC	1.703 ± 0.022 <sup>†</sup>	1.111 ± 0.071 <sup>†</sup>	0.723 ± 0.022 <sup>†</sup>	1.006 ± 0.069 <sup>†</sup>	0.872 ± 0.035 <sup>†</sup>	-	-	-

Note. DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay; ABTS<sup>rad+</sup>: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical cation assay); HO: hydroxyl radical scavenging assay; NO: nitric oxide radical scavenging assay; XO: xanthine oxidase assay; ORAC: Oxygen radical absorbing capacity assay; TPC: Total phenolic content assay; FRAP: ferric reducing antioxidant power. Values ± SEM (standard mean error of 3 assays). Positive Control (PC): <sup>1</sup>DPPH assay-ascorbic acid; <sup>2</sup>ABTS assay-ascorbic acid; <sup>3</sup>XO assay-allopurinol; <sup>4</sup>OH assay-ascorbic acid; <sup>5</sup>NO assay-trolox; <sup>6</sup>Anti-tyrosinase assay-Kojic acid. CC: *Cymbopogon citratus*; CGp: *Citrus grandis* (peel); CGI: *Citrus grandis* (leaves); CH: *Citrus hystrix*; CM: *Cupressus macrocarpa*; CR: *Citrus reticulata*; CZ: *Cinnamomum zeylanicum*; LI: *Lavandula x intermedia*; LN: *Laurus nobilis*; MQ: *Melaleuca quinquenervia*; PA: *Psidium arguta*; PB: *Piper betle*; PD: *Pimentadjoica*; PG: *Psidium guajava*; PT: *Psidium terebinthina*; RO: *Rosmarinus officinalis*; SO: *Salvia officinalis*; ST: *Schinusterebinthifolius*; TT: *Triphasia trifolia*. IC<sub>50</sub>: concentration (µg/mL).

<sup>†</sup> Values significantly lower than control with  $p < 0.05$ .

<sup>\*\*</sup> Values comparable to the control.

**Table 2**  
Correlation between antioxidant and antiglycation assays.

	DPPH	XO	OH	NO	ABTS	AtG
DPPH	1					
XO	0.360 <sup>**</sup>	1				
OH	0.395 <sup>**</sup>	0.729 <sup>†</sup>	1			
NO	0.581 <sup>†</sup>	0.562 <sup>†</sup>	0.721 <sup>†</sup>	1		
ABTS	0.457 <sup>†</sup>	0.340 <sup>**</sup>	0.628 <sup>†</sup>	0.943 <sup>†</sup>	1	
AtG	0.120 <sup>**</sup>	0.189 <sup>**</sup>	0.134 <sup>**</sup>	0.392 <sup>**</sup>	0.165 <sup>**</sup>	1

Note. DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay; ABTS<sup>rad+</sup>: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical cation assay); HO: hydroxyl radical scavenging assay; NO: nitric oxide radical scavenging assay; XO: xanthine oxidase assay; AtG: antiglycation assay.

<sup>†</sup> Correlation is statistically significant at an  $\alpha = 0.01$  (bilateral).

<sup>\*\*</sup> Correlation is not statistically significant.

1993) such as Parkinson disease, Alzheimer disease, Huntington disease and amyotrophic lateral sclerosis (Bredt, 1999). Results from the present study showed (Table 1) that PD, SO, LN, PB and PT have antioxidant properties towards NO radicals with IC<sub>50</sub> values 0.928 ± 0.031, 0.971 ± 0.072, 0.982 ± 0.097, 0.956 ± 0.061 and 0.902 ± 0.052 µg/mL respectively and hence are potential EOs for reducing oxidative stress. On the other hand, trolox was found to be a better inhibitor (IC<sub>50</sub>: 0.872 ± 0.035) of NO radical compared to the EOs, but, the IC<sub>50</sub> of PT and PD were found to be comparable to that of trolox. NO is the only assay which displayed significant ( $p = 0.015, 0.0001, 0.019$  and  $0.001$  for DPPH, ABTS, XO and NO respectively as indicated in Table 2) positive correlations to all the other antioxidant assays.

In the FRAP assay, the potential of the EOs were studied for their ability to reduce Fe<sup>3+</sup> ions to Fe<sup>2+</sup>. Similar to the DPPH, ABTS, OH and NO assays, it was found that the EOs of PD, SO, LN, PB and PT exhibited significant activity in the FRAP assay (Table 1). The difference in reducing capacity of CGp and CGI was significant ( $p = 0.0002$ ), with CGp as a moderate reducing agent while the activity of CGI was very low in the FRAP assay, which may be explained by the difference in the phytochemical composition of the EOs (Othman et al., 2007).

The oxygen radical absorbance capacity (ORAC) assay was performed as described by Dávalos et al. (2004). The mechanism of the ORAC assay is based on the capacity of the antioxidant to donate a proton to the pro-oxidant AAPH radical. As shown in Table 1, CZ (2.015 ± 0.169 gTE/gEO), PD (1.756 ± 0.052 gTE/gEO), PB (1.715 ± 0.216 gTE/gEO), LN (1.602 ± 0.540 gTE/gEO), SO (1.471 ± 0.256 gTE/gEO), PT (1.294 ± 0.406 gTE/gEO) and CR (1.307 ± 0.641 gTE/gEO) showed the highest antioxidant capacity based on the ORAC assay while the rest of the EOs showed moderate to low antioxidant capacity. The results of the total antioxidant capacity of the EOs tally with those demonstrated by the other antioxidant assays studied.

Overall, the EOs of PB, PD, LN and CH were among the most potent EOs. These results are in concordance with those found in the literature. Dwivedi and Tripathi (2014) and Prakash et al. (2010) reported the strong (IC<sub>50</sub> close to that of ascorbic acid) antioxidant capacity of the EO extracted from PB. Padmakumari et al. (2011) and Dharmadasa et al. (2015) demonstrated that the EO of PD possessed high radical scavenging activities. Goudjil et al. (2015) on one hand reported mild antioxidant activity for the EO of LN while on the other hand, Cherrat et al. (2014) reported

potential antioxidant activity. The antioxidant activity of CH was demonstrated by Saleh et al. (2010). However, the antioxidant activity of the EO of CH was described previously by Wungsintaweekul et al. (2007) as being moderate.

EOs are variable in nature due to the variation in their major active components, which also renders their oxidation mechanism complex. There are different pathways for the action for antioxidants, among which, there is the inactivation of oxygen singlet, the inhibition of the pro-oxidant enzymatic pathways, the enzymatic inactivation of ROS and RNS, the chelation through scavenging of transition metals as well as the stabilisation of ROS, among others. Hence using different assays for the assessment of the antioxidant capacity of EOs is warranted. As recommendation for future studies of EO, we would recommend the ABTS, ORAC and DPPH assays which are commonly used to assess the antioxidant properties of natural products as well as NO assays due to the correlation that exists between these tests. However, it is to be noted that the combination of different results tends to decrease the error margin, allowing a more reliable deduction while establishing the antioxidant capacity.

### 3.3. Anti-glycation assay

In Mauritius, diabetes mellitus is a very common chronic disease which is known to be linked to oxidative stress and non-enzymatic protein glycation (Dávalos et al., 2004). The formation of advanced glycation endproducts is accelerated in hyperglycaemic conditions, which alter the structure and function of long-lived proteins.

Diabetes associated health problems, such as retinopathy, nephropathy, neuropathy and vascular complications (Nagmoti and Juvekar, 2013) are devastating to the patients and treatment modalities are costly to the health care system. Thus, the use of natural medicinal alternatives as complementary therapies for the management of diabetes might be an interesting step.

Furthermore, the relevance of AGEs in the pathogenesis of diabetic complications warrants the search of natural alternative to the inhibition of AGE. In addition to that, it has been purported in the literature that natural products having both antioxidant and antiglycation properties can be beneficial (Nakagawa et al., 2002). The results of this study demonstrated that some of the EOs inhibited glycation of BSA by glucose in a dose-dependent manner after week 2 and that the inhibition was more important on week 3 (Table 3). Compared to aminoguanidine, a known good inhibitor of the glycation reaction, the active EOs were moderate inhibitors except for EOs of CM, LN, CZ and PG, which were found to be good inhibitors ( $IC_{50}$ :  $451.53 \pm 3.00$ ,  $387.04 \pm 1.53$ ,  $348.59 \pm 3.34$  and  $401.49 \pm 2.86$   $\mu\text{g/mL}$  respectively) compared to aminoguanidine ( $IC_{50}$ :  $351.58 \pm 3.57$   $\mu\text{g/mL}$ ).

Additionally, EOs of PA and CC demonstrated  $IC_{50}$  values ( $623.78 \pm 3.15$  and  $649.55 \pm 4.53$   $\mu\text{g/mL}$  respectively) which tend to classify them as moderate glycation inhibitors, when compared to aminoguanidine. Advanced glycation end products, (AGEs) are believed to play important roles in pathogenesis of diabetic and aging complications (Dávalos et al., 2004). Agents that inhibit the formation of AGEs are purported to have therapeutic potentials in patients with diabetes or age-related diseases. These EOs can thus be considered as good candidates as they possess antioxidant and anti-glycation properties. Therefore, these oils might be of therapeutic efficacy against diabetes and cardiovascular disease. A correlation study revealed that there is no correlation between the antioxidant assays and the antiglycation properties of the EOs (Table 2).

### 3.4. Total phenolic content

It is well established that phenolic compounds found in EOs represent one of the most numerous and widely distributed group of plant secondary metabolites possessing therapeutic benefits such as anti-microbial, anti-carcinogenic and anti-diabetic. Some

**Table 3**  
Summary of the antiglycation activity of 19 selected EOs after 3 weeks.

EOs	% Inhibition at different EOs concentration ( $\mu\text{g/mL}$ ) <sup>a</sup>				$IC_{50}$ ( $\mu\text{g/mL}$ )
	2000	1000	500	250	
PD	63.16 $\pm$ 10.6**	40.66 $\pm$ 2.65	27.11 $\pm$ 2.02	17.15 $\pm$ 2.17	1254.38 $\pm$ 0.50
LI	50.23 $\pm$ 3.11	49.01 $\pm$ 0.98	33.34 $\pm$ 2.77	25.35 $\pm$ 2.66	1484.79 $\pm$ 1.79
SO	42.11 $\pm$ 3.63	38.60 $\pm$ 3.31	25.78 $\pm$ 3.07	11.52 $\pm$ 2.22	2551.80 $\pm$ 6.51
CM	76.18 $\pm$ 2.25*	61.02 $\pm$ 4.12*	50.09 $\pm$ 3.66*	42.41 $\pm$ 1.19*	451.53 $\pm$ 3.00 <sup>1</sup>
CGp	52.65 $\pm$ 3.36	40.09 $\pm$ 2.22	38.15 $\pm$ 2.54	25.49 $\pm$ 3.33	1655.12 $\pm$ 3.75
CGl	44.32 $\pm$ 2.65	41.19 $\pm$ 2.09	30.04 $\pm$ 1.66	22.15 $\pm$ 1.77	2691.89 $\pm$ 1.00
LN	70.11 $\pm$ 9.66	63.42 $\pm$ 3.15*	55.12 $\pm$ 4.52*	43.05 $\pm$ 3.85*	387.04 $\pm$ 1.53 <sup>1</sup>
PB	66.23 $\pm$ 5.88**	59.05 $\pm$ 1.72*	42.47 $\pm$ 3.81**	29.01 $\pm$ 2.22	738.07 $\pm$ 4.66
CH	42.71 $\pm$ 6.51	36.60 $\pm$ 2.47	29.88 $\pm$ 4.48	18.35 $\pm$ 1.03	3278.14 $\pm$ 1.92
RO	56.08 $\pm$ 3.05	51.09 $\pm$ 3.70**	42.58 $\pm$ 6.51**	35.45 $\pm$ 1.25**	1014.41 $\pm$ 3.26
CC	63.21 $\pm$ 4.05**	59.17 $\pm$ 2.11*	49.85 $\pm$ 2.62*	33.06 $\pm$ 1.96**	649.55 $\pm$ 4.53 <sup>2</sup>
MQ	54.72 $\pm$ 3.55	44.29 $\pm$ 2.21	33.19 $\pm$ 1.23	22.15 $\pm$ 2.54	1462.70 $\pm$ 1.80
CZ	79.86 $\pm$ 9.55*	66.52 $\pm$ 4.21*	53.21 $\pm$ 2.47*	47.10 $\pm$ 2.98*	348.59 $\pm$ 3.34 <sup>1</sup>
ST	51.23 $\pm$ 1.17	47.92 $\pm$ 1.98	39.24 $\pm$ 2.68	27.54 $\pm$ 8.61	1425.22 $\pm$ 4.82
PG	83.44 $\pm$ 2.07*	70.61 $\pm$ 1.95*	55.37 $\pm$ 3.14*	39.12 $\pm$ 4.08*	401.49 $\pm$ 2.86 <sup>1</sup>
PA	66.48 $\pm$ 3.64**	59.85 $\pm$ 1.73*	45.29 $\pm$ 2.85**	33.01 $\pm$ 1.85**	623.78 $\pm$ 3.15 <sup>2</sup>
PT	54.61 $\pm$ 2.55	44.58 $\pm$ 1.63	38.12 $\pm$ 1.79	25.09 $\pm$ 2.53	1388.31 $\pm$ 3.26
TT	33.79 $\pm$ 2.69	25.19 $\pm$ 2.52	20.42 $\pm$ 1.77	16.80 $\pm$ 9.27	15637.48 $\pm$ 2.14
CR	40.09 $\pm$ 1.66	35.15 $\pm$ 2.74	23.19 $\pm$ 1.38	15.71 $\pm$ 2.96	3343.69 $\pm$ 1.88
PC	78.12 $\pm$ 1.79	65.05 $\pm$ 1.48	55.90 $\pm$ 1.66	44.95 $\pm$ 1.69	351.58 $\pm$ 3.57

**Note.** Values  $\pm$  SEM (standard mean error of 3 assays). PC – Positive Control (aminoguanidine). CC: *Cymbopogon citratus*; CGp: *Citrus grandis* (peel); CGl: *Citrus grandis* (leaves); CH: *Citrus hystrix*; CM: *Cupressus macrocarpa*; CR: *Citrus reticulata*; CZ: *Cinnamomum zeylanicum*; LI: *Lavandula x intermedia*; LN: *Laurus nobilis*; MQ: *Melaleuca quinquenervia*; PA: *Psidium arguta*; PB: *Piper betle*; PD: *Pimenta dioica*; PG: *Psidium guajava*; PT: *Psidium terebinthina*; RO: *Rosmarinus officinalis*; SO: *Salvia officinalis*; ST: *Schinusterebinthifolius*; TT: *Triphasia trifolia*.

<sup>a</sup> Concentration ( $\mu\text{g/mL}$ ) at which the inhibition% were calculated.  $IC_{50}$ : concentration ( $\mu\text{g/mL}$ ).

<sup>1</sup> Values significantly ( $p < 0.05$ ) lower than the  $IC_{50}$  of aminoguanidine.

<sup>2</sup> Values comparable to the  $IC_{50}$  of aminoguanidine ( $p > 0.05$ ).

\* Values significantly lower than control with  $p < 0.05$ .

\*\* Values comparable to positive control, with  $p < 0.05$ .

**Table 4**  
Chemical composition of EOs.

EO components	CC	CGp	CGI	CH	CR	CM	LI	MQ	PG % Abundance	TT	CZ	LN	PA	PB	PD	PT	RO	ST	SO	RI <sup>a</sup>	RI <sup>b</sup>	Ref
$\alpha$ -Pinene	–	–	–	–	4.7	63.2	–	–	–	–	2.01	0.07	–	0.52	–	3.74	15.36	11.65	0.1	917	939	(Awad and Abdelwahab, 2016)
Camphene	–	–	–	–	–	–	–	–	–	–	0.67	37.83	–	–	–	–	3.59	–	–	958	954	(Awad and Abdelwahab, 2016)
Sabinene	–	0.78	–	–	–	1.56	12.14	–	–	12.04	–	–	–	0.01	–	–	–	2.7	–	961	976	(Awad and Abdelwahab, 2016)
$\beta$ -Pinene	–	1.57	0.27	0.78	13.24	4.1	1.17	–	–	59.18	0.55	–	0.11	–	0.11	1.3	0.18	4.51	0.23	978	970	(Bendahou et al., 2008)
$\beta$ -myrcene	14.7	3.52	0.13	0.89	6.85	4.2	1.08	–	–	–	–	–	0.6	0.19	0.07	7.85	1.04	–	–	981	991	(Awad and Abdelwahab, 2016)
$\alpha$ -Phellandrene	–	–	–	–	–	–	–	–	–	–	1.1	–	–	–	1.04	–	0.17	11.62	–	1005	999	(Awad and Abdelwahab, 2016)
3-Carene	–	–	–	–	3.11	–	–	–	–	–	–	–	–	2.1	–	–	–	21.18	–	1010	1010	(Shellie et al., 2002)
Limonene	–	75.43	–	83.89	37.55	–	–	–	11.62	5.59	–	–	4.96	–	–	–	7.25	–	–	1030	1030	(Awad and Abdelwahab, 2016)
1,8-Cineol	–	–	–	–	–	–	1.17	40.3	–	–	–	4.15	2.2	–	0.45	–	47.5	–	9.79	1033	1034	(Shellie et al., 2003)
$\gamma$ -Terpinene	–	–	0.16	–	9.11	3.1	–	–	–	–	–	–	–	–	–	–	–	–	–	1054	1060	(Awad and Abdelwahab, 2016)
Linalool	1.64	–	26.01	–	–	–	47.33	–	–	–	4.05	13.21	–	–	–	–	4.54	–	–	1080	1098	(Awad and Abdelwahab, 2016)
Thujone	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	30.2	1102	1086	(Bendiabdellah et al., 2012)
Camphor	–	–	–	–	–	–	–	–	–	–	–	–	–	4.88	–	–	–	–	29.1	1140	1144	(Hudaib et al., 2002)
Terpinen-4-ol	–	2.35	5.24	–	6.32	–	3.72	–	–	0.15	0.17	–	–	–	–	–	7.22	0.32	–	1161	1178	(Awad and Abdelwahab, 2016)
Borneol	–	–	42.24	–	–	–	6.61	–	–	–	–	–	–	–	–	–	1.18	–	–	1165	1167	(Mondello et al., 2002)
Neral	37.88	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1242	1241	(Mondello et al., 2002)
Carveol	–	–	–	–	0.05	–	–	27.15	–	–	–	–	–	–	–	–	–	–	–	1252	1252	(Jordán et al., 2006)
Linalyl acetate	–	–	19.89	–	–	–	14.87	–	–	–	–	–	–	–	–	–	–	–	–	1253	1257	(Mondello et al., 2002)
Cinnamaldehyde	–	–	–	–	–	–	–	–	–	–	10.8	–	–	–	–	–	–	–	–	1270	1273	(Leela et al., 2009)
Geranial	34.19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1278	1255	(Awad and Abdelwahab, 2016)
Safrole	–	–	–	–	–	–	–	–	–	–	–	–	–	48.96	–	–	–	–	–	1290	1287	(Zachariah et al., 2008)
Eugenol	–	–	–	–	–	–	–	–	–	–	58.10	13.29	–	14.8	79.9	–	–	–	–	1359	1374	(Eyres et al., 2007)
Isoeugenol	–	–	–	–	–	–	–	–	–	–	–	–	50.26	–	0.02	–	–	–	–	1402	1457	(Zachariah et al., 2008)
Vanillin	–	–	–	–	–	–	–	–	–	–	–	–	10.47	–	–	–	–	–	–	1404	1404	(Watcharananun et al., 2009)
Methyl eugenol	–	–	–	–	–	–	–	–	–	–	–	12.92	11.21	–	9.3	–	–	–	–	1405	1405	(Mondello et al., 2002)
$\beta$ -Caryophyllene	–	–	–	–	–	–	–	–	–	–	6.01	–	0.63	15.01	4.72	–	0.54	1.17	5.3	1423	1418	(Grujic-Jovanovic et al., 2004)
Aromadendrene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.16	–	–	–	16.3	1441	1443	(Shellie et al., 2003)
$\alpha$ -Curcumene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1481	1473	(Kalemba and Thiem, 2004)
Germacrene	–	2.09	–	–	–	1.9	–	–	–	14.25	–	–	–	–	–	–	–	2.04	–	1519	1480	(Shellie et al., 2003)
Acetyl eugenol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.78	–	10.9	–	–	–	1521	1525	(Usman et al., 2010)
Caryophyllene oxide	–	–	–	–	–	–	–	0.38	15.39	–	0.70	–	13.11	–	–	–	0.37	–	–	1589	1580	(Awad and Abdelwahab, 2016)
Cedrol	–	–	–	–	–	7.21	–	–	–	–	–	–	–	–	–	–	–	–	–	1602	1596	(El Amine Dib et al., 2010)

**Note.** Table adapted from Ameeruddy-Elalfi et al. (2015); Ameeruddy-Elalfi et al., 2016), representing the abundance of the major components of the EOs, identified by GC–MS; CC: *Cymbopogon citratus*; CGp: *Citrus grandis* (peel); CGI: *Citrus grandis* (leaves); CH: *Citrus hystrix*; CM: *Cupressus macrocarpa*; CR: *Citrus reticulata*; CZ: *Cinnamomum zeylanicum*; LI: *Lavandula x intermedia*; LN: *Laurus nobilis*; MQ: *Melaleuca quinquenervia*; PA: *Psiadia arguta*; PB: *Piper betle*; PD: *Pimenta dioica*; PG: *Psidium guajava*; PT: *Psiadia terebinthina*; RO: *Rosmarinus officinalis*; SO: *Salvia officinalis*; ST: *Schinus terebinthifolius*; TT: *Triphasia trifolia*; RI<sup>a</sup>: Retention Index of identified compounds; RI<sup>b</sup>: Retention Index of compounds from the literature.



of these biological properties are correlated to the radical scavenging potential of phenolic compounds (Kim et al., 2002; Othman et al., 2007). These compounds can impede or prevent the oxidative damage of lipids or other molecules caused by free radicals. The role of free radicals in the etiology and development of a wide range of clinical disorders has continued to fuel the idea that natural phenolic antioxidants can play a potential role in reducing the incidence of a number of pathologies involving oxidative stress such as coronary heart disease, Alzheimer disease, and cancer. The total phenolic content of the EOs were found to range between 121 and 1223  $\mu\text{g GAE}/\mu\text{g EO}$  (Table 1). The TPC of the EOs of the citrus species (CG, CH and CR) were evaluated as moderate with  $349.5 \pm 6.65$ ,  $228.5 \pm 1.31$  and  $306.5 \pm 1.51$   $\mu\text{g GAE}/\mu\text{g EO}$  respectively. Also, no significant difference ( $p = 0.25$ ) was observed between the phenolic content of the peel of CG ( $349.5 \pm 6.65$   $\mu\text{g GAE}/\mu\text{g EO}$ ) and that of the leaves of CG ( $322.6 \pm 7.40$   $\mu\text{g GAE}/\mu\text{g EO}$ ). CM, CC, PD and LN with  $1223.1 \pm 8.67$ ,  $1203.6 \pm 6.11$ ,  $867.2 \pm 1.72$  and  $806.5 \pm 8.20$   $\mu\text{g GAE}/\mu\text{g EO}$  respectively were the EOs with the highest TPC.

### 3.5. GC–MS profile

Eugenol,  $\beta$ -caryophyllene, methyl-eugenol, 1,8-cineol, and linalool are among the components that can be found in EOs. They have been identified as major components in the EOs of PB, PD, LN and CH (Table 4). These components have been established in the literature as having antioxidant capacities (Saleh et al., 2010; Dahham et al., 2015). For the EOs of different botanical material (leaves, fruits, flowers and others) extracted from the same plant, a difference in antioxidant capacity can be observed. The EOs of CGP and CGI ( $\text{IC}_{50} = 1.622 \pm 0.418$  and  $1.054 \pm 0.098$   $\mu\text{g}/\text{mL}$  respectively in the XO assay) may be accounted by the presence of borneol in CG<sub>1</sub> (leaves of CG), a reported antioxidant component (Saleh et al., 2010) and its absence in CG<sub>p</sub>. Vanillin, a reported (Saleh et al., 2010) antioxidant exhibiting component has been identified in the EO of PA, which may explain the low  $\text{IC}_{50}$  ( $\text{IC}_{50} = 1.105 \pm 0.094$   $\mu\text{g}/\text{mL}$ , which is comparable to that of ascorbic acid,  $\text{IC}_{50} = 1.111 \pm 0.071$   $\mu\text{g}/\text{mL}$ ) of PA in the ABTS assay. The high TPC value of PD, LN and CZ are due to the presence of eugenol and methyl eugenol as major compounds in these EOs. The Folin-Ciocalteu colorimetric reaction by which the EOs were assessed is based on the chemical reducing capacity of the EOs towards gallic acid, which is a phenolic acid. This assay therefore allowed one to elaborate on the potential of the EOs to reduce gallic acid, thereby their antioxidant potential.

## 4. Conclusion

In conclusion, the selected EOs of this study, belonging to the rich microcosm of Mauritius have proved to be potential candidates as natural antioxidants, some of which successfully also inhibited advanced glycation end products. Our results also showed that the NO assay was highly correlated to the ABTS and DPPH assays which are commonly used to assess the antioxidant properties of natural products. We also confirmed the correlation that exists between biological activities exhibited and the chemical composition of the EOs. Further purification, isolation and identification of individual phenolic and active compounds are warranted. These steps, together with the *in vivo* evaluation of antioxidant activities will add to the understanding of the mechanism of action of EOs and their major components as antioxidants. Also, our results allowed a rationale for the exploration of the molecular mechanism of the antiglycation potency of EOs exhibiting both antioxidant and antiglycation properties.

## Conflict of interest

The authors report no declarations of interest.

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