

Phytochemical Profile and Antioxidant Properties of Six Medicinal Plants Traditionally Used in the Management of Diabetes in Mauritius

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ABSTRACT

Background: Diabetic patients tend to have more oxidative internal environments compared to healthy individuals. Recently, the antioxidant potential of medicinal plants has gained momentum. This study was aimed at studying the anti-radical potential of 5 medicinal plants of Mauritius traditionally used for the management of diabetes namely *Erythroxylum laurifolium* (EL), *Elaeodendron orientale* (EO), *Antidesma madagascariensis* (AM), *Stillingia lineata* (SL) and *Faujasiaopsis flexuosa* (FF). **Materials and Methods:** Crude aqueous extracts prepared following traditional decoction method and methanolic extracts were evaluated for their ability to scavenge 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH[•]), nitric oxide (NO[•]), hypochlorous acid (HOCl), hydroxyl (OH[•]) radicals and xanthine oxidase (XO) inhibition using various *in-vitro* antioxidant assays. The total phenolic, flavonoid and anthocyanin content, reducing power (Ferric Reducing Antioxidant Power (FRAP), Trolox Equivalent Antioxidant Capacity (TEAC) and the phytochemical profile was established. **Results:** Extracts having high phenolic content exhibited significant ($p < 0.05$) reducing capacity (correlation coefficient values being 0.92 between FRAP and TPC and 0.73 between TEAC and TPC). Both crude methanolic and aqueous extracts of EL showed high Fe³⁺ reducing capacity (23.79 ± 0.43 and 29.12 ± 1.22 TE (mM mg^{-1}) crude extract, respectively). TEAC values showed that EL, EO, AM and SL were potent hydrogen-donating compounds with values ranging from 1124.44 ± 50.48 – 2960.00 ± 53.33 TE ($\mu\text{M mg}^{-1}$) crude extract. On the other hand, aqueous extracts of EL, AM and FF (IC_{50} : 105.57 ± 1.88 , 135.76 ± 3.04 and 29.91 ± 4.92 $\mu\text{g mL}^{-1}$, respectively) proved to be more effective than the positive control, ascorbic acid (IC_{50} : 189.91 ± 6.18 $\mu\text{g mL}^{-1}$) in scavenging DPPH[•]. The extracts were found to scavenge NO[•] and OH[•] but were significantly ($p < 0.05$) less active than positive controls (IC_{50} : 333.98 ± 4.75 $\mu\text{g mL}^{-1}$ ascorbic acid and 122.83 ± 0.88 $\mu\text{g mL}^{-1}$ α -tocopherol for respective assays). Interestingly, all extracts, except methanolic extracts of EO and AM significantly ($p < 0.05$) scavenged HOCl acid (IC_{50} ranging from 155.82 ± 6.56 to 7162.85 ± 29.98 $\mu\text{g mL}^{-1}$). Only methanolic extracts of EL, EO and AM (IC_{50} ranged from 6158.42 ± 6.51 to 31409.38 ± 25.36 $\mu\text{g mL}^{-1}$) inhibited XO but were less potent than the standard, allopurinol (IC_{50} : 444.48 ± 3.64 $\mu\text{g mL}^{-1}$). **Conclusion:** The phenolic content and antioxidant activities of the plant extracts could be part of the mechanism by which the plants could manage and/or delay diabetes and related complications.

Key words: Oxidative stress, antioxidant properties, phytochemical

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INTRODUCTION

It has been estimated that more than 385 million people suffer from diabetes worldwide and forecasted that 439 million adults will develop this disease in 2030 with high prevalence in developing countries (Azmi and Qureshi, 2012; IDF, 2013). Despite significant advances in treatment and preventive measures, Mauritius has one of the highest prevalence of diabetes in the world with nearly one in five adults above 30 years old being diabetics or pre-diabetics (MHQL, 2009). It has been reported that diabetic patients tend to have more

oxidative internal environments compared to healthy subjects (Rains and Jain, 2011). Oxidative stress mechanism involves an increased production of Reactive Oxygen Species (ROS) and impaired antioxidant defense mechanisms (Patel *et al.*, 2012). Several reports have stressed on the role of oxidative stress in the onset and progression of micro-vascular complications linked to diabetes such as retinopathy, neuropathy and nephropathy (Kangralkar *et al.*, 2010; Rajeshwari and Andallu, 2011; Jaiswal *et al.*, 2012). Chronic blood sugar imbalances or dysglycaemia has been found to both induce and exacerbate the oxidative condition. Persistent hyperglycaemia contribute to the progression and maintenance of an oxidative environment through

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glucose oxidation and the production of Advanced Glycation End products (AGE) (Goycheva *et al.*, 2006; Pazdro and Burgess, 2010; McCune and Johns, 2007). Additionally, evidence from scientific literature has shown that plants exhibiting antioxidant properties contributed in maintaining antioxidant level in diabetic subjects, thus delaying and managing diabetes complications (Brewer, 2011; Shori and Baba, 2011). Indeed, plants remain one of the best sources for the discovery of novel nutraceuticals (Brewer, 2011; Shori and Baba, 2011). Furthermore, the supplementation of antioxidants together with existing glycaemic control strategies might be beneficial in the treatment and management of the pathology.

From the ancestral shamanic cultures of Africa and South America to the highly modernised societies of today, several medicinal plants have been used for the treatment of various human ailments, including diabetes. Knowledge of the therapeutic potential of traditional medicinal plants has been the essence of search for new drugs. Furthermore, phytochemicals present in plants such as flavonoids, phenolic acids, coumarins, anthraquinones have been reported to be potent oxidant scavengers (Rajeshwari and Andallu, 2011; Goycheva *et al.*, 2006). Emerging research has begun to investigate the antioxidant potential of local plants. Although, a free advanced health care system exists, many Mauritians still rely on the use of folk medicine. Many indigenous and endemic plant species of Mauritius are used in folkloric medicine to treat various ailments including diabetes and related complications (Mahomoodally *et al.*, 2010; Gurib-Fakim *et al.*, 1993). However, the antioxidant potential of the majority of traditional anti-diabetic medicinal plants has not been scientifically evaluated yet.

The present study evaluated the phytochemical and antioxidant profile of six traditional medicinal plants of the island using *in-vitro* bio-assay models. The reducing capacities along with the total phenolic, flavonoid and anthocyanin content and phytochemical profile of the extracts were also determined.

MATERIALS AND METHODS

Plant materials and extraction: Leaves of traditional medicinal plants; *Stillingia lineata* Lam. (Euphorbiaceae) (SL), *Faijasiopsis flexuosa* Lam. (Asteraceae) (FF), *Erythroxylum laurifolium* Lam. (Erythroxylaceae) (EL), *Elaeodendron orientale* Jacq. (Celastraceae) (EO) and *Antidesma madagascariensis* Lam. (Euphorbiaceae) (AM) were collected from the upper humid regions of the island. The identity of the plants was confirmed by the natural reserve curator. The harvested plant materials were thoroughly washed under running tap water and air dried until a constant weight was obtained. Subsequently,

the dried samples were ground (Pacific mixer grinder, India) and stored in a cool-dry place prior to extraction. Crude methanolic extracts were obtained by soaking the dry powdered material into 70% methanol (1:10, sample: solvent w/v) for 72 h. Crude aqueous extracts were prepared following traditional decoction method. Briefly, dried powdered material (50 g) was boiled into distilled water (200 mL) for 30 min. The filtrates were concentrated *in vacuo* using a rotary evaporator (Rotavap Stuart Scientific Ltd, Staffordshire, UK). The resulting paste like material was stored at -20°C or dissolved in appropriate solvents.

Phytochemicals screening: Concentrated extracts were subjected to phytochemical screening using standard protocols (Tiwari *et al.*, 2011; Andzouana and Mombouli, 2011) to detect the presence of alkaloids, saponins, phenols, anthraquinones, steroids and flavonoids.

Determination of total phenol content: Total Phenolic Content (TPC) was determined according to the modified Folin Ciocalteu assay described by Nickavar and Esbati (2012). The reaction mixture containing a tenfold diluted Folin Ciocalteu reagent solution (2500 µL), plant extract (500 µL) and sodium carbonate (2000 µL, 7.5%) was allowed to react for 30 min at room temperature. The TPC was then spectrophotometrically determined at 760 nm. Results obtained were expressed as µg Gallic Acid Equivalent (GAE) mg⁻¹ crude extract.

Determination of total flavonoid content: Total Flavonoid Content (TFC) was determined following the aluminium chloride colorimetric method (Amaeze *et al.*, 2011). The reaction mixture consisted of plant extract (2 mL) and 2% aluminium chloride solution (2 mL). The mixture was allowed to react for 30 min at room temperature and the absorbency was read at 420 nm. Results so obtained were expressed as µg Rutin Equivalent (RE) mg⁻¹ crude extract.

Determination of total anthocyanin content: Total Anthocyanin Content (TAC) was determined based on the pH differential method (Sutharut and Sudarat, 2012). Briefly, 1 mL of plant extract (100 mg mL⁻¹) was transferred into 10 mL volumetric flask and the volume was adjusted with buffer pH 1.0 and pH 4.5. Mixtures were allowed to equilibrate for 15 min. Absorbance of each dilution was spectrophotometrically determined at 510 and 700 nm. Absorbance of diluted samples was determined using the following equation:

$$A = (A_{510} - A_{700}) \text{pH}_{1.0} - (A_{510} - A_{700}) \text{pH}_{4.5}$$

The monomeric anthocyanin pigment concentration in the original sample was calculated according to the following equation:

$$\text{Anthocyanin content (mg mL}^{-1}\text{)} = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times l}$$

where, MW is the molecular weight of cyanidin-3-glucoside (484.5), DF the dilution factor and ϵ the molar extinction coefficient (26,900).

Ferric Reducing Antioxidant Power (FRAP) assay:

The capacity of the extracts to reduce iron was determined according to the modified method of Benzie and Strain (1996) using trolox as standard. Plant extracts of varying concentrations were mixed with 2850 μL FRAP solution (25 mL acetate buffer (300 mM, pH 3.6), 2.5 mL 2-4-6 tripyridyl-s-triazine (10 in 40 mM hydrochloric) (TPTZ; Sigma-Aldrich, Sydney, Australia) and 2.5 mL hydrated ferric chloride solution (20 mM) previously equilibrated for 30 min in the dark at 37°C). The reaction was allowed for 30 min in the dark and absorbance was read at 593 nm. Data obtained were expressed as mM Trolox Equivalent (TE) mg^{-1} crude extract.

Nitric oxide radical scavenging assay: Nitric Oxide (NO) was generated from sodium nitroprusside (SNP) (Sigma-Aldrich, Sydney, Australia) and was measured by the Griess Illosvoy reagent (Mandal *et al.*, 2009), using 0.1% w/v naphthylethylene-diamine-dihydrochloride (Sigma-Aldrich, Sydney, Australia) instead of 5% 1-naphthylamine. The reaction mixture contained SNP (2 mL), Phosphate Buffer Saline (PBS) (0.5 mL, pH 7.4) and plant extract (0.5 mL) was incubated for 2½ h at 25°C. Following incubation, 0.5 mL of the reaction mixture was added to 1 mL suphanilic acid (0.33 in 20% glacial acetic acid) (Sigma-Aldrich, Sydney, Australia) and the mixture was allowed to stand for 5 min. Naphthylethylene-diamine-dihydrochloride (1 mL, 0.1% w/v) was then added to the mixture vortexed and allowed to stand for further 30 min. The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylene-diamine-dichloride was read at 546 nm. Percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \times 100$$

and the IC_{50} was calculated.

DPPH free radical scavenging assay: The free radical scavenging activity of the different extracts was measured by using 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) (Sigma-Aldrich, Sydney, Australia) using the modified

method of Umamaheswari and Chatterjee (2008). Briefly, 200 μL freshly prepared DPPH solution (100 μM in methanol) and 100 μL plant extract at different concentration in methanol was incubated at 37°C for 30 min. After incubation, absorbance was measured at 517 nm. The percentage inhibition of DPPH was calculated following the equation mentioned above and the IC_{50} was calculated.

Hydroxyl radical (OH•) scavenging assay: The reaction mixture (1 mL) consisted of hydrogen peroxide (150 $\mu\text{mol L}^{-1}$), iron chloride (30 M), EDTA (30 M), ascorbic acid (30 M), 2deoxy-D-ribose (25.8 M), PBS (pH 7.4) and extracts of differing concentration was incubated at 37°C for 30 min (Halliwell *et al.*, 1987). Subsequently, 1 mL trichloroacetic acid (60 g L^{-1}) and 0.5 mL thiobarbituric acid (1 g in 100 mL of 0.05 mol L^{-1} NaOH) were added and the reaction mixture was boiled for 20 min. The solution was allowed to cool and the absorbance was read at 532 nm. Percentage inhibition was calculated from the equation above and the IC_{50} was generated.

Hypochlorous acid radical scavenging assay: Stable hypochlorous acid was prepared according to the method described by Wang *et al.* (2007). To 100 μL of plant extract in 1 mL PBS (pH 7.4), 100 μL HOCL (600 $\mu\text{mol L}^{-1}$) and 100 μL taurine (150 mmol L^{-1}) were added. The mixture was vortexed and allowed to stand for 10 min at 25°C. Potassium iodide (100 μl , 20 mmol L^{-1}) was then added to each tube and absorbency was read at 350 nm. The chlorination of taurine was used to measure hypochlorous acid (HOCL) released (Weiss *et al.*, 1982).

Trolox Equivalent Antioxidant Capacity (TEAC) assay:

A modified TEAC method involving 2, 2-azinobis (3, ethyl benz-thiazoline-6-sulfonic acid) diammonium salt and manganese (IV) oxide was used to evaluate the free radical scavenging activity of the plant extracts as described by Rummun *et al.* (2013). ABTS (2, 2 azinobis- 3-ethylbenzthiazoline-6-sulfonic acid) radical was generated between ABTS (0.5 mM) and activated MnO_2 (1 mM) in phosphate buffer (0.1 M, pH 7.0). The absorbance of the resulting blue solution was adjusted to $0.500 \text{ nm} \pm 0.010$. To 3 mL of the ABTS^{•+} solution, 0.5 mL of extract was added and the decay in absorbance at 734 nm was monitored for 15 min. Calculations were made with respect to a dose-response curve of trolox and the results were expressed in μmol Trolox Equivalent (TE) mg^{-1} crude extracts.

Xanthine oxidase inhibition assay: Crude methanolic and aqueous extracts were assayed for their Xanthine Oxidase (XO) inhibitory activity as described by Abdullahi *et al.* (2012). The assay mixture consisted of 1 mL extract at varying concentration, 2.9 mL phosphate

buffer (pH 7.5), 0.1 mL xanthine oxidase (0.1 units mL⁻¹ in phosphate buffer pH 7.5). After preincubation at 25°C for 15 min, 2 mL xanthine (150 mM in phosphate buffer pH 7.5) was added to initiate the reaction. The mixture was incubated at 25°C for 30 min and the reaction was stopped by the addition of 1 mL 1 N hydrochloric acid. Uric acid produced was measured at 290 nm using a UV spectrophotometer. Allopurinol a known inhibitor of xanthine oxidase was used as positive control.

Statistical analysis: Results are expressed as mean of triplicates \pm SD of independent determinations. Difference between the samples and controls was determined using one way ANOVA with statistical significance $p < 0.05$ using SPSS 16.0 and correlation coefficients were computed using Microsoft Office Excel 2007.

RESULTS

Phytochemical screening: Table 1 shows the phytochemical profile of the methanolic and aqueous plant extracts of six traditional medicinal plants of Mauritius. Alkaloids, flavonoids and phenolic compounds were found to be present in all extracts.

Total phenolic, flavonoids and anthocyanin content: Table 2 shows the TPC, TFC and TAC of plant extracts. Methanolic extracts yielded higher TPC and TAC as compared to their corresponding aqueous extracts.

Reducing power and antioxidant potential: Table 3 summarises the reducing power and oxidant scavenging activity of methanolic and aqueous plant extracts. It was found that the extracts exhibited variable reducing potential on Fe³⁺. It is noteworthy to highlight that EL extracts (23.79 \pm 0.43 and 29.12 \pm 1.22 TE (mM mg⁻¹ crude extract) for methanolic and aqueous fractions, respectively) showed highest Fe³⁺ reducing capacity. Similarly, TEAC values showed that EL, EO, AM and SL were potent hydrogen-donating compounds. EL, AM and FF aqueous extracts (IC₅₀: 105.57 \pm 1.88, 135.76 \pm 3.04 and 29.91 \pm 4.92 μ g mL⁻¹, respectively) significantly ($p < 0.05$) scavenged DPPH[•] compared to positive control, ascorbic acid (IC₅₀: 189.91 \pm 6.18 μ g mL⁻¹). Results in Table 3 also demonstrated the ability of active plant extracts to scavenge NO[•] and OH[•]. The plant extracts scavenged NO[•] (IC₅₀ ranged from 440.67 \pm 29.44 to 4680.84 \pm 52.40 μ g mL⁻¹) and OH[•] (IC₅₀ ranged from 2147.08 \pm 14.68 to 5701.92 \pm 72.71 μ g mL⁻¹) but were significantly ($p < 0.05$) less active than ascorbic acid and α -tocopherol, respectively (IC₅₀ value: 333.98 \pm 4.75 μ g mL⁻¹ and 122.83 \pm 0.88 μ g mL⁻¹, respectively). On the other hand, IC₅₀ values of extracts that significantly ($p < 0.05$) scavenged HOCl acid radical compared to ascorbic acid (IC₅₀: 6455.28 \pm 9.33 μ g mL⁻¹) ranged from 161.14 \pm 5.02-4576.23 \pm 37.26 μ g mL⁻¹. Moreover, the inhibitory activity of the plant extracts on XO was assayed. It was observed that crude methanolic extracts of EL, EO and AM (IC₅₀ ranged from

Table 1: Qualitative analysis of phytochemical of plant extracts

Plant extracts	Alkaloids	Phenols	Flavonoids	Saponins	Steroids	Anthraquinones
EL	[+] (+)	[+] (+)	[+] (+)	[-] (-)	[-] (+)	[+] (+)
EO	[+] (+)	[+] (+)	[+] (+)	[-] (-)	[+] (+)	[+] (+)
AM	[+] (+)	[+] (+)	[+] (+)	[+] (-)	[+] (+)	[+] (+)
SL	[+] (+)	[+] (+)	[+] (+)	[+] (-)	[-] (-)	[+] (+)
FF	[+] (+)	[+] (+)	[+] (+)	[+] (-)	[-] (-)	[+] (+)

SL: *Stillingia lineata*, FF: *Faijasiopsis flexuosa*, EL: *Erythroxylum laurifolium*, EO: *Elaeodendron orientale*, AM: *Antidesma madagascariensis*, +: Detected. -: Not detected. []: Methanolic extracts, (): Aqueous extracts

Table 2: Total phenolic, flavonoid and anthocyanin content of plant extracts

Plant extracts	TPC (GAE μ g mg ⁻¹ crude extract)	TFC (RUE μ g mg ⁻¹ crude extract)	TAC (cyanidin-3-glucoside mg mL ⁻¹)
EL	[454.68 \pm 0.71] (160.25 \pm 0.74)	[20.11 \pm 0.59] (23.17 \pm 0.79)	[52.95 \pm 4.02] (1.80 \pm 1.00)
EO	[335.20 \pm 3.03] (313.47 \pm 4.88)	[14.67 \pm 0.96] (16.86 \pm 0.32)	[44.01 \pm 8.04] (2.58 \pm 0.68)
AM	[273.87 \pm 3.06] (58.10 \pm 0.18)	[11.04 \pm 0.45] (5.12 \pm 0.48)	[34.70 \pm 5.25] ND
SL	[299.77 \pm 1.65] (114.87 \pm 1.27)	[34.93 \pm 2.87] (19.47 \pm 0.90)	[6.96 \pm 1.89] ND
FF	[228.13 \pm 5.92] (131.51 \pm 4.10)	[22.30 \pm 0.70] (24.73 \pm 1.47)	[36.08 \pm 0.73] ND

SL: *Stillingia lineata*, FF: *Faijasiopsis flexuosa*, EL: *Erythroxylum laurifolium*, EO: *Elaeodendron orientale*, AM: *Antidesma madagascariensis*, TPC: Total phenolic content, TFC: Total flavonoid content, TAC: Total anthocyanin content. All data are the mean of triplicate measurements \pm standard deviation. []: Methanolic extracts, (): Aqueous extracts, ND: Not detected

Table 3: Reducing capacities and radical scavenging properties (DPPH•, NO•, OH• and HOCl scavenging XO inhibitory activity) of plant extracts

Plants	Trolox equivalent		IC ₅₀ (μg mL ⁻¹)				
	FRAP (mM mg ⁻¹ crude extract)	TEAC (μM mg ⁻¹ crude extract)	DPPH•	NO•	OH•	HOCl	XO
EL	[23.79±0.43] (29.12±1.22)	[2363.51±26.49] (3062.22±53.89)	[195.28±6.86**] (105.57±1.88*)	[1025.18±21.52**] (604.18±15.58**)	[2459.50±42.63**] NA	[1359.50±33.06*] (3325.52±5.44*)	[31409.38±25.36**] NA
EO	[21.48±0.41] (19.36±0.83)	[2633.68±21.05] (3191.11±20.37)	[255.52±8.91**] (258.24±6.63**)	[1090.19±40.46**] (889.22±21.70**)	NA NA	NA (4576.23±37.26*)	[23331.74±45.49**] (86779.18±22.13**)
AM	[21.08±0.77] (11.33±0.26)	[2342.46±6.08] (1124.44±50.48)	[296.33±9.48**] (135.76±3.04*)	[717.28±24.67**] (780.11±12.10**)	[5701.92±72.71**] NA	[230.91±5.71*] (7162.85±29.98**)	[6158.42±6.51**] NA
SL	[21.80±0.80] (13.95±0.36)	[2960.00±53.33] (1644.44±55.51)	[322.26±11.19**] (297.81±7.90**)	[440.67±29.44**] (3458.42±38.01**)	[4414.83±12.32**] NA	[203.57±2.87*] (557.93±27.20*)	NA (15317.15±16.74**)
FF	[18.91±0.30] (23.83±0.60)	[968.89±55.51] (1484.44±40.73)	[632.68±11.72**] (29.91±4.92*)	[1461.93±19.15**] (574.48±6.78**)	[5553.76±21.62**] NA	[161.14±5.02*] (1160.14±49.20*)	NA NA
Control	NA	NA	189.91±6.18 ^a	333.98±4.75 ^a	122.83±0.88 ^b	6455.28±9.33 ^a	444.48±3.64 ^c

SL: *Stillingia lineate*, FF: *Fajasiopsis flexuosa*, EL: *Erythroxylum laurifolium*, EO: *Elaeodendron orientale*, AM: *Antidesma madagascariensis*, FRAP: Ferric reducing antioxidant power, TEAC: Trolox equivalent antioxidant capacity, DPPH•: 2, 2-diphenyl-2-picrylhydrazyl radical, NO•: Nitric oxide radical, OH•: Hydroxyl radical, HOCl acid: Hypochlorous acid radical, XO: Xanthine oxidase. All data represent the mean ± standard deviation of three independent analyses. *Values significantly (p<0.05) higher than control. **Values significantly (p<0.05) lower than control. NA: No activity. Control: ^aascorbic acid or ^bα-tocopherol or ^callopurinol, []: Methanolic extracts, (): Aqueous extracts.

Table 4: Correlation coefficients between quantitative phytochemical parameters (TPC, TFC and TAC) and antioxidant assays (DPPH•, NO•, OH• and HOCl acid scavenging XO inhibitory activity)

	TPC	TFC	TAC
FRAP	[0.93] (0.34)	[0.05] (0.78)	[0.25] NA
TEAC	[0.46] (0.83)	[0.17] (0.39)	[-0.30] NA
DPPH•	[-0.78] (0.36)	[0.20] (-0.22)	[-0.22] NA
HOCl	[0.83] (-0.01)	[0.01] (-0.84)	[0.50] NA
NO•	[-0.10] (-0.19)	[-0.36] (0.05)	[-0.65] NA
OH•	[-0.96] NA	[-0.23] NA	[-0.39] NA
XO	[0.93] NA	[0.95] NA	[0.98] NA

FRAP: Ferric reducing antioxidant power, TEAC: Trolox equivalent antioxidant capacity, DPPH•: 2, 2-diphenyl-2-picrylhydrazyl radical, NO•: Nitric oxide radical, OH•: Hydroxyl radical, HOCl acid: Hypochlorous acid radical, XO: Xanthine oxidase, TPC: Total phenolic content, TFC: Total flavonoid content, TAC: Total anthocyanin content, []: Methanolic extracts, (): Aqueous extracts, NA: Not applicable

6158.42±6.51 to 31409.38±25.36 μg mL⁻¹) inhibited XO but were significantly (p<0.05) much less potent than allopurinol (IC₅₀: 444.48±3.64 μg mL⁻¹).

Correlation coefficients between TPC, TFC, TAC and antioxidant assays: From Table 4, a correlation coefficient close to 1 was obtained between TPC and FRAP for methanolic fractions only. TEAC for aqueous extracts was correlated to the TPC (0.83) rather than TFC (0.39). Table 4 highlight the positive correlation coefficients between TPC, TFC, TAC and XO inhibition in the presence of methanolic extracts.

DISCUSSION

Oxidative stress has been extensively linked to the pathogenesis of diabetes and of its related complications

(Kangralkar *et al.*, 2010; Rajeshwari and Andallu, 2011; Jaiswal *et al.*, 2012). Indeed, an imbalance between the formation of ROS and the innate antioxidant defense mechanisms has been suggested to contribute to the onset and progression of several chronic diseases, including diabetes. There have been a growing number of investigations on the possible anti-radical potential of traditional used medicinal plants (Senthil Kumar *et al.*, 2012). Furthermore, studies geared towards finding natural antioxidants have gained increased importance due to potential side effects such as carcinogenicity of synthetic antioxidants (Senthil Kumar *et al.*, 2012). In the present investigation, the antioxidant properties along with the TPC, TFC and TAC of six traditionally used medicinal plants of Mauritius were evaluated. To evaluate oxidant scavenging capacity of these extracts, standard bioassays such as FRAP, TEAC, NO•, OH•, DPPH• and HOCl acid radical potentials were used. Data from the study tend to show that methanolic extracts of EL, AM, SL and FF were active scavengers of NO•, OH•, DPPH• and HOCl acid radical and reduced Fe³⁺ and ABTS+*in-vitro*. However, variable inhibition was observed and few extracts gave IC₅₀ values lower than standard positive controls (ascorbic acid and α-tocopherol).

Active oxygen species such as hydrogen peroxide, hydroxyl radical and peroxy nitrite anions are known to contribute to oxidative stress and to the exacerbation of diabetes and its related health problems (Young and Woodside, 2001; Maritim *et al.*, 2003). Recently, an increased interest has emerged in finding naturally occurring oxygen scavengers due to their safety and their ability to shift in balance towards an adequate antioxidant status. Particular importance has been given to plants due to their rich phytochemical content. In the present study, FRAP and TEAC assays demonstrated that EL, EO, AM, SL and FF possess reducing potential. The TEAC and

FRAP method reflected the hydrogen-donating ability of the plant extracts through the scavenging of preformed radical cation ABTS⁺ and Fe³⁺ relative to that of the standard trolox. It was also observed that the extracts having high phenolic content exhibited significant ($p < 0.05$) reducing capacity (positive correlation coefficient values obtained between FRAP/TEAC and TPC). This finding was consistent with Guruvaiah *et al.* (2012), who previously reported the strong reductive capacity of phenol-rich extracts. On the other hand, DPPH, a stable free radical, was used for estimating free radical scavenging activities of antioxidants. The reduction of DPPH[•] to a stable diamagnetic molecule was monitored by a decrease in absorbance at 517 nm. Experimental data revealed that EL, AM and FF aqueous extracts significantly ($p < 0.05$) scavenged DPPH[•] compared to ascorbic acid. Other studies tend to suggest that the DPPH method measures the ability of hydrophilic antioxidant molecules to scavenge the radical (Ang *et al.*, 2012; Senthil Kumar *et al.*, 2012). This might explain the pronounced activity of the aqueous extracts compared to the organic fractions. Moreover, it can be argued that the DPPH[•] scavenging activity of aqueous extracts might be due to flavonoids and phenolic compounds (Senthil Kumar *et al.*, 2012) rather than anthocyanin due to the relatively low TAC of the aqueous extracts. Besides, it was observed that plant extracts exhibited variable inhibitory action against NO[•] but were less potent than the positive control ascorbic acid. These extracts might be less beneficial in scavenging NO[•] which according to Amaeze *et al.* (2011), has been associated to inflammatory conditions such as juvenile diabetes. Apart from NO[•], OH[•] is also a reactive radical species (Young and Woodside, 2001; Sathya and Siddhuraju, 2012) associated with most free radical induced tissue damage *in-vivo* (Manian *et al.*, 2008). Due to its high instability, OH[•] was found to react with almost every type of molecules *in vivo* (Young and Woodside, 2001). Non-radical molecules such as H₂O₂ and HOCl contribute in the formation of OH[•], increasing its concentration (Halliwell, 2005). Hence, scavenging HOCl might be beneficial in delaying and/or preventing the formation of OH[•]. Results from the present investigation indicated that the extracts (except EO methanolic and AM aqueous extracts) were found to be potent scavengers of HOCl, thereby suggesting that they might prevent the formation of OH[•] *in-vivo*.

Multiple lines of evidence support the antioxidant capacities of medicinal plants and such effects could be ascribed to secondary metabolites or phytochemicals. From the present investigation, it was found the presence of at least alkaloids, flavonoids, phenols and anthraquinones which are known to possess antioxidant properties (Senthil Kumar *et al.*, 2012). The variability in composition of phytochemicals might be attributed to

various factors including, geographical location, environment and species (Andzouana and Mombouli, 2011). Hence, it can be suggested that the observed antioxidant activity of these extracts might be due to these phytochemicals or to their concerted action. Besides, the inhibitory activity of crude methanolic and aqueous extracts on Xanthine Oxidase (XO) was assessed. XO is a pro-oxidant enzyme and has been related to the increased production of ROS and to the onset of secondary complications such as cataract (Miric *et al.*, 2013) and vascular complications (Matsumoto *et al.*, 2003). Another study reported that XO was a major source of hyperglycemia-induced ROS production in muscles. Furthermore, it was observed that inhibition of XO reduced oxidative stress and improved mitochondrial alterations in muscles cells of diabetics (Bravard *et al.*, 2011). Data gathered from this study has shown that methanolic extracts of EL, EO and AM inhibited XO, although, to a much less extent than allopurinol. The inhibitory action of these extracts might be due to the concerted action of phytochemicals present (positive correlation observed between XO inhibitory activity and TPC, TFC and TAC).

CONCLUSION

The results of this study demonstrated that both methanolic and aqueous extracts contain phytochemical compounds that exhibit antioxidant activities. Further studies are required to determine the safety of the plants prior to their use as adjuncts in the treatment of inflammatory conditions like diabetes mellitus. Moreover, the isolation and purification of active compounds present in the plant extracts might be beneficial in the development of novel targets against diabetes.

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