

## Research Article

# Phenolic Constituents and Antioxidant Efficacies of some Mauritian Traditional Preparations Commonly Used Against Cardiovascular Disease

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### ABSTRACT

The surge of interest in traditional medicine has prompted research into the phenolic composition and antioxidant activity of *Adansonia digitata*, *Catharanthus roseus*, *Eugenia uniflora*, *Phyllanthus emblica* and *Terminalia arjuna* which are commonly used as traditional remedies against cardiovascular diseases in Mauritius. The polyphenolic profiles of these 5 herbal preparations (prepared according to traditional medicine sellers' recipe) were screened. HPLC analyses showed that the plant extracts had complex flavonoid profiles with interesting amounts of quercetin, myricetin and kaempferol conjugates. Flavan-3-ol derivatives such as (+) catechin, (-) epicatechin, catechin gallates, procyanidins B1 and B2 were identified in most preparations. The highest total phenolic ( $34.9 \pm 0.92$  mg/g FW) and proanthocyanidin ( $15.0 \pm 0.416$  mg/g FW) contents were measured in *T.arjuna* whilst *E.uniflora* had the highest flavonoid composition ( $7.8 \pm 0.04$  mg/g FW). The antioxidant and free radical scavenging potential were evaluated using trolox equivalent antioxidant assay (TEAC), ferric reducing antioxidant power (FRAP), scavenging of hypochlorous acid, deoxyribose assay and inhibition of microsomal lipid peroxidation. *T.arjuna* extract exhibited the most powerful antioxidant capacity in the systems studied with a TEAC value of  $306.92 \pm 2.37$   $\mu$ mol/g FW and calculated IC<sub>50</sub>s of  $1.5 \pm 0.09$  mg/ml and  $0.2 \pm 0.01$  mg/ml in scavenging hypochlorous acid and in inhibiting microsomal lipid peroxidation respectively. The antioxidant activities correlated strongly with the total phenolic content of the plant extracts.

**Key words:** antioxidants, cardiac dysfunctions, free radicals, oxidative damage, phenolics, traditional medicine in Mauritius

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### INTRODUCTION

Cardiovascular diseases (CVD) have taken pandemic proportions and are the major causes of death in the Western world as well as in developing countries. Changes in human behaviour and lifestyle such as sedentarity, overly rich nutrition, obesity in conjunction with genetic susceptibility have resulted in dramatic increase in the incidence of CVD worldwide. A high prevalence of chronic diseases of the circulatory system among the Mauritian population, which accounted for 35 % of deaths in 2008, has been reported. Out of this, acute myocardial infarction and other ischaemic heart diseases represented 35.7 % of the total number of deaths resulting from CVDs with a further 19.3 % caused by other heart diseases<sup>1</sup>.

Biochemical, physiological and pharmacological evidence support direct and/or indirect role of oxidative stress in cardiovascular tissue injury<sup>2-4</sup>. Oxidative stress induced-damage includes acceleration of cell death through apoptosis and necrosis while it can play a pivotal role in many cardiac

disorders primarily vascular smooth muscle proliferation, angiogenesis, cardiac hypertrophy, fibrosis and atherosclerosis<sup>5, 6</sup>. In addition, oxidative modification of low-density lipoprotein (LDL) is recognized as an important step in the initiation of atherogenic plaque formation and oxidised-LDL triggers the up-regulation of surface and soluble cell adhesion molecule and the release of cytokines by vascular endothelium which induces the expression of genes involved in cell growth, survival, adhesion and inflammatory responses<sup>7, 8</sup>. Thus there is much interest in antioxidants in particular from dietary and plant sources for counteracting the deleterious effect of free radicals in cardiovascular disorders.

The use of traditional and alternative medicine in CVD is strongly anchored in various parts of the world as well as in Mauritius. For instance St John's wort, *Ginkgo biloba*, *Panax ginseng*, *Eugenia uniflora*, *Terminalia arjuna* and kava kava extracts are widely used for cardiac and vascular indications and the beneficial effects have been attributed to

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Table 1. Details of plants used in the present study.

Plant species	Family	Vernacular name	Site of collection	Part used
<i>Terminalia arjuna</i>	Combretaceae	-	Réduit	Bark
<i>Eugenia uniflora</i>	Myrtaceae	<i>Roussaille</i>	Rose-Hill	Leaves
<i>Phyllanthus emblica</i>	Euphorbiaceae	<i>Bilimbi madras</i>	Triolet	Seeds
<i>Adansonia digitata</i>	Bombaceae	<i>Baobab</i>	Eglise St-Anne	Leaves
<i>Catharanthus roseus</i>	Apocynaceae	<i>Saponaire</i>	Rose-Hill	Leaves

the presence of bioactive secondary metabolites like alkaloids and polyphenols<sup>9,10</sup>. The pleiotropic effects along with the modulatory actions of plant phytochemicals have made them valuable prophylactic compounds. Epidemiological studies have consistently shown an inverse association between consumption of phenolic-rich dietary food like vegetables, fruits and teas and CVD and certain forms of cancer<sup>11-15</sup>. A significant number of flavonoids mostly from the flavonols, flavan-3-ols, flavanones, flavones and isoflavones classes are purportedly known to have beneficial cardiovascular health effects<sup>16</sup> which may in part stem from their antioxidative capacities. These compounds reaching only 1–5  $\mu\text{M}$  in blood levels are bioavailable to various degrees and although they have a relatively short half-life, measurable tissue levels in humans appear to be sufficient for biological activity, and there do not appear to be any safety risks from typical dietary intakes of flavonoid-rich foods. This indicates that flavonoids rich extracts can find application for the management of CVD. Plant remedies in Mauritius have long been described in the traditional pharmacopoeias and 460 medicinal plants from 118 families have been described till date, taken as infusions or decoctions of the plant's various parts<sup>17</sup>. In view of the growing interest in traditional medicines, plant extracts are being re-evaluated to indicate their mechanism of action by extensive research with reference to their therapeutic principles. In this line, five plant-based formulations commonly used against cardiovascular diseases in Mauritius namely *Terminalia arjuna*, *Eugenia uniflora*, *Adansonia digitata*, *Catharanthus roseus* and *Phyllanthus emblica* were screened for their phenolic constituents and antioxidant potential using five independent in vitro redox assays. The data generated will provide an overview of the antioxidant efficacies of the medicinal plant extracts and possible

mechanistic insights into their prophylactic action thus strengthening the traditional knowledge systems on the use of these plants.

## MATERIALS AND METHODS

### Chemicals

2,2'-azino-bis(3-ethylbenzthiozoline-6-sulphonic acid (ABTS), 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), trolox C (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E and (2,4,6-tri(2-pyridyl) s-triazine (TPTZ) were purchased from Sigma Co. (St. Louis, MO, USA). HPLC grade of (+)-catechin ((+)-C), (-)-epicatechin ((-)-EC), (-)-epigallocatechin ((-)-EGC), (-)-epicatechin-3-gallate ((-)-ECG), (-)-epigallocatechin-3-gallate ((-)-EGCG), procyanidin dimers B1 and B2, myricetin, kaempferol, quercetin, cyanidin chloride and gallic acid (GA) were purchased from Extrasynthèse (Genay, France). All other reagents used were of analytical grade.

### Plant material

The five traditional medicinal plants used were: *Terminalia arjuna*, *Eugenia uniflora*, *Adansonia digitata*, *Catharanthus roseus* and *Phyllanthus emblica* (Table 1). Voucher specimens were deposited at the Mauritius Herbarium, Mauritius Sugar Industry Research Institute, Réduit and Department of Biosciences, University of Mauritius for identification purposes.

### Herbal preparation

The plant extracts were prepared as indicated by the traditional practitioner and as per the way it is consumed in traditional medicine (Table 2).

### Total phenolic content

Table 2: Preparation of the herbal extracts as indicated by their traditional use.

Plant species	Method of herbal preparation
<i>T. arjuna</i>	Sheets of the bark were allowed to dry in darkness for 14 days. The bark was then crushed into a fine powder. 50 g of powder was placed in 500 mL water and brought to the boil. Boiling was maintained for 4 hrs under a low flame. The decoction was then strained and the extract freeze-dried.
<i>P. emblica</i>	The fruits were boiled and the pulp was removed. The seeds were allowed to dry for 14 days. The seeds were crushed into a fine powder. 50 g of seed powder was then brought to the boil in 500 mL of water and boiling was maintained for 2 hrs under a low flame. The decoction was then strained and the extract freeze-dried.
<i>E. uniflora</i>	50g of fresh leaves were allowed to infuse in 300 mL of boiled water. The infusion was allowed to stand overnight in the fridge. The infusion was then strained and the aqueous extract freeze-dried.
<i>A. digitata</i> and <i>C. roseus</i>	50 g of fresh leaves were boiled in 500 mL water. Boiling was maintained under low flame for 2 hrs. The decoction was then strained and the extract freeze-dried.

The total phenolic content of the extract was estimated using the Folin-Ciocalteu method adapted from Singleton and Rossi<sup>18</sup>. To 0.25 ml of diluted extract, 3.5 ml of distilled water was added followed by 0.25 ml of Folin-Ciocalteu solution (Merck). After 3 minutes, 1 ml sodium carbonate (20% w/v) was added. The whole mixture was incubated at 40 °C for 40 minutes. A deep blue colouration developed whose absorbance was read at 685 nm. Results are expressed in mg of gallic acid equivalent g<sup>-1</sup> fresh weight of plant material.

#### **Total flavonoid content**

The AlCl<sub>3</sub> method adapted from Lamaison et al.<sup>19</sup> was used for the determination of the total flavonoid content of the extracts. 1.5 ml methanolic extract was added to an equal volume of a solution of 2% AlCl<sub>3</sub>.6H<sub>2</sub>O. After proper mixing, the mixture was incubated for 10 minutes at ambient temperature. The absorbance of the solution was read at 440 nm. Flavonoid contents are expressed in mg quercetin equivalent g<sup>-1</sup> fresh weight of plant material.

#### **Total proanthocyanidin content**

The modified acid/butanol assay of Porter et al.<sup>20</sup> was used to quantify the total proanthocyanidin content. 0.25 ml aliquot of extract was added to 3 ml of a 95% solution of n-butanol/HCl (95:5 v/v) followed by 0.1 ml of a solution of NH<sub>4</sub>Fe(SO<sub>4</sub>)<sub>2</sub>.12H<sub>2</sub>O in 2 M HCl. The tightly capped tubes were incubated for 40 min at 95 °C. The absorbance of the cooled extract was read at 550 nm. Results are expressed in mg of cyanidin chloride g<sup>-1</sup> fresh weight.

#### **High-performance liquid chromatography (HPLC)**

Chromatographic analyses of total and hydrolysed plant extracts were carried out using a HP1100 series HPLC (Germany) equipped with a vacuum degasser, quaternary pump, autosampler, thermostatted column compartment, diode array detector and HP Chemstation for data collection and manipulation. After filtration on Millipore (0.22 µm), 30 µl of extract was injected on a Waters Spherisorb ODS-2 column (5µm particle size, 80 Å pore size, 4.6 mm id x 150 mm) eluted by an acidified acetonitrile-water gradient. Chromatographic separation carried out at 25°C with a flow rate of 0.7 ml/min was as follows: 0-30 minutes, 0-15% B in A; 30-50 minutes, 15% B in A; 50-60 minutes, 15-25% B in A; 60-90 minutes, 15-100% B in A; 90-100 minutes, 100-0% B in A (Solvent A: acetonitrile/water, 1/9 v/v, pH 2.6; Solvent B : acetonitrile/water, 1/1 v/v, pH 2.6). The identification and quantification of the compounds were carried out from (i) the retention time and (ii) peak area in comparison with authentic standards.

#### **Quantitative analysis of phenolic acid and flavan-3-ols in the plant extracts**

Gallic acid, (+) catechin, (-) epicatechin, (-) epicatechin gallate, (-) epigallocatechin, (-) epigallocatechin gallate, procyanidins B1 and B2 were identified and quantified by comparison with authentic standard in duplicates. Absorption wavelength was selected at 280 nm with a 65 minutes running time.

#### **Hydrolysis of flavonoid conjugates and quantitative analysis of aglycones released**

Hydrolysis of flavonoid conjugates was carried out essentially as described by Crozier et al.<sup>21</sup>. An aliquot of 1g fresh weight equivalent freeze dried extract was dissolved in 20 ml 60% methanol containing 100 µg morin as internal standard. 5 ml of 6 M HCl was added such that the resulting solution contained 1.2 M HCl. The mixture was refluxed at 90 °C for two hours. After cooling the flavonoid aglycones were exhaustively extracted using ethyl acetate (2 x 20 ml). The latter phase was evaporated to dryness and taken in absolute methanol prior to analysis by HPLC. The hydrolysed extract was subjected to HPLC analysis and quercetin, myricetin, kaempferol, apigenin and luteolin were identified and quantified within the plant extracts. Absorption wavelength was selected at 360 nm with a 100 minutes running time.

#### **Trolox equivalent antioxidant capacity (TEAC) assay**

The total antioxidant activity of the extracts was assessed by the TEAC assay according to the method of Campos and Lissi<sup>22</sup>. To 3 ml of ABTS<sup>+</sup> solution, generated by a reaction between ABTS (0.5 mM) and activated MnO<sub>2</sub> (1 mM) in phosphate buffer (0.1 M, pH 7), 0.5 ml diluted plant extract was added. The decay in absorbance at 734 nm was monitored for 15 minutes. Data are expressed in µmol Trolox equivalent g<sup>-1</sup> fresh weight.

#### **Ferric reducing antioxidant power (FRAP) assay**

The method of Benzie and Strain<sup>23</sup> was employed to assess the reducing power of the extracts. 100 µl of plant sample was added to 300 µl water followed by 3 ml FRAP reagent. The mixture was incubated at room temperature for 4 minutes and the absorbance was read at 593 nm. Results were expressed in µmol Fe<sup>2+</sup> g<sup>-1</sup> fresh weight.

#### **Scavenging of preformed hypochlorous acid**

The ability of the sample to scavenge hypochlorous acid was assessed according to the method<sup>24</sup> involving chlorination of the amino acid taurine. HOCl was prepared by adjusting the pH of a 1% (v/v) solution of NaOCl to 6.2 with dilute sulphuric acid. The working concentration of the stock solution was determined spectrophotometrically by measuring its absorbance at 235 nm and applying a molar extinction coefficient of 100 M<sup>-1</sup> cm<sup>-1</sup> (Weiss et al, 1982). The reaction mixture contained taurine (10 mM), HOCl (1 mM), plant extract (variable concentrations), phosphate saline buffer (pH 7.4) in a final volume of 1 ml. The solution was mixed thoroughly and incubated for 10 minutes at ambient temperature followed by the addition of 10 µl of potassium iodide (2 mM). A yellow coloration was developed and the absorbance was read at 350 nm. The results were expressed as IC<sub>50</sub> values (mg FW/ml).

#### **Potential to inhibit deoxyribose degradation (Deoxyribose assay)**

The hydroxyl radical scavenging potential of the extracts was determined using the deoxyribose assay<sup>25,26</sup>. The reacting mixture contained in a final volume of 1 ml the following reagents: 200 µl KH<sub>2</sub>PO<sub>4</sub>-KOH (100mM), 200 µl deoxyribose (15 mM), 200 µl FeCl<sub>3</sub> (500 µM), 100 µl EDTA (1mM), 100 µl sample, 100 µl H<sub>2</sub>O<sub>2</sub> (10 mM) and 100 µl ascorbic acid (1 mM). Reaction mixtures were incubated at 37 °C for 1 h. At the end of the incubation

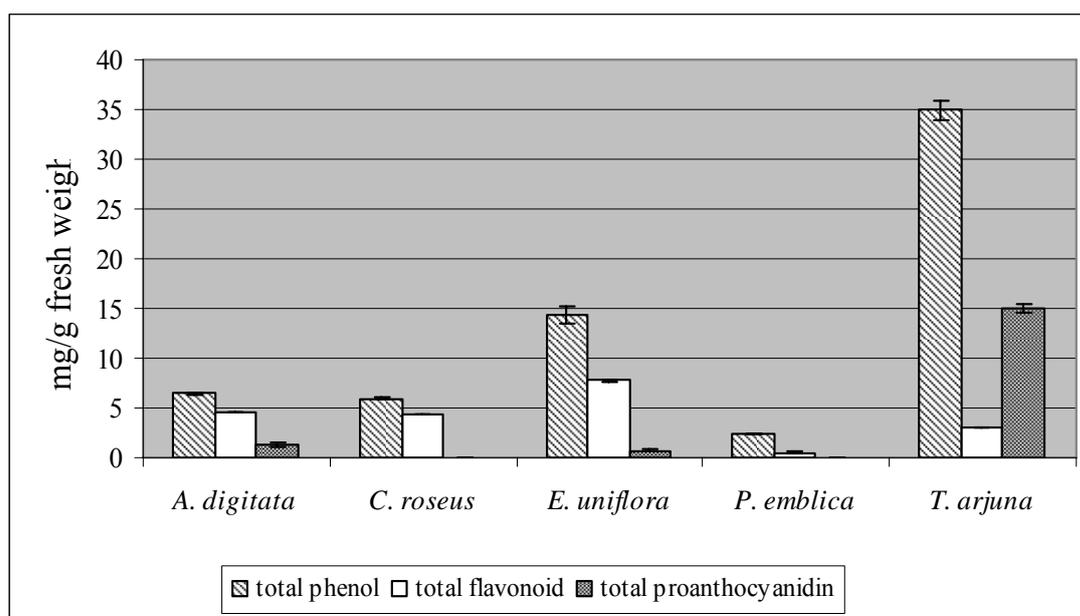


Figure 1: Total phenol<sup>a</sup>, total flavonoid<sup>b</sup> and total proanthocyanidin<sup>c</sup> content of the herbal extracts. Data expressed as mean values  $\pm$  standard deviation ( $n = 3$ ); <sup>a</sup>mg gallic acid/g fresh weight; <sup>b</sup>mg quercetin /g fresh weight, <sup>c</sup>mg cyanidin chloride /g fresh weight

Table 3: HPLC data of (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, procyanidin B1, procyanidin B2 from the herbal preparations. Data expressed as mean values  $\pm$  standard deviation ( $n = 3$ )  $\mu\text{g/g}$  FW.

Plant species	(+) C	(-) EC	(-) EGC	B 1	B 2
<i>A. digitata</i>	544 $\pm$ 25.1	-	286 $\pm$ 10.6	-	348 $\pm$ 15.8
<i>C. roseus</i>	1751 $\pm$ 8.9	635 $\pm$ 2.6	-	-	26 $\pm$ 5.6
<i>E. uniflora</i>	928 $\pm$ 9.5	978 $\pm$ 3.8	-	426 $\pm$ 32.6	219 $\pm$ 9.8
<i>P. emblica</i>	51 $\pm$ 2.5	1798 $\pm$ 7.2	55 $\pm$ 1.2	-	6 $\pm$ 0.9
<i>T. arjuna</i>	530 $\pm$ 12.0	-	408 $\pm$ 31.2	619 $\pm$ 25.4	-

Table 4: Antioxidant capacity of the plant extracts as measured by TEAC, FRAP, HOCl scavenging, deoxyribose assay and inhibition of microsomal lipid peroxidation

Plant Species	TEAC <sup>a</sup>	FRAP <sup>b</sup>	HOCl <sup>c</sup>	Deoxyribose Assay <sup>d</sup>	Lipid peroxidation <sup>e</sup>
<i>T. arjuna</i>	306.9 $\pm$ 2.4	442.6 $\pm$ 1.4	1.5 $\pm$ 0.1	45.1 $\pm$ 4.5	0.2 $\pm$ 0.01
<i>E. uniflora</i>	203.8 $\pm$ 0.6	128.5 $\pm$ 0.3	2.2 $\pm$ 0.1	38.2 $\pm$ 4.2	0.8 $\pm$ 0.01
<i>A. digitata</i>	60.5 $\pm$ 0.1	67.0 $\pm$ 1.0	4.3 $\pm$ 0.3	14.4 $\pm$ 1.8	1.0 $\pm$ 0.01
<i>C. roseus</i>	32.8 $\pm$ 0.4	36.3 $\pm$ 1.7	10.4 $\pm$ 0.6	28.0 $\pm$ 3.4	1.3 $\pm$ 0.05
<i>P. emblica</i>	26.8 $\pm$ 1.5	25.5 $\pm$ 0.6	9.3 $\pm$ 0.3	8.0 $\pm$ 1.1	0.9 $\pm$ 0.01

period, 1 ml 1% (w/v) thiobarbituric acid (TBA) was added to each mixture followed by the addition of 1 ml 2.8% (w/v) trichloroacetic acid (TCA). The solutions were heated in a water bath at 80 °C for 20 min to develop the pink coloured MDA-(TBA)<sub>2</sub> adduct. As turbidity was encountered, the MDA-(TBA)<sub>2</sub> chromogen was extracted into 2 ml butan-1-ol and its absorbance measured at 532 nm. Results were expressed as the percentage inhibition of deoxyribose degradation g<sup>-1</sup> fresh weight of plant material.

**Inhibition of microsomal lipid peroxidation induced by 2, 2'-azobis(2-amidinopropane) hydrochloride (AAPH)**

Beef liver microsomes were prepared by tissue homogenization with 4 volumes of ice-cold 0.25 M

containing 10 mM Tris buffer and 1 mM EDTA, pH 7.4. Microsomal fractions were isolated by removal of the nuclear fractions after centrifugation at 1500 rpm for 5 minutes and removal of mitochondrial fraction at 5000 rpm for 15 minutes. The microsomal fraction was then sedimented in a Beckman Optima™ ultracentrifuge at 100 000 x g for 60 minutes at 4°C. The pellet was then washed in 0.25 M NaCl, pH 7.5. The membranes, suspended in saline (0.25 M, pH 7.5), were stored in aliquots at -20°C. Microsomal proteins were estimated by the Lowry-Folin method using bovine serum albumin as standard.

The reaction mixture contained in a final volume of 0.8 ml the following reagents: 200  $\mu\text{l}$  of beef liver microsomes (0.5 mg/ml microsomal protein final concentration) suspended in

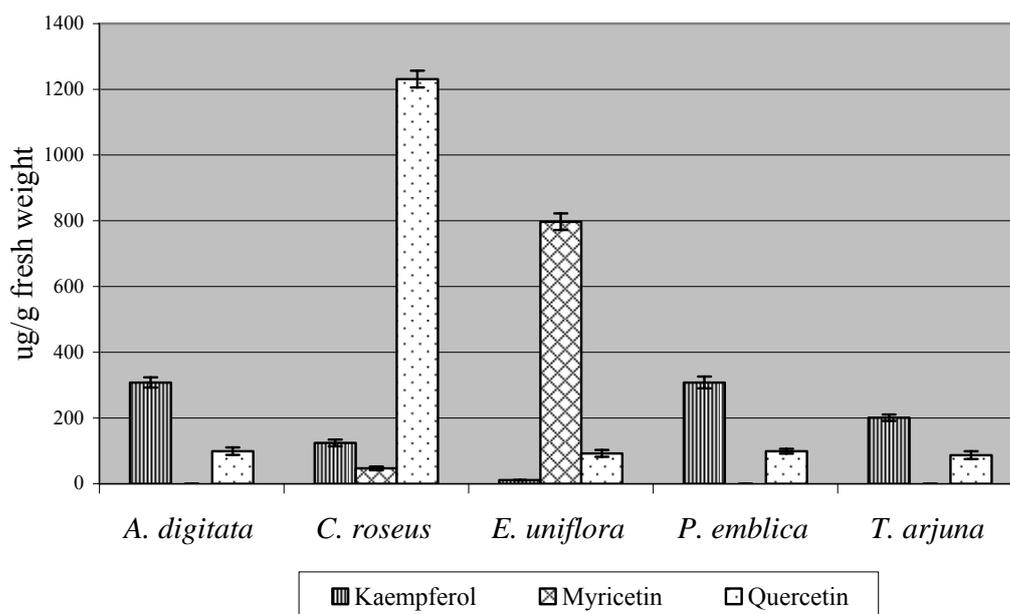


Figure 2: HPLC data of aglycones namely kaempferol, myricetin and quercetin from herbal extracts. Values are expressed mean values  $\pm$  standard deviation ( $n = 3$ )  $\square$   $\mu$ g/g fresh weight.

0.1 M potassium phosphate buffer, pH 7.5, 400  $\mu$ l sample and 200  $\mu$ l 2,2'-azobis(2-amidinopropane hydrochloride) (20 mM) to initiate peroxidation. The mixture was incubated at 37  $^{\circ}$ C for 1 hour and the solution gently shaken at 10 min interval. After incubation, 1.6 ml TCA-TBA-HCl stock solution (15 % w/v trichloroacetic acid, 0.375 % thiobarbituric acid, 0.25N HCl) was added. The solution was heated in a boiling water bath for 15 min. After cooling, the precipitate was removed by centrifugation and the absorbance of the resulting supernatant measured at 532 nm. Results are expressed as IC<sub>50</sub> (mg FW/ml).

### Statistical analysis

All spectrophotometric measurements were made on a Unicam spectrophotometer interfaced with Unicam UV Series Vision 32 software (Version 1.22, Unicam Instruments, Cambridge, UK). Results are expressed as mean value  $\pm$  standard deviation ( $n = 3$ ). Simple regression analysis was performed to calculate the dose-response relationship of standard solutions used for calibration as well as for the test samples. Analysis of data was done with SPSS statistical analysis software (version 10.0J, SPSS Inc., Tokyo Japan). A nominal two-sided  $P$  value of  $< 0.05$  was used to assess significance

## RESULTS

### Phenolic contents of the herbal extracts

Figure 1 depicts the phenolic contents of the traditional plant preparations. The total phenolic content of the plant extracts varied from 2.4 to 34.9 mg/g FW. *T. arjuna* showed the highest levels followed *E. uniflora* while a comparable amount was measured in *A. digitata* and *C. roseus*. *P. emblica* contained the lowest phenolic content with  $2.4 \pm 0.06$  mg/g FW.

The proanthocyanidin content followed a similar trend to that of the total phenolic content with the maximum amount in *T. arjuna* ( $15.0 \pm 0.42$  mg/g FW). Lower levels were detected in the other plant extracts (*A. digitata*:  $1.2 \pm 0.19$

mg/g FW, *E. uniflora*:  $0.76 \pm 0.11$  mg/g FW), while only trace amount was detected in *C. roseus* and *P. emblica*. HPLC analyses showed the presence of procyanidins B1 and B2 dimers (Table 3). The highest B1 level was measured in *T. arjuna* ( $619 \pm 25.4$   $\mu$ g/g FW) while *A. digitata* contained the maximum level of B2 dimer ( $348 \pm 15.8$   $\mu$ g/g FW).

The total flavonoid content ranged from 0.5 to 7.8 mg/g FW with optimum amounts assayed in *E. uniflora*. The amount of flavonoids measured in the extracts were in the following order *E. uniflora*  $>$  *A. digitata*  $\geq$  *C. roseus*  $>$  *T. arjuna*  $>$  *P. emblica*. Among the other flavan-3-ols derivatives screened using HPLC, (+)-C was ubiquitously distributed in all the extracts while (-)-EC and (-)-EGC had a much restricted distribution. The highest (+)-C level was measured in *C. roseus* with  $1751 \pm 8.9$   $\mu$ g/g FW. The maximum amount of (-)-EC and (-)-EGC were observed in *P. emblica* and *T. arjuna* respectively (Table 3).

HPLC analyses of the acid hydrolysed extract showed that quercetin and kaempferol were the main flavonol aglycones with their levels varying among the different extracts. The highest quercetin content was measured in *C. roseus* ( $1231 \pm 25.5$   $\mu$ g/g FW) while comparable level ( $\approx 90$   $\mu$ g/g FW) was detected in the other extracts. Myricetin was detected only in *C. roseus* ( $47 \pm 5.6$   $\mu$ g/g FW) and *E. uniflora* ( $797 \pm 25.3$   $\mu$ g/g FW) under the present experimental conditions. Kaempferol was ubiquitously distributed with amounts ranging from 11 to 308  $\mu$ g/g FW (Figure 2).

### Free radical scavenging activities and reducing power of the plant extracts

The antioxidant activities of the traditional preparations based on TEAC, FRAP, anti-peroxidative, and their abilities to scavenge hypochlorous acid and hydroxyl radicals are shown in Table 4. The scavenging potential of the preparations as assayed by the TEAC method varied between 26.8 and 306.9  $\mu$ mol/g FW. *T. arjuna* exhibited the

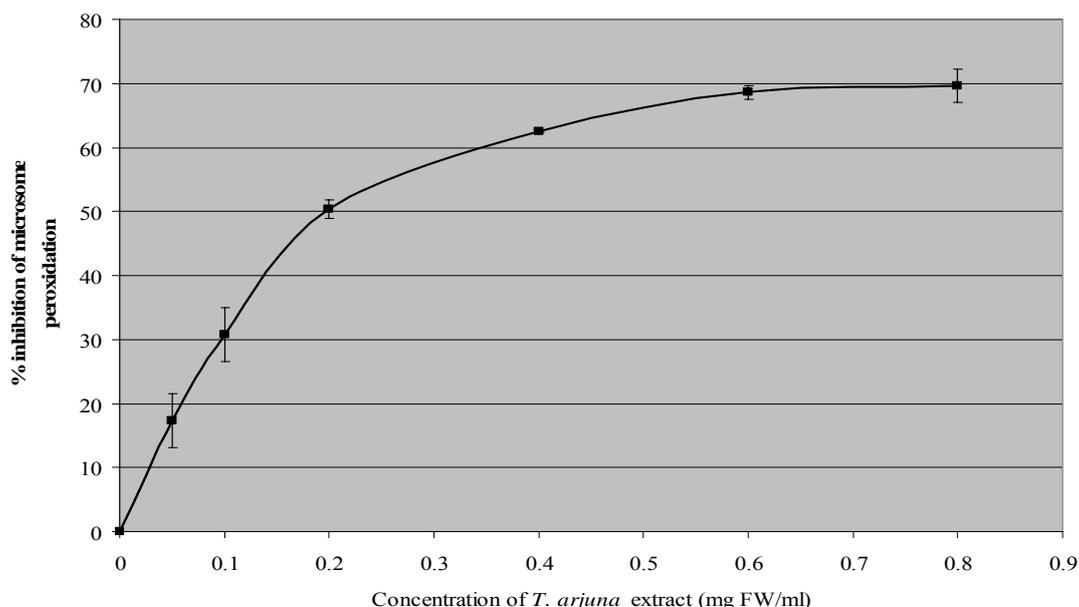


Figure 3: Concentration dependent inhibition of AAPH-induced microsome lipid peroxidation by *T. arjuna* extract.

highest activity (306.9  $\mu\text{mol/g}$  FW) followed by *E. uniflora* (203.8  $\mu\text{mol/g}$  FW). The other extracts had much lower TEAC values ( $\leq 60$   $\mu\text{mol/g}$  FW). *T. arjuna* and *E. uniflora* were characterised by higher ferric reducing activity compared to *A. digitata*, *C. roseus* and *P. emblica* showed low reducing potencies ranging from 25 to 67  $\mu\text{mol/g}$  FW. The calculated  $\text{IC}_{50}$  values indicated that *T. arjuna*, *E. uniflora* and *A. digitata* were powerful scavengers of hypochlorous acid compared to *P. emblica* and *C. roseus*. The ability of the extracts to scavenge hydroxyl radicals was assessed using the deoxyribose assay (Aruoma, 1994; Soobrattee et al., 2005) and the data showed that the plant extracts were potent inhibitors of deoxyribose damage except *P. emblica* (Table 4). The order of activity was *T. arjuna* > *E. uniflora* > *C. roseus* > *A. digitata* > *P. emblica*. The extracts were characterized by high antioxidant activities in the lipid peroxidation system. In the AAPH-induced microsome lipid peroxidation assay the antioxidant hierarchy based on the calculated  $\text{IC}_{50}$  was *T. arjuna* > *E. uniflora* > *P. emblica* > *A. digitata* > *C. roseus*. Most of the plant extracts inhibited microsomal lipid peroxidation in a concentration dependent manner and *T. arjuna* showed the highest activity with a calculated  $\text{IC}_{50}$  value of 0.2 mg FW/ml (Figure 3) while the other plant extracts were weaker inhibitors of AAPH-induced beef liver microsomes peroxidation. Control experiments showed that the extracts did not interfere with the colour development stage of the assay.

All analyses were mean of triplicates measurements  $\pm$  standard deviations. <sup>a</sup> Results expressed in units  $\mu\text{mol}$  trolox equivalent/g fresh weight. <sup>b</sup> Values are expressed in units of  $\mu\text{mol}$  Fe (II)/ g fresh weight. <sup>c</sup>  $\text{IC}_{50}$  is defined as the concentration sufficient to obtain 50 % of a maximum scavenging activity and expressed in mg FW/ml. <sup>d</sup> Results are expressed as % inhibition of deoxyribose oxidation by 1 g FW/L plant extract.

## DISCUSSION

Traditional medicine has always had an important place in the therapeutic armoury of mankind and several plant extracts have been reported for their cardioprotective effects. The purported mechanism of action of these extracts in the management of cardiovascular dysfunctions extends from cholesterol-lowering properties, hypolipidemic effects, regulation of blood pressure, improving cardiac muscle strength to antioxidant effects. Multiple lines of evidence support the traditional use of plants in the preventive or therapeutic treatment of CVD and such effects have been mostly ascribed to the presence of secondary metabolites including alkaloids, phenolics and terpenoids<sup>27,28</sup>.

The present study describes the polyphenolic composition and the antioxidant efficacy of five traditional preparations used against cardiovascular dysfunction in Mauritius primarily *T. arjuna*, *E. uniflora*, *A. digitata*, *P. emblica*, *C. roseus* from the Combretaceae, Myrtaceae, Bombaceae, Euphorbiaceae and Apocynaceae family respectively. Research studies have shown the antihypertensive effects of *E. uniflora* and *T. arjuna* which can be mediated by a direct vasodilating effect or by cholinergic mechanisms. The anti-ischaemic and hypolipidaemic properties of *T. arjuna* have also been reported<sup>9, 29-31</sup>. The protective effects of *T. arjuna* in patients with severe refractory heart failure associated with cardiomyopathy have also been shown<sup>32</sup>.

The data showed significant amounts of phenolic and proanthocyanidins measured in the herbal extracts. *T. arjuna* (34.9  $\pm$  0.9 mg/g FW) had the highest total phenolics content while *E. uniflora* (14.9  $\pm$  0.42 mg/g FW) had a significant level of flavan-3-ols derivatives. Kaempferol and quercetin aglycones were most abundant in the plant extracts compared to myricetin suggesting that the extracts were rich in kaempferol and quercetin glycosides. Similarly elevated levels of phenolic compounds in a wide range of plant sources used in traditional medicine have been reported<sup>28,33,34</sup>. Eddouks et al<sup>35</sup> reported the antihypertensive and diuretic effects of the aqueous extract of *Retama raetam* Forssk. leaves in both normotensive (WKY) and

spontaneously hypertensive rats (SHR) while similar effects of bioflavonoid rich azuki bean extract were detected in hypertensive rats<sup>34</sup>.

A multiplicity of antioxidant assays were used in order to provide a comprehensive prediction of the antioxidant efficacy of the plant extracts primarily because the mechanism of action of naturally occurring antioxidant can be diverse. Although several screening approaches have been proposed, an estimation of plant extracts antioxidant potential by using the oxygen radical absorption assay (ORAC), ferric reducing antioxidant power (FRAP), total oxyradical scavenging capacity (TOSC), the deoxyribose assay, assays involving oxidative DNA damage, assays involving reactive nitrogen intermediates (e.g. ONOO<sup>-</sup>), Trolox equivalent antioxidant capacity (TEAC) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay can provide preliminary information, giving a basis for further characterization procedures<sup>36-38</sup>. In this study TEAC, FRAP, scavenging of hypochlorous acid, deoxyribose assay and inhibition of microsomal lipid peroxidation were used to assess the antioxidant capacity of the plant extracts. The plant extracts studied had varied antioxidant potencies and their order of potency in TEAC, FRAP and deoxyribose assay was *T. arjuna* > *E. uniflora* > *A. digitata* > *C. roseus* > *P. emblica* while a slightly different trend of activity *T. arjuna* > *E. uniflora* > *A. digitata* > *P. emblica* > *C. roseus* was observed against HOCl acid scavenging and inhibition of microsomal lipid peroxidation. Investigating the antioxidant potentials of nine plants namely *Terminalia arjuna*, *Aegle marmelos*, *Sida rhombifolia*, *Piper longum*, *Vitex negundo*, *Cassia fistula*, *Boerhaavia diffusa*, *Zingiber officinale* and *Calotropis gigantean* used in Ayurvedic formulations for CVD therapy, Munasinghe et al<sup>39</sup> reported that *T. arjuna* demonstrated the highest antioxidant activity. This is in accordance with the present results where *T. arjuna* showed the strongest antioxidant activity in the different systems studied. In addition, the bark powder of *T. arjuna* is reported to exert hypocholesterolaemic and antioxidant effect in humans<sup>40</sup>.

Phenolic constituents are known to exert their antioxidant effects by directly intercepting ROS/RNS, by recycling other antioxidant in vivo, such as  $\alpha$ -tocopherol. Furthermore, phenolics can chelate transition metal ions, such as iron and copper, thus preventing ROS formation from these metal ions while simultaneously retaining their free-radical scavenging capacity. In this study the total phenol content strongly correlated with the TEAC ( $r = 0.96$ ,  $p < 0.05$ ), FRAP activity ( $r = 0.99$ ,  $p < 0.05$ ), scavenging of HOCl ( $r = 0.75$ ,  $p < 0.05$ ), hydroxyl radical scavenging capacity of the extracts ( $r = 0.85$ ,  $p < 0.05$ ) and the inhibition of microsomal lipid peroxidation ( $r = 0.9$ ,  $p < 0.05$ ). The ability of the extracts to react with HOCl further showed good correlation with the monomeric flavan-3-ol derivatives (-)-EC ( $r = 0.54$ ,  $p < 0.05$ ) and (-)-EGC ( $r = 0.59$ ,  $p < 0.05$ ) whereas a moderate correlation was observed between the anti-peroxidative activity of the extracts and (+)-C ( $r = 0.51$ ,  $p < 0.05$ ) and (-)-ECG ( $r = 0.73$ ,  $p < 0.05$ ).

Literature data has greatly highlighted the cardioprotective effects of dietary phenolic constituents<sup>41</sup>. For instance, resveratrol was shown to mediate cardioprotection by activating heme-oxygenase-1 which in turn generates a survival signal by upregulating the phosphorylation of p38MAP kinase  $\beta$  and Akt as well as inhibiting p38MAP

kinase  $\alpha$ <sup>42</sup>. Inhibitors of oxidative damage of cholesterol-rich low density lipoproteins (LDL) have been associated with a reduced progression of coronary artery disease. Quercetin and its conjugates have been reported as potent inhibitors of LDL oxidation<sup>43</sup> and can thus prevent oxidative damage in a variety of systems. The flavonoid catechins may also be active in the regenerations of  $\alpha$ -tocopherol<sup>44</sup>. Synergistic activities of rutin, a derivative of quercetin, with the antioxidants ascorbic acid and  $\gamma$ -terpinene have been found in the inhibition of LDL oxidation<sup>45</sup>. In addition to their antioxidant, vasorelaxant and anti-inflammatory properties, the effects of phenolic on cholesterol homeostasis have been indicated to contribute to cardioprotection<sup>46</sup>. The pleiotropic effects along with the modulatory actions of plant phenolics have made them valuable prophylactic compounds. Plant phenolics interact with a wide spectrum of molecular targets central to the cell signaling machinery. For instance, apigenin and EGCG are known as potent inhibitor of growth factor-induced rat aortic vascular smooth muscle cell growth, an activity mediated by Ras/JNK and the downregulation of c-jun thereby halting the development of atherosclerotic plaque.

Thus, the data indicated the phenolic richness of the herbal preparations used traditionally against CVD in Mauritius. The protective effects of these extracts may stem at least in part from the antioxidative properties of the bioactive components as suggested by the antioxidant activity of the extracts. However, this research work sets the stage for further establishing the cellular and molecular mechanism of action of these traditionally used plants in cardioprotection.

#### ACKNOWLEDGEMENT

We thank the staff of the Biosciences laboratory, University of Mauritius and the Tertiary Education Commission, Mauritius.

#### DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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