MAURITIUS RESEARCH COUNCIL

POLYPHENOLICS, VITAMINS AND ANTIOXIDANT STATUS OF THE MAURITIAN DIET

Final Report

Year 2004

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MAURITIUS RESEARCH COUNCIL, UNSOLICITED RESEARCH GRANT SCHEME

POLYPHENOLICS, VITAMINS AND ANTIOXIDANT STATUS OF THE MAURITIAN DIET

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FINAL REPORT

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INTRODUCTION

We are hereby submitting the final report of the MRC funded project entitled **"Polyphenolics, vitamins and antioxidant status of the Mauritian diet"**. Data relating to the polyphenolic, vitamin C composition and antioxidant capacities of 17 exotic fruits, 10 commonly consumed Mauritian vegetables and 9 brands of local teas are reported and are presented in papers that have been either published (Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits, *Journal of the Science of Food and Agriculture*, **(2003) 83: 496-502**), or accepted for publication (Total phenol, flavonoid, proanthocyanidin, vitamin C levels and antioxidant activities of Mauritian vegetables, *Journal of the Science of Food and Agriculture*, **(2004) in press**) or as drafts submitted for publication (Assessment of the total phenol, proanthocyanidin, flavonoid, catechin and gallic acid contents and antioxidant activities of Mauritian commercial black teas : Important contributor to their medicinal properties, submitted to *The Journal of Nutritional Biochemistry*). 5 independent antioxidant assays (Trolox equivalent antioxidant assay (TEAC), Ferric reducing antioxidant power (FRAP), Hypochlorous Acid Scavenging Assay (HOCl), Hydroxyl scavenging assay, The Copper-Phenanthroline assay (Prooxidant assay) have been used to evaluate the antioxidant efficacies of food extracts. Unpublished additional data on teas using HOCl, hydroxyl scavenging and Copper-Phenanthroline assays are also included. It is noteworthy that the main objectives of the project (spelt out below) have been successfully achieved within the time frame allotted (June 2000- June 2003). We would like to present our apologies to the Mauritius Research Council for the delay in the writing up of this report as a number of results had to be reconfirmed before final analysis and compilation.

OBJECTIVES OF PROJECT

Our broad objectives were to:

- survey and select local exotic fresh and processed fruits, vegetables and beverages commonly consumed in Mauritius,
- determine the polyphenolic and vitamin contents of food items,
- develop and optimise techniques to analyse these metabolites in food extracts,
- determine the antioxidant capacity of food extracts.

Our specific objectives were to:

- estimate the polyphenol (total phenolics, flavonoids, anthocyanins, catechins and flavan-3-ol derivatives) and vitamins contents of locally consumed fruit, vegetable and tea extracts,
- characterise and quantify main polyphenolic derivatives from the above mentioned classes of compounds,
- evaluate antioxidant activities of food extracts
- investigate the existing correlation between polyphenol and vitamin contents and antioxidant activities of food extracts

PREVIOUS REPORTS

A first progress report compiling research activities conducted from **April 2000 to January 2001** was submitted to the Mauritius Research Council in February 2001. The report gave a summary of the extensive literature review carried out on the analytical techniques (extraction, qualitative analyses, quantitative determinations etc…) used to study vitamins and polyphenols together with antioxidant assessment methodologies currently used to evaluate antioxidant capacities of food extracts. The methodological approaches and the optimization and standardisation of procedures were fully addressed.

The materials and method section gave a list of all the Mauritian exotic fruits (17 types) and vegetables (10 varieties) selected for analyses of antioxidant Vitamin C, Vitamin E, Vitamin A and polyphenols. It also indicated the list of commercially fermented tea (9 brands) and fresh tea leaves to be studied. The protocols for Total Phenols and Total proanthocyanidins determination from food extracts were elaborated since they were the only metabolites analysed. Finally, a detailed protocol for the setting up of the TEAC antioxidant assay at the Biological Science Department and the expression of the results as exemplified by a sample were also indicated. Preliminary results relating to total phenolic and total proanthocyanidin contents of fruits and vegetables were also given.

A second progress report gave an account of research activities conducted during the period **May 2001-December 2002.** Studies relating to the polyphenolic, vitamin C composition and antioxidant capacities of 10 commonly consumed Mauritian vegetables were reported. Moreover the antioxidant and a detailed analysis of polyphenolic classes (total phenols, catechins (detailed analysis), proanthocyanidins, flavonoids and phenolic acid) of 9 common Mauritian tea brands were emphasized. The relationship between the polyphenolic composition and antioxidant capacities of vegetable and tea samples was detailed.

1. POLYPHENOLS, VITAMINS AND ANTIOXIDANT CAPACITIES OF MAURITIAN EXOTIC FRUITS

The findings related to this research work have been published in the *Journal of the Science of Food and Agriculture*, (2003), 83: 496-502 A copy of the paper is appended

Journal of the Science of Food and Agriculture

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Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits

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Abstract: Seventeen commonly consumed exotic fruits from Mauritius were analysed for their antioxidant capacity, total phenolics, proanthocyanidins, flavonoids and vitamin C content. Two independent methods were used to evaluate the antioxidant potential of total fruit extracts. The antioxidant activities of the fruits ranged from 1 to 47 μmol Trolox equivalent antioxidant capacity (TEAC) g^{-1} fresh weight and from 0.3 to 34 micro/mol g fresh weight (FRAP) g^{-1} fresh weight. Total phenolics in the fruits ranged from 118 to 5638 μg g^{-1} fresh weight, proanthocyanidins from 7 to 2561 μg g^{-1} fresh weight, flavonoids from 21 to 712 μg g^{-1} fresh weight and vitamin C content from 8 to 1426 μg g^{-1} fresh weight. There were strong correlations between antioxidant activity (assessed by both TEAC and FRAP) and total phenolics and proanthocyanidins. Flavonoids seemed to contribute less to the antioxidant potential of the fruits, while very poor correlations were observed between ascorbate content and antioxidant activity. The highest antioxidant capacities were observed in red and yellow *Psidium cattleianum* Sabine 'Chinese guava', sweet and acid *Averrhoa carambola* L 'starfruit', *Syzygium cumini* L Skeels 'jamblon' and white *Psidium guajava* L 'guava'. These fruits were also characterised by high levels of total phenolics. Mauritian exotic fruits are thus a significant source of phenolic antioxidants, which may have potential beneficial effects on health.

Keywords: exotic fruits; antioxidant activity; TEAC; FRAP; total phenols; proanthocyanidins; flavonoids; vitamin C

INTRODUCTION

There is convincing evidence of the beneficial role of fruits and vegetables in the diet for the maintenance of health and prevention of disease.[1–3] Cellular damage caused by exposure to high levels of free radicals induces cardiovascular disorders, neurological dysfunctions and various cancers.[4–6] It is believed that fruits and vegetables provide protection against these disorders because they are rich sources of antioxidants, which scavenge free radicals and thereby reduce the incidence of degenerative pathologies.[7–9] The compounds thought to be responsible for the protective effects of a fruit-and-vegetable-rich diet include carotenoids and antioxidant vitamins such as ascorbic acid and tocopherols. However, there is growing evidence that other phytochemicals contribute to varying degrees to the antioxidant capacity of individual fruits or vegetables. In this regard, interest has focused on the significance of phenolics such as catechins, phenolic acids, flavonoids, proanthocyanidins and anthocyanins.[10,11] These compounds have exhibited a range of biological effects including antibacterial, antiviral, anti-inflammatory, antithrombotic and vasodilatory actions.[12–14] They also exert pronounced antioxidant and free radical-scavenging activities.[15–19] It is important to note that many biological functions such as antimutagenicity, anticarcinogenicity and anti-aging stem from this property.[20–22]

Interest in the role of antioxidants in human health, particularly with regard to the relatively high incidence of cardiovascular diseases, cancers and diabetes in Mauritius,[23] has prompted research in the field of horticulture and food science to study the phytochemistry and antioxidant capacity of Mauritian fruits and vegetables. The current investigation examined the relationship between the *in vitro* antioxidant capacity and the total phenolic, proanthocyanidin, flavonoid and vitamin C contents in some selected exotic Mauritian fruits.

MATERIALS AND METHODS

Standards and chemicals

ABTS (2,2′-azino-bis(3-ethylbenzthiozoline-6-sulphonic acid) and TPTZ (2,4,6-tri(2-pyridyl)-*s*-triazine) were from Sigma Co (St Louis, MO, USA). Trolox

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(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E, was purchased from Sigma-Aldrich (DeisenhaBen Germany). HPLC grade quercetin and cyanidin chloride were obtained from Extrasynthèse (Genay, France). All other reagents used were of analytical grade.

Fruit samples

All fruit samples were collected during the year 2000 fruit-bearing season. Samples of red and yellow *Psidium cattleianum* fruits were obtained from Bigarra and Petrin (central Mauritius) respectively, while *Syzygium cumini* fruits were from Black River (west Mauritius). All other mature ripe fruits were harvested at random from 'La Compagnie Agricole de Labourdonnais' at Mapou in northern Mauritius. The determination of fruit maturity and ripeness was based on fruit firmness and surface colour. Table 1 lists the names of the studied species, their fruit types/subcultivars, harvest sites and parts used for analysis. Voucher specimens of fruit samples have been deposited in the Department of Biological Sciences, Faculty of Science, University of Mauritius.

Extraction

Polyphenols

Portions (100 g) of the edible parts of fresh fruits were homogenised in acetone/water (70:30 v/v; 2 × 300 ml) using a Waring blender and left to macerate for 24 h at 4 °C. After filtration the residue was homogenised in absolute methanol (2 × 300 ml) and again left to macerate for 24 h at 4 °C. Acetone was removed from the combined filtrates *in vacuo* at 37 °C and the aqueous residue was washed with dichloromethane (3 × 150 ml) to remove fat-soluble substances. The aqueous extract was concentrated *in vacuo* at 37 °C and divided into two equal aliquots. One was freeze-dried and redissolved in methanol at a final 1:5 fresh weight/volume ratio and was used for the quantitative analysis of phenolic compounds. The second aliquot was used to determine the antioxidant activity.

Vitamin C

A modified method of Daood *et al*[24] was used for the extraction of vitamin C from fresh fruits. Portions (10 g) of fruit material were homogenised with 40 ml of a solution of 30 g l^{-1} metaphosphoric acid in 80 g l^{-1} glacial acetic acid (pH 1.5) for 1 min using a Waring blender. The extracts were then mechanically shaken for 15 min in darkness. After filtration the clear extracts were stored at −40 °C for subsequent analysis.

Total phenolic content analysis

Total phenolics were determined by the method of Singleton and Rossi[25] using the Folin–Ciocalteu reagent. Results were expressed as μg gallic acid equivalent g^{-1} fresh weight.

Total proanthocyanidin content analysis

The HCl/butan-1-ol assay of Porter *et al*[26] was used to quantify total proanthocyanidins. Result were expressed as μg cyanidin chloride equivalent g^{-1} fresh weight.

Total flavonoid content analysis

The $AlCl_3$ method adapted from Ref 27 was used to determine the total flavonoid content of the methanolic extracts. Results were expressed as μg quercetin equivalent g^{-1} fresh weight.

Vitamin C content analysis

The vitamin C content of the fruit extracts was determined by the 2,6-dichloroindophenol titrimetric method (AOAC).[28] Results were expressed as μg ascorbic acid equivalent g^{-1} fresh weight.

Measurement of antioxidant activity

Trolox equivalent antioxidant capacity (TEAC)

The antioxidant activity of total fruit extracts was measured in terms of radical-scavenging ability

Table 1. Details of 17 Mauritian exotic fruits studied

Common name	Scientific name	Fruit type/subcultivar	Harvest site	Part used
Starfruit	*Averrhoa carambola* L	Acid	Mapou	Whole
		Sweet	Mapou	Whole
Chinese guava	*Psidium cattleianum* Sabine	Red	Bigarra	Whole
		Yellow	Petrin	Whole
Guava	*Psidium guajava* L	Pink	Mapou	Whole
		White	Mapou	Whole
Hogplum	*Spondias dulcis* Sonn	—	Mapou	Skin + pulp
Pineapple	*Ananas comosus* (L) Merrill	Bourgault	Mapou	Pulp
Banana	*Musa acuminata* (diploides)	Gingeli	Mapou	Pulp
Avocado	*Persea americana* P Miller	—	Mapou	Pulp
Jamalac	*Syzygium samarangense* (Blume) Merr et Perry	—	Mapou	Pulp
Jamblon	*Syzygium cumini* (L) Skeels	—	Black River	Pulp
Passion fruit	*Passiflora edulis* Sims	Orange	Mapou	Pulp + seed
Mango	*Mangifera indica* L	Maison Rouge	Mapou	Pulp
Papaya	*Carica papaya* L	Exotica	Mapou	Pulp
Litchi	*Litchi chinensis* Sonnerat	—	Mapou	Pulp
Longanberry	*Euphoria longan* (Lour) Steud	—	Mapou	Pulp

TEAC assay, in the following order for the highest antioxidant activities: red *Psidium cattleianum* > *yellow Psidium cattleianum* > *Syzygium cumini* > *sweet Averrhoa carambola* > *white Psidium guajava* > *acid Averrhoa carambola* (Fig 1). However, fruits of *Spondias dulcis, Mangifera indica, Litchi chinensis, Ananas comosus* and *Passiflora edulis* exhibited higher antioxidant activities in the FRAP than in the TEAC assay (Fig 1). Both assays showed relatively low antioxidant activities in fruits such as banana, avocado and longanberry.

Total phenols, proanthocyanidins and flavonoids were determined for all 17 fruit species. Total phenolic contents in the fruit extracts varied from 118 ± 4 to $5638 \pm 364\,\mu g\,g^{-1}$ fresh weight (Table 2). A striking correlation between total phenolics and antioxidant activity of the fruit extracts was noted (TEAC, $r = 0.98$, FRAP, $r = 0.95$) (Table 3). Fruits with the highest phenolic contents—red *Psidium cattleianum* ($5638 \pm 364\,\mu g\,g^{-1}$), yellow *Psidium cattleianum* ($5372 \pm 186\,\mu g\,g^{-1}$), sweet *Averrhoa carambola* ($2099 \pm 104\,\mu g\,g^{-1}$), white *Psidium guajava* ($2473 \pm 45\,\mu g\,g^{-1}$), *Syzygium cumini* ($2359 \pm 47\,\mu g\,g^{-1}$) and acid *Averrhoa carambola* ($1429 \pm 71\,\mu g\,g^{-1}$)—had the highest antioxidant potentials in both the TEAC and FRAP assays (Table 2). On the other hand, fruit extracts characterised by low total phenolic levels exhibited poor antioxidant capacities (Table 2).

A relatively good correlation was observed between proanthocyanidin content and antioxidant activity of the fruit extracts (TEAC, $r = 0.96$; FRAP, $r = 0.92$) (Table 3). The pattern of variation in proanthocyanidin content was similar to that observed for total phenolics, with maximum levels occurring in red ($2561 \pm 101\,\mu g\,g^{-1}$) and yellow ($2409 \pm 89\,\mu g\,g^{-1}$) *Psidium cattleianum*, sweet *Averrhoa carambola* ($1321 \pm 61\,\mu g\,g^{-1}$), acid *Averrhoa carambola* ($896 \pm 23\,\mu g\,g^{-1}$) and *Syzygium cumini* ($453 \pm 85\,\mu g\,g^{-1}$), which displayed the highest antioxidant capacities (Table 2). The other fruit extracts contained too low amounts of proanthocyanidins to significantly influence the antioxidant activity.

Flavonoid levels ranged between 21 ± 0 and $712 \pm 32\,\mu g\,g^{-1}$, with the highest amounts being recorded in red *Psidium cattleianum* ($712 \pm 32\,\mu g\,g^{-1}$), *Carica papaya* ($376 \pm 15\,\mu g\,g^{-1}$), yellow *Psidium cattleianum* ($308 \pm 13\,\mu g\,g^{-1}$), *Mangifera indica* ($281 \pm 28\,\mu g\,g^{-1}$) and white *Psidium guajava* ($209 \pm 10\,\mu g\,g^{-1}$) (Table 2). Much lower levels of flavonoid derivatives were present in the other fruits. Compared with total phenols and proanthocyanidins, flavonoids appear to exert less effect on the antioxidant potential of the fruits (Table 3).

Table 3 indicates that ascorbate content and antioxidant capacity were poorly correlated (TEAC, $r = 0.07$; FRAP, $r = 0.04$), since in many cases vitamin C levels were low in the fruits where antioxidant capacity was high (Table 2). Vitamin C contents ranged from 8 ± 1 to $1426 \pm 26\,\mu g\,g^{-1}$, with maximum values observed in white *Psidium guajava* ($1426 \pm 26\,\mu g\,g^{-1}$), *Carica papaya* ($929 \pm 19\,\mu g\,g^{-1}$), pink *Psidium guajava* ($722 \pm 6\,\mu g\,g^{-1}$), *Mangifera indica* ($605 \pm 15\,\mu g\,g^{-1}$) and *Ananas comosus* ($275 \pm 0\,\mu g\,g^{-1}$) (Table 2). Relatively lower amounts were found in the other fruits. Mauritian banana and avocado were almost devoid of vitamin C (Table 2).

Table 3. Correlation coefficients of TEAC and FRAP with respect to total phenolic, total flavonoid, total proanthocyanidin and vitamin C contents of Mauritian fruits as evaluated by linear regression analysis

	Total phenolics	Total flavonoids	Total proanthocyanidins	Vitamin C
TEAC	0.98	0.77	0.96	0.07
FRAP	0.95	0.69	0.92	0.04

DISCUSSION

Free radicals, more particularly their excessive production, appear to feature in many human disorders such as cardiovascular disease, diabetes and cancer, all of which have high and increasing incidences and mortality rates in Mauritius.[23] As such, dietary antioxidants may have an important role in combating these pathologies through their protective effect against free radical damage to cellular constituents. The literature abounds with examples where fruits from temperate regions have been reported to be good sources of natural dietary antioxidants.[31–33] Data relating to tropical fruits are more limited and in Mauritius there has previously been no investigation of the phenolic and vitamin C contents and antioxidant capacity of commonly consumed local fruits.

The present study determined the antioxidant capacities of 17 Mauritian fruits and analysed their extracts for compounds, namely total phenolics, proanthocyanidins, flavonoids and vitamin C, that may contribute to this antioxidant activity. Total phenolics were measured by the Folin–Ciocalteu assay, which is based on an oxidation–reduction reaction. This method determines not only phenolic compounds but also other chemical components such as carotenoids, amino acids, sugars and vitamin C.[25,34] Using the 6-dichloroindophenol titrimetric method,[28] the phenolic extracts gave a response corresponding to 20% of the vitamin C content measured in fresh homogenised fruit extracts (data not shown). In spite of various precautions taken to limit the interference of lipid-soluble compounds such as carotenoids by repeated washing with dichloromethane, it is obvious that the total phenolic content measured by the Folin–Ciocalteu procedure does not give a full picture of the quantity and quality of the phenolic constituents of the extracts. In the light of the above limitations it is perhaps more appropriate to use the term Folin–Ciocalteu index rather than total phenolics. Nevertheless, this widely used method provides a rapid and useful overall evaluation of the phenolic content of extracts.

above total phenolic and flavonoid values. Similarly, it is speculated that the dietary needs for vitamin C are met by a minimum intake of 60 mg day^{-1} for an adult.[64] However, a review related to epidemiological studies of antioxidants and disease suggests that a daily intake of 150 mg of vitamin C in association with other vitamins (vitamin E, β-carotene) is linked to a reduced incidence of cancer and cardiovascular disease.[65] Interestingly, 105 g of white guava (representing a single whole fruit) corresponds to this daily intake.

This study shows the potential antioxidant properties of certain Mauritian fruits, notably *Psidium cattleianum*, *Psidium guajava*, *Averrhoa carambola* and *Syzygium cumini*, that could be used as supplements in a balanced diet within existing nutrition programmes. This could prove to be a more effective and economical means of protecting the body against various oxidative stresses than supplementation with individual antioxidants such as vitamin C or α-tocopherol. This could be of interest to commercial growers, as it provides them with an opportunity to market these fruits for their health benefits. The critical question, however, remains the bioavailability of such plant-derived antioxidants. Presently, data on the absorption, assimilation and metabolism of polyphenolics are limited, despite the fact that much attention is being paid to the study of the bioavailability and metabolism of these molecules.[66,67] Such data would appear to be a *sine qua non* condition for potential health benefits associated with the high antioxidant capacity of certain fruits.

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2. POLYPHENOLS, VITAMINS AND ANTIOXIDANT CAPACITIES OF MAURITIAN VEGETABLES

The results related to this research work have been accepted to be published in the *Journal of the Science of Food and Agriculture*. A copy of the accepted paper is appended

Total phenol, flavonoid, proanthocyanidin, vitamin C levels and antioxidant activities of Mauritian vegetables

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ABSTRACT

Mauritian vegetables, broccoli, cauliflower, white cabbage, lettuce, Chinese cabbage, mugwort, carrot, onion, tomatoes and chili pepper were analyzed for their total phenols, flavonoids, proanthocyanidins, vitamin C contents and antioxidant capacity. Antioxidant activities of vegetables ranged from 0.43 to 3.68 μmol Trolox equivalent g^{-1} fresh weight (TEAC) and from 0.60 to 8.47 μmol g^{-1} fresh weight ferric reducing antioxidant power (FRAP). The levels of total phenols in vegetables varied between 132 and 1189 $\mu g\ g^{-1}$ fresh weight, those of total flavonoids between 45 and 944 $\mu g\ g^{-1}$ fresh weight while proanthocyanidins were detected at very low levels in only a few vegetables. Vitamin C contents varied between 25 and 748 $\mu g\ g^{-1}$ fresh weight. Quercetin was the dominant flavonoid aglycone in the hydrolysed vegetable extract with values in the range 15 to 390 $\mu g\ g^{-1}$ fresh weight. There were strong correlations between the antioxidant activity and total phenolics (TEAC: r = 0.91; FRAP: r = 0.83) and total flavonoids (TEAC: r = 0.89; FRAP: r = 0.82). Vitamin C contents show poor correlation with TEAC values (r = 0.33) while no correlation was observed with FRAP values. Highest antioxidant capacities were observed in Chinese cabbage (*Brassica sinensis* L.), onion (*Allium cepa* L.), mugwort (*Artemisia vulgaris* Cantley) and broccoli (*Brassica oleracea* L. *var. botrytis* L. *sub. var. cymosa*). Mauritian vegetables present a significant source of phenolic antioxidants of which the quercetin derivatives are more abundant, and this may contribute to their potential health benefits.

Keywords: Vegetables, quercetin, vitamin C, total phenols, flavonoids, proanthocyanidins, antioxidant capacity, TEAC and FRAP

INTRODUCTION

Plant-based diets are widely suggested to contribute to the reduction of the risk for the development of chronic diseases such as cancer, atherosclerosis, cardiac dysfunctions, diabetes, hypertension and neurodegenerative disorders.[1–5] This function is largely due to their antioxidant effects of their bioactive components. One common denominator in the pathogenesis of most chronic diseases is the implication of oxidative stress mechanisms.[6–8] Polyphenols are bioactive molecules, ubiquitously distributed in plant species, influencing their morphology, growth, and reproduction as well as their resistance against parasites and environmental stresses.[9] The anti-mutagenic, antibacterial, anti-viral, anti-inflammatory and antithrombotic actions of flavonoids are well characterized.[10–12] Flavonoids can act as vasodilators,[13] platelet disagregators,[14] possess efficient antioxidant and free radical scavenging abilities.[15–18]

Mauritius is a tropical island in the Indian Ocean with a relatively high prevalence of cardiovascular diseases, cancers and diabetes.[19] This has triggered interest for the study of the phytochemistry and the antioxidant capacity of the Mauritian diet that comprises a wide variety of exotic fruits, vegetables and beverages. Studies by Kusamran *et al* [20] examined the fruits and vegetables consumed in Malaysia for their efficacy as antimutagenic and anticarcinogenic agents. This paper report on the examination of the relationship between the *in vitro* antioxidant capacity (evaluated by using the Ferric

Reducing Antioxidant Power (FRAP)[21] and Trolox Equivalent Reducing Capacity (TEAC)[22]) and the total phenolic, proanthocyanidin, flavonoid and vitamin C contents in selected Mauritian vegetables.

MATERIALS AND METHODS

Standards and Chemicals

ABTS (2,2'-azino-bis(3-ethylbenzthiozoline-6-sulfonic acid) and TPTZ (2,4,6-Tri (2-pyridyl)-s-triazine) were from Sigma Co. (St Louis, MO). Trolox C (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), HPLC grade of myricetin, kaempferol, apigenin, luteolin and ascorbic acid were purchased from Sigma-Aldrich (Germany). HPLC grade of quercetin and cyanidin chloride were obtained from Extrasynthèse (Genay, France). All other reagents used were of analytical grade.

Vegetable cultivars

Lettuce, mugwort and Chinese cabbage samples were collected at random from commercial gardens at Vacoas (Central region of Mauritius) while samples of the local variety of onion were obtained from the wholesale distributor, the Agricultural Marketing Board (AMB) of Mauritius. All other vegetables were purchased from the farms of the Ministry of Agriculture, Food Technology and Natural Resources (Mauritius).

Scientific Names*	Common names	Sample Type/Variety	Collection sites	Parts used
Brassica oleracea L. *var. botrytis* L. *sub. var. cymosa*	Broccoli	Packman	Richelieu	Flower
Brassica oleracea L. *var. botrytis* L.	Cauliflower	Kashmere	Réduit	Flower
Brassica oleracea L. *var. capitata* L.	White cabbage	KKCross	Réduit	Leaves
Lactuca sativa L.	Lettuce	Mignonette	Vacoas	Leaves
Brassica chinensis L.	Chinese cabbage	Shantung	Vacoas	Leaves
Artemisia vulgaris Cantley	Mugwort	Green Boy	Vacoas	Leaves
Daucus carota L. *subsp. sativus* (Hoffm) *Arcang.*	Carrot	Kuroda	Réduit	Root tuber
Allium cepa L.	Onion	Local Red	Belle-Mare	Bulb
Lycopersicon esculentum Mill.	Tomatoes	MST/32	Quatre-Bornes	Whole
Capsicum annum L.	Chili pepper	Cypaye	Réduit	Whole

*Source: G Rouillard and J Guého (2000). Les plantes et leur histoire a l'ile Maurice.

Table 1 lists the names, sample types, harvest sites as well as the parts of the vegetables used for analysis. Prior to the extraction outer dryer scales of onions were removed while tomatoes and other vegetables were thoroughly washed. Voucher specimens have been deposited in the Department of Biological Sciences, Faculty of Science, University of Mauritius.

Extraction

Polyphenols. 100 g of the edible parts of fresh vegetables were homogenized using a Waring blender in acetone/water (70/30 v/v) (2 x 300 ml) and left to macerate for 24 h at 4 °C. After filtration the residue was homogenized in methanol 100% (2 x 300 ml) and left again to macerate for 24 h at 4 °C. The combined filtrates were reduced to the aqueous phase *in vacuo* at 37 °C before being washed with dichloromethane (3 x 150 ml) to remove fat-soluble substances. The aqueous extract was concentrated and divided into two equal aliquots. The first part was freeze-dried and re-dissolved in methanol at a final 1:5 fresh weight:volume ratio. This was used for the quantitative analysis of phenolic compounds. The second aliquot was used to determine antioxidant activity.

Vitamin C. A modified method of Daood *et al* [23] was used for the extraction of Vitamin C from fresh vegetables. 10 g of vegetable material was homogenized with 40 ml of a solution of 3% metaphosphoric acid in 8% glacial acetic acid, pH 1.5 for 1 minute, using a Waring blender. The extracts were then mechanically shaken for 15 minutes in darkness. After filtration, the clear extract was stored at –40 °C prior to analysis by the 2,6-dichloroindophenol titrimetric method. [24]

Total phenolic content analysis

Total phenolics were determined by the method of Singleton and Rossi [25] using the Folin-Ciocalteu reagent. An aliquot 0.25 ml of diluted samples was added to 3.5 ml of distilled water in screw-capped test tubes followed by 0.5 ml Folin-Ciocalteu solution. After 3 minutes, 1 ml of sodium carbonate (1 %) was added and the contents of the test tubes were thoroughly mixed before being incubated in boiling water bath for 1 min. The tubes were allowed to cool in the dark. The absorbance of the blue colour that developed was read at 685 nm using gallic acid as standard. Results were expressed in mg of gallic acid g^{-1} fresh weight.

Total proanthocyanidin content analysis

The HCl/butan-1-ol assay of Porter *et al*[26] was used to quantify the total proanthocyanidins. Aliquots of 0.25 ml of extract was added to 3 ml of a 95 % solution of n-Butanol/HCl (95:5 v/v) in stoppered test tubes followed by 0.1 ml of a solution of $NH_4Fe\ (SO_4)_2.12H_2O$ in 2 M HCl. The tubes were incubated for 40 minutes at 95°C. The absorbance of the red colouration was read at 550 nm and the data expressed in mg of cyanidin chloride g^{-1} fresh weight.

Total Flavonoid content analysis

The $AlCl_3$ method adapted from Lamaison[27] was used for the determination of the total flavonoid content of the methanolic extracts. Aliquots of the extracts (1.5 ml) were added to equal volumes of a solution of 2 % $AlCl_3.6H_2O$ (2 g in 100 ml methanol) and

thoroughly mixed. The mixture was vigorously shaken and absorbance was read at 367.5 nm after 10 minutes incubation. Data were expressed in mg quercetin equivalents g^{-1} fresh weight.

High performance liquid chromatography

HPLC analyses of vegetable extracts were carried out using a Hewlett Packard 1100 series liquid chromatography system equipped with a vacuum degasser, quaternary pump, auto-sampler, temperature controlled column compartment and diode array detector. After filtration on Millipore (0.22 μm) 30μl of 25% (v/v) aqueous methanolic extracts was injected onto a Spherisorb ODS 2 RP 18 column (5 μm pore size, 4.6 mm id x 150 mm) eluted by an acidified acetonitrile-water gradient. Elution with a flow rate of 0.7 ml/min at 25 °C 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). Myricetin, quercetin, kaempferol, apigenin and luteolin were identified and quantified in the extracts after acid hydrolysis of flavonoid conjugates essentially as described in Crozier *et al* [28] with morin as an internal standard. An aliquot of 3 g fresh mass of vegetables were extracted with 20 ml of 60 % aqueous methanol containing 50 μg morin as an internal standard. 5 ml of 6 M HCl was added to each extract, which were then refluxed at 90 °C for 2 hrs. Samples of cabbage, chili pepper and carrot were refluxed for 3 hrs. After cooling the flavonoid aglycones were extracted using 2 x 25ml of ethyl acetate. The organic phase was evaporated to dryness and taken in absolute methanol prior to analysis by HPLC. Absorption wavelengths were selected at 280 and 360 nm.

Measurement of antioxidant activity

The TEAC assessment was performed in terms of radical scavenging ability according to the ABTS/MnO_2 method.[22] This is based on the ability of an antioxidant to scavenge the preformed radical cation ABTS+ [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] relative to that of the standard antioxidant Trolox C. The ABTS+ radical was generated by a reaction between ABTS (0.5 mM) and activated MnO_2 (1 mM) in phosphate buffer (0.1 M, pH 7). To 3 ml of the ABTS+ solution 0.5 ml of diluted extracts was added and the decay in absorbance at 734 nm was followed for 15 minutes on an Helios-alpha Spectrophotometer maintained at 20° C by a Peltier thermostator. Distilled water was used in the blank test and values were expressed in μmol Trolox g^{-1} fresh weight from triplicates. The FRAP assay measures the antioxidant potentials of "antioxidants" to reduce the Fe^{3+} /tripyridyl-s-triazine complex present in stoichiometric excess to the blue coloured ferrous form.[21] The FRAP reagent was freshly prepared by mixing together 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM ferric chloride in 0.25 M acetate buffer, pH 3.6. 100 µl of sample was added to 300 µl water followed by 3 ml FRAP reagent at 1 min intervals. The absorbance was read at 593 nm after 4 minutes incubation at ambient temperature against a water blank on an Helios-alpha Spectrophotometer equipped with a water bath and a Peltier thermostator to maintain the temperature at 37°

C. A calibration curve of ferrous sulphate (0.1–1.0 mM) was used and results expressed in terms of μmol Fe^{2+} g^{-1} fresh weight from three determinations. Data are expressed in μmol Trolox g^{-1} fresh weight for the TEAC values and in μmol Fe^{2+} g^{-1} fresh weight for the FRAP values.

Statistical Analysis

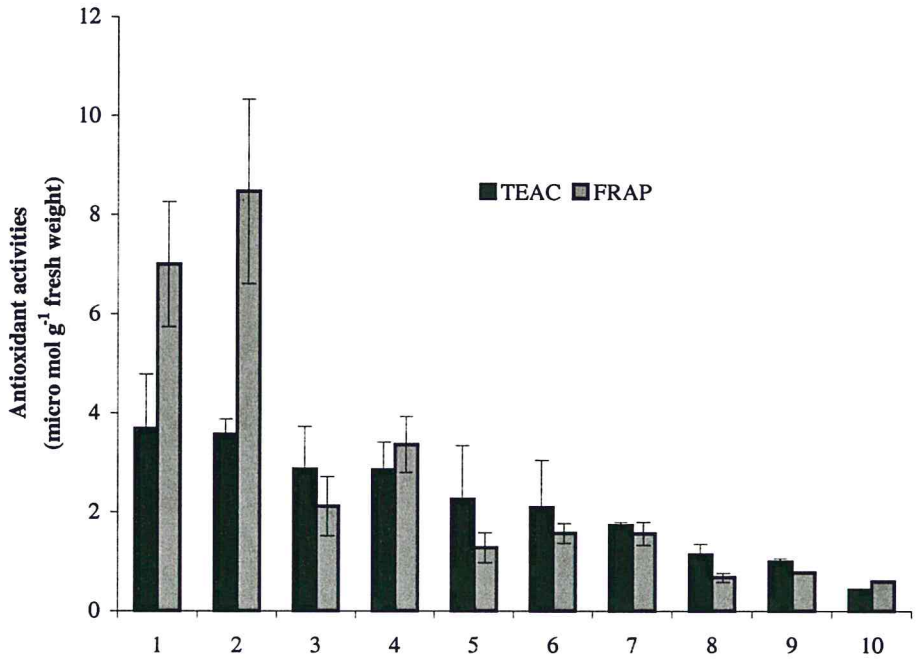
Simple regression analysis was performed to calculate the dose-response relationship of standard solutions used for calibration as well as test samples. Linear regression analysis was performed, quoting the correlation coefficient r_{xy} between antioxidant activities, phenolic classes and vitamin C. The Unicam Vision 32 software was used to evaluate initial and final antioxidant rate values for TEAC assay while the ChemStation software was used for the HPLC determinations. All results are expressed as mean value ± standard deviation (n = 3).

RESULTS

TEAC values varied between 0.43 ± 0.01 and 3.68 ± 1.10μmol g^{-1} fresh weight. Vegetables exhibiting highest activities were Chinese cabbage, onion, mugwort, broccoli, chili pepper and cauliflower (Table 2). Lettuce, tomato, white cabbage and carrot had low TEAC values. FRAP values ranged from 0.60 ± 0.01 to 8.47 ± 1.86 μmol g^{-1} fresh weight. The FRAP and TEAC values were similar in the vegetable extracts tested with the following antioxidant profiles in increasing order: onion > Chinese cabbage > broccoli > mugwort > cauliflower > white cabbage (Figure 1 and Table 2). The FRAP values for onion, broccoli, cauliflower, white cabbage and tomatoes were higher than the corresponding TEAC values. Tomato, lettuce and carrot extracts were poor antioxidants in both systems however.

Values of antioxidant activities measured by the TEAC and FRAP method of total vegetable extracts are shown in Figure 1 and Table 2. TEAC values varied between 0.43 ± 0.01 and 3.68 ± 1.10μmol g^{-1} fresh weight. Vegetables exhibiting highest activities were Chinese cabbage, onion, mugwort, broccoli, chili pepper and cauliflower (Table 2). Lettuce, tomato, white cabbage and carrot had low TEAC values. FRAP values ranged from 0.60 ± 0.01 to 8.47 ± 1.86 μmol g^{-1} fresh weight. The FRAP and TEAC values were similar in the vegetable extracts tested with the following antioxidant profiles in increasing order: onion > Chinese cabbage > broccoli > mugwort > cauliflower > white cabbage (Figure 1 and Table 2). The FRAP values for onion, broccoli, cauliflower, white cabbage and tomatoes were higher than the corresponding TEAC values. Tomato, lettuce and carrot extracts were poor antioxidants in both systems however.

Figure 1: Antioxidant activities of vegetables as assessed by TEAC and FRAP assay:



1: Chinese cabbage; 2: Onion; 3: Mugwort; 4: Broccoli; 5: Chili pepper; 6: Cauliflower; 7: White cabbage; 8: Lettuce; 9: Tomatoes; 10: Carrot

The total phenol contents ranged from 132 ± 9 in carrot to 1189 ± 125 $\mu g\ g^{-1}$ fresh weight, in Chinese cabbage (Table 2). In view of the large variation in the total phenol contents, we propose 3 groupings of the total phenolic contents of the vegetables:

1) high level: > 800 $\mu g\ g^{-1}$ fresh weight including Chinese cabbage, mugwort, broccoli;
2) medium level: 275-425 $\mu g\ g^{-1}$ fresh weight including chili pepper, tomatoes, cauliflower and
3) low level: < 275 $\mu g\ g^{-1}$ fresh weight comprising white cabbage, lettuce and carrot).

Table 2: Total phenol, flavonoid, proanthocyanidin, vitamin C contents and antioxidant activities as assessed by the TEAC and FRAP assays of commonly consumed vegetables. Data expressed as mean values ± standard error (n = 3); [a]μg gallic acid g^{-1} fresh weight; [b]μg quercetin g^{-1} fresh weight; [c]μg cyanidin chloride g^{-1} fresh weight; [d]μg ascorbic acid g^{-1} fresh weight; [e]μmol Trolox g^{-1} fresh weight; [f]μmol FeII g^{-1} fresh weight.

Common names	[a]Total Phenols	[b]Total Flavonoids	[c]Proantho cyanidins	[d]Vitamin C	[e]TEAC	[f]FRAP
Chinese cabbage	1189 ± 125	944 ± 73	-	253 ± 20	3.68 ± 1.10	7.00 ± 1.26
Onion	1010 ± 75	514 ± 42	116 ± 11	187 ± 7	3.57 ± 0.30	8.47 ± 1.86
Mugwort	956 ± 71	435 ± 65	-	351 ± 26	2.86 ± 0.86	2.12 ± 0.60
Broccoli	822 ± 89	316 ± 45	12 ± 1	748 ± 62	2.85 ± 0.56	3.36 ± 0.56
Chili pepper	412 ± 8	321 ± 16	-	344 ± 23	2.26 ± 1.08	1.28 ± 0.30
Tomatoes	350 ± 11	79 ± 8	-	86 ± 3	1.00 ± 0.06	0.78 ± 0.02
Cauliflower	278 ± 15	172 ± 11	7 ± 1	499 ± 53	2.09 ± 0.95	1.57 ± 0.20
White cabbage	153 ± 21	102 ± 9	-	188 ± 13	1.73 ± 0.06	1.56 ± 0.23
Lettuce	134 ± 28	87 ± 5	-	25 ± 3	1.14 ± 0.21	0.68 ± 0.09
Carrot	132 ± 9	45 ± 2	4 ± 0	298 ± 14	0.43 ± 0.01	0.60 ± 0.01

Flavonoids were the dominating phenolic class in the vegetables studied and their variation was analogous to that of total phenols except for tomato. Levels of flavonoids in the vegetables were in the order: Chinese cabbage > onion > mugwort > broccoli > chili pepper > cauliflower > white cabbage. Lettuce, tomato and carrot were relatively poor in flavonoids. Free flavonoids were not detected in the vegetable extracts, however after hydrolysis HPLC analysis showed that quercetin, kaempferol and myricetin were the main flavonol aglycones present.

Table 3: Quercetin, kaempferol, myricetin, apigenin and luteolin contents in vegetable hydrolysed extracts. Values are represented in μg g^{-1} fresh weight from 3 determinations

Common names	Myricetin	Quercetin	Kaempferol	Apigenin	Luteolin
Chinese cabbage	1 ± 0	390 ± 53	96 ± 23	45 ± 2	12 ± 3
Onion	32 ± 3	311 ± 57	45 ± 8	21 ± 6	11 ± 2
Mugwort	-	302 ± 37	125 ± 14	73 ± 9	-
Broccoli	-	137 ± 21	46 ± 3	-	-
Chili pepper	12 ± 2	105 ± 6	-	14 ± 3	14 ± 4
Lettuce	9 ± 0.8	74 ± 2	-	23 ± 5	-
White cabbage	-	51 ± 9	-	8 ± 1	-
Cauliflower	-	39 ± 8	12 ± 1	2 ± 0.1	-
Tomatoes	-	38 ± 4	7 ± 1	-	-
Carrot	4 ± 0. 2	15 ± 2	6 ± 0.4	-	8 ± 1

Quercetin was the predominant flavonol aglycone detected in all extracts (Table 3). Its level varied from 15 ± 2 $\mu g\ g^{-1}$ fresh weight in carrot to 390 ± 53 $\mu g\ g^{-1}$ fresh weight in Chinese cabbage. Kaempferol derivatives were present in all vegetable extracts except chili pepper, lettuce and white cabbage with amounts ranging between 6 $\mu g\ g^{-1}$ fresh weight and 125 $\mu g\ g^{-1}$ fresh weight. Low levels of myricetin were recorded in onion, chili pepper, lettuce, carrot and Chinese cabbage (Table 3). Apigenin and luteolin were the only flavones detected in hydrolysed vegetable extracts. Levels of Apigenin were between 2 ± 1 $\mu g\ g^{-1}$ and 73 ± 9$\mu g\ g^{-1}$ fresh weight with highest amounts recorded for mugwort and Chinese cabbage. Trace amounts of luteolin were present in Chinese cabbage, onion, chili pepper and carrot (Table 3). The levels of vitamin C ranged from 25 ± 2 $\mu g\ g^{-1}$ fresh weight to 748 ± 62 $\mu g\ g^{-1}$ fresh weight in the vegetables studied (Table 2). Broccoli, cauliflower, mugwort and chili pepper were the highest vitamin C–containing vegetables. This was followed by carrot, Chinese cabbage, white cabbage and onion. Tomato and lettuce are poor sources of vitamin C with levels of 86 ± 4 and 25 ± 2 $\mu g\ g^{-1}$ fresh weight respectively (Table 2). Proanthocyanidins were found mainly in onion (116 ± 23 $\mu g\ g^{-1}$ fresh weight). Levels in broccoli, cauliflower and carrot were insignificant and were absent in all the other vegetables.

DISCUSSION

Several reports exist where the contribution of phenolics of whole vegetables to antioxidant status have been examined.[29–32] The phenol contents in vegetables such as cauliflower, lettuce, tomato [31] were essentially in the same range as in Table 4. Among 38 Asian vegetables studied by Kaur *et al* [30] the highest total phenol levels (in terms of catechol equivalence) were found in green chili pepper, cauliflower, cabbage, broccoli, tomato, onion and carrot (Table 4).

Table 4: Comparative literature data on the total phenol, quercetin and kaempferol contents of vegetable extract. UD: Undetected. * Unit expressed in catechol equivalent g^{-1} fresh weight.

	Cauliflower	Lettuce	Tomato	Broccoli	Onion
Total Phenols (µg g^{-1} FW)					
Present study	278	134	350	822	1010
Proteggente *et al*[31]	300	140	300	1280	880
Kaur *et al*[30*]	960	-	680	875	568
Quercetin derivatives (µg g^{-1} FW)					
Present study	UD	74	38	137	311
Crozier *et al*[44]	-	94	23-203	-	185
Hertog *et al*[45]	-	1.9-30	4.6^{-1} 1	30	284
Hollman and Arts[46]	-	14-79	-	30-370	340
Kaempferol derivatives (µg g^{-1} FW)					
Present study	12	UD	7	46	45
Crozier *et al*[44]	-	UD	UD	-	UD
Hermann *et al*[52]	-	-	-	30	21-235
Price *et al*[50]	-	140	300	94	300

Total phenolic contents are indicative of the amount of polyphenols in vegetables. The Folin-Ciocalteu method is known to overestimate the content of phenolic compounds primarily because other agents present in food, such as carotenoids, amino-acids, sugars and vitamin C, can interfere.[25, 33] We have suggested the term "Folin Ciocalteu index" rather than total phenols.[34] The correlation coefficient between antioxidant activities and "Folin Ciocalteu index" for the antioxidant assays are TEAC: r = 0.91 and FRAP: r = 0.83. The "phenol-antioxidant index", indicate a combined measure of the quality and quantity of antioxidants present in vegetables. Although phenolic compounds have different responses in the Folin-Ciocalteu method, such responses depend on their chemical nature. Maximum antioxidant potentials were obtained for Chinese cabbage, onion, mugwort, broccoli and chili pepper on a fresh weight basis. Vegetable flavonoid contents were much higher than any other phenolic subclasses and therefore contributed significantly to the antioxidant capacity of vegetables. This was indicated by the high correlation coefficients between antioxidant capacity and total flavonoid levels (TEAC: r = 0.89; FRAP: r = 0.82). Similar linear relationship between antioxidant activities and phenolic contents have been reported in onions and green leaves of sweet potatoes,[30, 35] in fruits,[36] in medicinal plants,[37] in fruit juices,[38] in wines,[39] in edible seaweeds [40] and in plant cell cultures.[41] However antioxidant activity might not always correlate with phenolic contents as reported in berries [42] and in plant extracts. [43]

The qualitative and quantitative determination of individual flavonoid glycosides in foods is difficult as most of the reference compounds are not commercially available. The hydrolysis of flavonoid glycosides to aglycones is a practical approach for the quantitative determination of flavonoids in plant extracts. Quercetin is the dominant flavonol in the hydrolysed methanolic vegetable extracts with maximum levels in Chinese cabbage (390 ± 53 μg g^{-1} fresh weight), onion (311 ± 57 μg g^{-1} fresh weight) and mugwort (302 ± 37 μg g^{-1} fresh weight). The levels of kaempferol, myricetin, apigenin and luteolin were less pronounced in Mauritian vegetables. The 311 ± 57 μg g^{-1} fresh weight quercetin concentration in the Mauritian onion cultivar compares with U.K onion data reported by Crozier *et al* [44] and in onions studied by Hertog *et al* [45] in the Netherlands (Table 4). The quercetin level of the Mauritian Mignonette lettuce (74 ± 2 μg g^{-1} fresh weight) was close to the value observed in "Lollo Bionda var. Cerieo" variety [44] and was within the range reported by Hollman and Arts [46] (Table 4). Some commercial (e.g. "Round" lettuce var. Cortina, "Lollo Rosso" var. Malibu, "Lollo Bionda" var. Cerieo from U.K) and home-grown (e.g. "Green Salad Bowl", "Marvel of Four Seasons" from Scotland) cultivars[44] had quercetin contents varying between 11 and 911 μg g^{-1} fresh weight. This compares with the 1.9 to 30 μg g^{-1} fresh weight observed in leaves of *Latuca sativa* var, Capitula in the Netherlands cultivars [45] and the 1 to 54 μg g^{-1} fresh weight detected in 13 varieties of U.S.A grown head and leaf lettuce.[47] Dutch tomatoes, Spanish tomatoes and Scottish tomatoes had relatively low quercetin contents while Spanish cherry tomatoes and English cherry tomatoes were the richest quercetin containing varieties depending on the period of purchase (Table 5). A study conducted on commercial tomatoes in the Netherlands showed that their quercetin content ranged from 4.6 to 11 μg g^{-1}.[48] Similarly, Martinez-Valverde *et al* [49] reported quercetin contents in the range of 7.19 - 43.59 μg g^{-1} fresh weight from 9 commercial Spanish varieties of tomato (Table 5). The quercetin content of the Mauritian tomatoes var. MST/32 (38 ± 4 μg g^{-1})

was found to be within the range of the quercetin rich varieties reported above. The level of quercetin in Mauritian broccoli (137 ± 2 μg g^{-1} fresh weight) was within the 30-370 μg g^{-1} range recorded by Proteggente *et al.*[31] Hertog *et al* [45] and Price *et al* [50] reported values at the lower end of the scale however, e.g., 30 μg g^{-1} in Broccoli cv. Italica L. and 43 μg g^{-1} in broccoli florets (cv. Marathon) respectively.

Table 5: Comparative literature data on the quercetin contents of tomato cultivars.

	Type	Date of Purchase	Quercetin (μg g^{-1} fresh weight)
Present study	Mauritian tomatoes MST/32	-	38
Crozier *et al* [44]			
	Spanish tomatoes	12 Jan 1995	4.4
	var. Assun	23 Jan 1995	3.5
	var. Assun	4 Feb 1995	2.0
	var. Daniella	3 Apr 1995	8.7
	Scottish tomatoes var. Spectra	9 Jun to 10 Aug 1995	4.6 to11.2
	Dutch beef tomatoes var. Trust	9 Jun to 10 Aug 1995	2.2 to 6.8
	Spanish cherry tomatoes var. Paloma	4 Feb to 10 Aug 1995	28 to 203
	English cherry tomatoes var. Favorita	4 Feb to 10 Aug 1995	17 to 77
Hertog *et al* [45]	Commercial tomatoes	Apr 1991 to Apr 1992	4.6 to 11
Martinez-Valverde *et al* [49]	Spanish Tomatoes		
	var. Rambo	-	7.19
	var. Senior	-	17.16
	var. Ramillete	-	28.66
	var. Liso	-	12.45
	var. Pera	-	10.34
	var. Canario	-	28.08
	var. Durino	-	22.28
	var. Daniella	-	43.59
	var. Remate	-	21.25

Kaempferol was present in the Mauritian onions at a concentration of 45 ± 8 μg g^{-1} fresh weight, an outcome that was in contrast to the findings of Crozier *et al* [44] who were unable to detect this compound in red and white onions. Kaempferol was also present in tomato, mugwort, Chinese cabbage, broccoli and carrot (6 –125 μg g^{-1} fresh weight). Nielsen *et al* [51] showed that cabbage contained a mixture of more than 20 compounds of which three have been identified as 3-O-sophoroside-7-O-β-D-glucosides of kaempferol and quercetin while Chu *et al*[34] detected traces of kaempferol in Chinese cabbage. Herrman *et al* [52] found significant levels of kaempferol in onion (21 to 235 μg g^{-1}) and

broccoli (30 μg g^{-1} fresh weight). In addition, Price *et al* [50] measured 94 μg g^{-1} fresh weight in broccoli florets while Hertog *et al* [53] observed 72 μg g^{-1} fresh weight in the Italica L. cultivar.

Myricetin occurred at relatively low levels or traces in Mauritian Chinese cabbage, onion, chili pepper, lettuce and carrot. The presence of myricetin in lettuce leaf, Chinese cabbage, white cabbage and onion was shown by Chu *et al.*[34] Among the flavones, apigenin was observed in Chinese cabbage, onion, mugwort, broccoli, cauliflower, tomato and carrot while luteolin occurred in trace amounts in Chinese cabbage, onion, chili pepper and carrot. However a study, which included the same types of vegetables grown in the U.K and the Netherlands [44, 45] were unable to detect these flavones.

Contents of flavonoid derivatives in Mauritian vegetables were in general high. Factors including differences in variety and high sunlight conditions (a characteristic feature of tropical Mauritius), which can induce the accumulation of flavonoids,[54] are probably responsible for the increased yield. Although geographical differences in the levels of phenolics are widely discussed for teas, we suggest that this may also be widespread for fruits and vegetables.

Proanthocyanidins are poorly distributed in the vegetable extracts. This agrees with data reported by De Pascual-Teresa *et al*[55] where total flavanol contents of low degree of polymerization were non-detectable in most of the 13 vegetables studied. Vitamin C levels of Mauritian vegetables varied between 22 ± 3 and 748 ± 62 μg g^{-1} fresh weight. Proteggente *et al* [31] reported comparable values for the vitamin C contents in lettuce (20 μg g^{-1} fresh weight) and higher amounts in tomato (180 μg g^{-1} fresh weight). The amount of vitamin C in Mauritian tomatoes was relatively low compared to literature data on the same. This is probably due to varietal differences and prevailing agricultural culture practices. Mauritian broccoli, onion and cauliflower were rich in vitamin C. These vegetables (broccoli, onion and cauliflower) together with Chinese cabbage and carrot contained higher vitamin C levels than those reported in the USDA FOOD Composition Database.[56] Ascorbate contents of vegetables correlated weakly with the TEAC values ($r = 0.33$) since in many cases vitamin C levels were low where antioxidant capacity was high. No correlation was found between vitamin C levels and FRAP data. Similar observations have been made where vitamin C makes little contribution or does not contribute at all to the total antioxidant capacity of fruits and vegetables.[57–59] Vitamin C and E supplement do not seem to reduce reactive oxygen species activity in *Helicobacter pylori* gastritis in the short term.[60]

The FRAP and TEAC data are consistent with literature data for Chinese cabbage, broccoli and onion, which are generally reported to have high antioxidant activities [31, 34, 61, 62] and vegetables such as carrot and lettuce to be weak antioxidants in *in vitro* assays .[31, 62] High antioxidant activities have however been reported in tomato, cabbage and carrot and lower activities for onion, chili pepper and cauliflower using a model system consisting of β-carotene and linoleic acid.[30] The low antioxidant activities of tomatoes and carrots may be attributed to the extraction process resulting in the assay of more water soluble extracts. Defatting and maceration have largely contributed to the removal

of the antioxidant carotenoids and tocopherols present in these vegetables. The emerging consensus of opinion is that use of one method to assess antioxidant action does not give universal answers. It is clear that the antioxidant efficacy of an extract should be evaluated by different methods rather than depending on the results of one method.[63, 64] Nevertheless some comparative comments are worthwhile. Proteggente *et al* [31] reported TEAC and FRAP values for cauliflower of the order of 2.95 and 2.59 μmol g^{-1} fresh weight respectively. The TEAC and FRAP data they measured in lettuce compared with the values of the Mauritian "Mignonette" lettuce but the antioxidant capacities of broccoli extracts were higher (Table 6). The FRAP evaluation reported by Halvorsen *et al* [65] for broccoli, cauliflower, cabbage and onion were similar to those in the Mauritian cultivars. FRAP values recorded by Szeto *et al* [59] as measured in lettuce "iceburg" cultivar and broccoli were of similar order as recorded in the Mauritian "Mignonette" lettuce and broccoli but showed higher values for cultivars of cauliflower, tomato and carrot (Table 6).

Table 6: Comparative data on the antioxidant capacities of vegetable extracts as expressed in TEAC and FRAP values (μmol g^{-1} fresh weight). NA: Not Applicable

	Present study		**Proteggente *et al* [31]**		**Halvorsen *et al* [65]**	**Szeto *et al* [59]**
	TEAC	**FRAP**	**TEAC**	**FRAP**	**FRAP**	**FRAP**
Cauliflower	2.09	1.57	2.95	2.59	1.3	2.84
Lettuce	1.14	0.68	1.71	1.24	0.7	0.88
Broccoli	2.85	3.36	6.48	8.33	3.5	2.94
Onion	3.57	8.47	5.32	3.69	7	4.32
Tomato	1.00	0.78	2.55	3.44	3.4	2.36
Carrot	0.43	0.60	NA	NA	NA	1.66

The outcome of several epidemiological studies that diets rich in fruits, vegetables, herbs and spices correlate with low incidence of cancer and heart disease. [66, 67] We report that the overall antioxidant capacity of Mauritian vegetables can be attributed to the high "Folin Ciocalteu index" with flavonoids making a major contribution. Phenolic acids, which may be present in the extracts, but not assayed, could add up to the overall antioxidant synergistic effect of Mauritian vegetables. Although the quantitative roles of antioxidants are not precisely known in relation to their health benefits, phenolic compounds, especially the plant flavonols like quercetin, myricetin and rutin have powerful antioxidants when compared with the traditional vitamins.[68] The health influences of flavonoids have yet to be fully established but there are grounds for encouraging the use of foods rich in flavonoids. Mauritian vegetables such as Chinese cabbage, onion, mugwort, broccoli and chili pepper are potent examples, particularly with regard to the relatively high incidence of cardiovascular diseases, cancers and diabetes in Mauritius.

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3. POLYPHENOLS AND ANTIOXIDANT CAPACITIES OF MAURITIAN TEAS

The results related to this research work have been submitted for publication in the *Journal of Nutritional Biochemistry*. A copy of the paper is appended.

Assessment of the total phenol, proanthocyanidin, flavonoid, catechin and gallic acid contents and antioxidant activities of Mauritian commercial black teas: Important contributor to their medicinal properties

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Running title: Antioxidant capacities of Mauritian teas

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ABSTRACT

Tea drinking is associated with an improved antioxidant status *in vivo* which may contribute to the lowering of the risk of certain types of cancer, coronary heart disease and stroke. The effects of different preparation methods on the polyphenolic bioactive composition and the antioxidant properties of 9 Mauritian commercial black teas were evaluated. Hot water infusates contained higher levels of total phenols, total proanthocyanidins and total flavonoids compared with the corresponding organic extracts. HPLC data of the individual compounds revealed remarkably high levels (+)-Catechin, (-)-epicatechin), (-)-epicatechin 3-gallate, epigallocatechin, (-)-epigallocatechin 3-gallate, gallic acid, and procyanidin dimers B1 and B2 in both types of extracts. In the hydrolysed organic extracts quercetin was the dominant flavonol aglycones followed by myricetin and kaempferol. Based on FRAP and TEAC assays the antioxidant potential of Mauritian teas ranked in the following order for infusates: Ouvagalia tea > Buccaneer's choice> Black Label >Red Label > Extra > Corson > Chartreuse > La Flora > 3-Pavillions and in the following order for organic extracts: Extra > Ouvagalia > Buccaneer's choice > Red Label > Chartreuse > Corson > Black Label > 3-Pavillions > La Flora. Linear regression analyses produced high correlation coefficient with total proanthocyanidin (TEAC r = 0.96 and FRAP r = 0.95) and total phenol contents (TEAC r = 0.90 and FRAP r = 0.92) in infusates while antioxidant capacity of organic extracts seem be strongly influenced by total phenols (TEAC: r = 0.95 and FRAP: r = 0.96) and to a lesser extent by total proanthocyanidin and total flavonoid contents. Catechins and gallic acid seem to add up to the overall antioxidant capacity of black tea extracts. Fresh tea leaves had high levels in total phenols, total flavonoids, total proanthocyanidin and exhibited greater antioxidant potentials when compared with black teas. Mauritian teas have high levels of polyphenolic compounds and important antioxidant activities, which are highly relevant to maintenance of normal health and disease management.

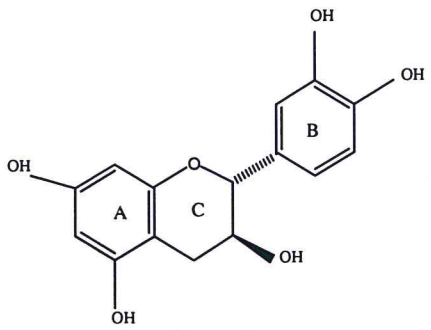
Keywords: *Black teas, hot water infusates, acetone/methanol tea extracts, total phenols, flavonoids, proanthocyanidins, catechins, antioxidant capacity, TEAC and FRAP*

INTRODUCTION

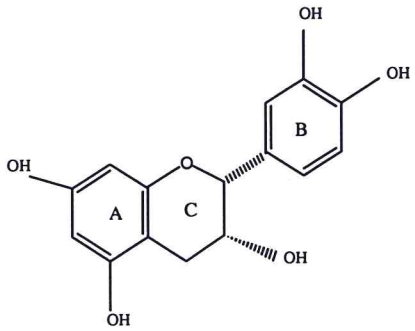
Tea plants are widely cultivated in Southeast Asia, including China, India, Japan, Taiwan, Sri Lanka, Indonesia and in many African countries including Mauritius. Regular intake of tea is associated with an improved antioxidant status *in vivo* which may contribute to the lowering risk of certain types of cancer, coronary heart disease and stroke (Block et al., 1992; Knekt et al., 1996; Hong et al., 2001; Uesato et al., 2001; Van het Hof et al., 1999; Yang and Wang, 1993; Lin et al., 1996, 1997; Rice-Evans 1999, Yoshida et al., 1999; Hollman and Katan, 1999; Weisburger 1997). In addition, antimutagenic effects (Shiraki et al., 1994, Kuroda et al., 1999; Gupta et al., 2002), inflammation (Katiyar et al., 1999) and protection against neurodegenerative diseases (Choi et al., 2000, 2001) are widely discussed. Hertog et al., (1993) reported an average intake of all flavonoids in the Dutch diet to be 23 mg/day, with tea accounting for about 48% of total intake.

Teas are classified into three major categories: the non-fermented or green tea which represents about 20% of world-wide tea consumption, the partially fermented oolong or paochong tea representing only 2 %, and the fully fermented black or pu-erh tea with an 80% consumption (Balentine, 1992; Gupta et al., 2002). Their composition of tea varies with species, season, age of leaf, climate, and horticultural practices (Lin et al., 1996). Polyphenols are the most abundant group of compounds in fresh tea leaves and are found in green and black tea beverages at 30-42% and 3-10% of the total dry matter, respectively (Graham, 1992). The major tea catechins are (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-gallate, (-)-gallocatechin, (-)-epigallocatechin, (-)-epigallocatechin-3-gallate **(Figure 1).**

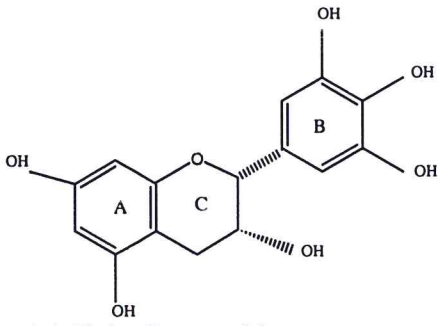
Figure 1: Common catechins present in teas



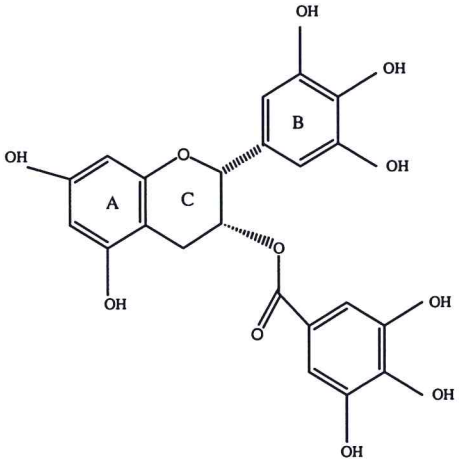
(+)-Catechin



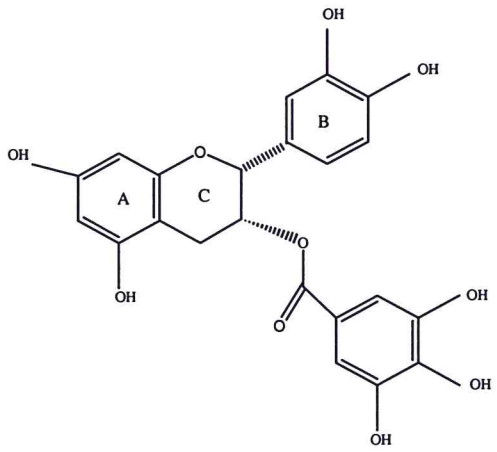
(–)-Epicatechin



(–)-Epigallocatechin



(–)-Epigallocatechin-3-gallate



(–)-Epicatechin-3-gallate

In the manufacturing of black tea, the monomeric flavan-3-ols undergo polyphenol oxidase-dependent oxidative polymerization leading to the formation of bisflavanols, theaflavins, thearubigins and other oligomers in the process commonly known as fermentation (Lin *et al.*, 1998). During the manufacture of oolong and black tea, the catechins (flavanols) are easily oxidized by polyphenol oxidase, and further polymerizations lead to theaflavins, thearubigins and compounds of higher molecular mass (Graham, 1992; Harbowy and Balentine, 1997; Wright et al., 2002). These polyphenols are responsible for the characteristic reddish color and the astringency of the black tea (Rider et al., 1992). The finest teas are often made from young shoots containing the highest catechin levels (Thanaraj and Seshadri, 1990).

Tea catechins can cause increased activities of phase II detoxifying enzymes (Lee et al., 1995), suppress extracellular signals and cell proliferation (Liang et al., 1997), inhibit the induction of nitric oxide synthase (Lin and Lin, 1997), inhibit cyclooxygenase and lipoxygenase-dependent metabolism of arachidonic acid in human colon mucosa and colon tumor tissues (Hong et al., 2001), inhibit the growth of cancerous human colon and hepatic epithelial cells (Uesato et al., 2001). (-)-Epigallocatechin-3-gallate (-)-EGCG has been shown to attenuate β-amyloid-induced neurotoxicity in cultured hippocampal neurons as well as inhibiting cloned rat brain Kv1.5 potassium channels (Choi et al., 2001). Datla et al., (2001) have shown that pre-treatment of rats with the citrus flavonoid tangeretin was neuroprotective in the 6-hrdroxydopamine animal model of Parkinson's disease. This is in line with the report of Levites et al., (2002) suggesting that tea extracts attenuated the neurotoxicity of 6-OHDA in rat pheochromocytoma (PC12) and human neuroblastoma (NB) SH-SY5Y cells *in vitro*.

This study was aimed at determining The polyphenolic contents of 9 different brands of commonly consumed Mauritian black teas using extraction by infusion with boiled water for 5-10 mins and organic solvent extraction with acetone/methanol. Antioxidant capacity of each of the extract component was assessed using the TEAC and FRAP assays. A comparative study was also conducted using fresh tea leaves collected from the central part of Mauritius.

MATERIALS AND METHODS

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

Tea samples

Nine commercially available black tea or black tea samples **(Table 1)** were purchased from a local Hypermarket in Mauritius and were used directly for polyphenolic extraction. Fine plucks of fresh tea leaves comprising the first 2–4 leaves with a bud were also collected in the region of Curepipe (Central Mauritius) during the month of March 2001, and were immediately used for extraction. The fresh leaves are generally used for the production of the Bois Chéri teas (3-Pavillons, Black Label, Red Label, Extra and Ouvagalia)

Extraction

Boiled water infusates

2 g (equivalent to 1 tea bag) of black tea and 10 g of fresh tea leaves were extracted with 150 ml (a standard cup equivalent) of boiled distilled water for 5–10 minutes. After filtration the aqueous filtrate was divided into parts. The first part was freeze-dried and re-dissolved in methanol at a final 1:5 fresh weight:volume ratio. This was used for the quantitative analysis of phenolic compounds. The second part was used to determine antioxidant activity.

Acetone/methanol extracts

10 g of black tea and 20 g of fresh green leaves were homogenized using a Waring blender in acetone/water (70/30 v/v) (2 x 300 ml) and left to macerate for 24 h at 4 °C. After filtration the residue was homogenized in methanol 100% (2 x 300 ml) and left again to macerate for 24 h at 4 °C. The combined filtrates were evaporated *in vacuo* at 37 °C before being washed with dichloromethane (3 x 150 ml) to remove fat-soluble substances. The aqueous extract obtained was concentrated and divided into two equal aliquots. One was freeze-dried, re-dissolved in methanol at a final 1:5 fresh weight: volume ratio and used for the quantitative analysis of phenolic compounds. The other aliquot was used to determine antioxidant activity.

Table 1: Commercial black tea brands with their cultivation sites and producers in Mauritius.

Manufacturer/Producer	Local Brands of Black tea	Region of Cultivation
Bois Chéri Tea	3-Pavillions	Grand-Bois and Curepipe
	Black Label	Grand-Bois and Curepipe
	Red Label	Grand-Bois and Curepipe
	Extra	Grand-Bois and Curepipe
	Ouvagalia	Grand-Bois and Curepipe
La Flora Tea	La Flora	Grand-Bois and Curepipe
La Chartreuse Tea Manufacturing Co. Ltd	Chartreuse	Curepipe
Corson Tea Estate Co. Ltd	Corson	Curepipe
Mauritius Tea Factories Co. Ltd	Buccaneer's Choice	Curepipe

Total phenolics

Total phenol estimation was determined by the method of Singleton and Rossi (1965) using the Folin-Ciocalteu reagent. 0.25 ml of diluted samples was added to 3.5 ml of distilled water in screw-capped test tubes followed by 0.5 ml Folin-Ciocalteu solution. After 3 mins, 1 ml of sodium carbonate (1 %) was added and the test tubes were properly shaked before incubating in boiling water bath for 1 min. The tubes were then allowed to cool in the dark. The absorbance of the blue color that developed was measured at an absorbance of 685 nm and results expressed in mg of gallic acid/g dry weight using appropriate standard curve.

Total Proanthocyanidins

The HCl/butan-1-ol assay of Porter et al., (1986) was used to quantify the total proanthocyanidins. 0.25 ml of extract was added to 3 ml of a 95 % solution of n-Butanol/HCl (95:5 v/v) in stoppered test tubes followed by 0.1 ml of a solution of $NH_4Fe\ (SO_4)_2.12H_2O$ in 2 M HCl. The tubes were incubated for 40 mins at 95°C. The absorbance of the red colour that developed was read 550 nm with data expressed as mg of cyanidin chloride/g dry weight.

Total Flavonoid

The $AlCl_3$ method adapted from Lamaison (1990) was used for the determination of the total flavonoid content of the methanolic extracts. 1.5 ml of extracts was added to equal volumes of a solution of 2 % $AlCl_3.6H_2O$ (2 g in 100 ml methanol). The mixture was thoroughly mixed and incubated for 10 min, after which absorbance was read at 367.5 nm. Data were expressed in mg quercetin equivalents/g dry weight.

High performance liquid chromatography

HPLC analysis of tea extracts were carried out using a Hewlett Packard 1100 series liquid chromatography system equipped with a vacuum degasser, quaternary pump, auto-sampler, thermostated column compartment and diode array detector. After filtration on Millipore filter paper (0.22 μm) 30μl of 25% (v/v) aqueous methanolic extracts were injected on a Spherisorb ODS 2 RP 18 column (5 μm pore size, 4.6 mm id x 150 mm) eluted by an acidified acetonitrile-water gradient. Elution with a flow rate of 0.7 ml/min at 25 °C 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). Recovery of compounds from acid hydrolysed extracts was monitored by use of internal standards.

Gallic acid, catechins and procyanidin dimers
GA, (+)-C, (-)-EC, (-)-ECG, (-)-EGC, (-)-EGCG, procyanidin dimer B1 and procyanidin dimer B2 were identified and quantified by comparison with authentic standards at 280 nm.

Flavonols and flavones
Myricetin, quercetin and kaempferol were identified and quantified in the tea extracts after acid hydrolysis of the flavonoid conjugates essentially as described in Crozier et al., (1997a) with morin as an internal standard. An aliquot 0.5 g black tea and 1 g fresh tea leafs were extracted with 20 ml of 60 % aqueous methanol containing 200 μg morin as an internal standard. 5 ml of 6 M HCl was added to each extract, which were then refluxed at 90 °C for 2 hrs. After cooling the flavonoid aglycones were extracted using 2 x 25ml of ethyl acetate. The organic phase was evaporated to dryness and taken in absolute methanol prior to analysis by HPLC. Absorption wavelength was selected at 360 nm.

Measurement of antioxidant activity

The Trolox Equivalent Antioxidant Capacity (TEAC) for the tea extracts was measured in terms of radical scavenging ability according to the ABTS/MnO_2 method of Campos and Lissi (1996). Data are expressed in μmol Trolox/g dry weight. The Ferric Reducing Antioxidant Power (FRAP) Assay of Benzie and Strain (1996) was used and results expressed in terms of μmol Fe (II)/g dry weight.

Statistical Analysis

Simple regression analysis was performed to calculate the dose-response relationship of standard solutions used for calibration as well as test samples. Linear regression analysis was performed, quoting the correlation coefficient r_{xy} between antioxidant activities, phenolic classes and vitamin C. The Unicam Vision 32 software (1.22 version) was used

to evaluate initial and final antioxidant rate values for TEAC assay. All results are expressed as mean value ± standard deviation (n = 3).

RESULTS

Phenolic contents of Mauritian black teas

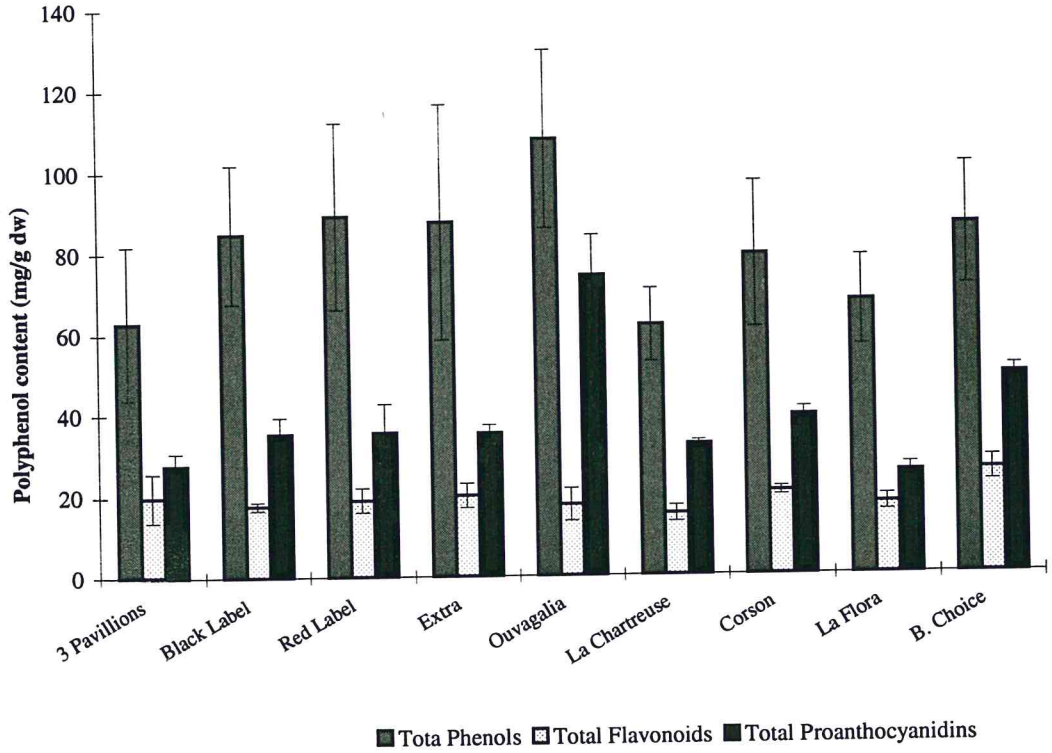
Boiled water infusates

The total phenol, total flavonoid and total proanthocyanidin contents of the 9 brands of Mauritian black tea infusates are shown in **(Figure 2).** The total phenol contents of the infusates ranged from 62 ± 9 to 107 ± 22 mg/g dry weight. The highest level was measured in Ouvagalia infusate of Bois Chèri tea. Comparable and significant amounts were also obtained in Red Label (89 ± 23 mg/g), Extra (87 ± 29 mg/g), Buccaneer's Choice (86 ± 15 mg/g) and Black Label (85 ± 17 mg/g dry weight) while Chartreuse contained the lowest amount.

The total flavonoid contents of the infusates were relatively low and ranged from 15 ± 2 to 26 ± 3 mg/g dry weight **(Figure 2).** Buccaneer's Choice contained the highest total flavonoids and Chartreuse the lowest. Similar moderate levels were obtained in 3-Pavillion tea (20 ± 6 mg/g dry weight), Extra (20 ± 3 mg/g dry weight) and Corson (20 ± 1 mg/g dry weight).

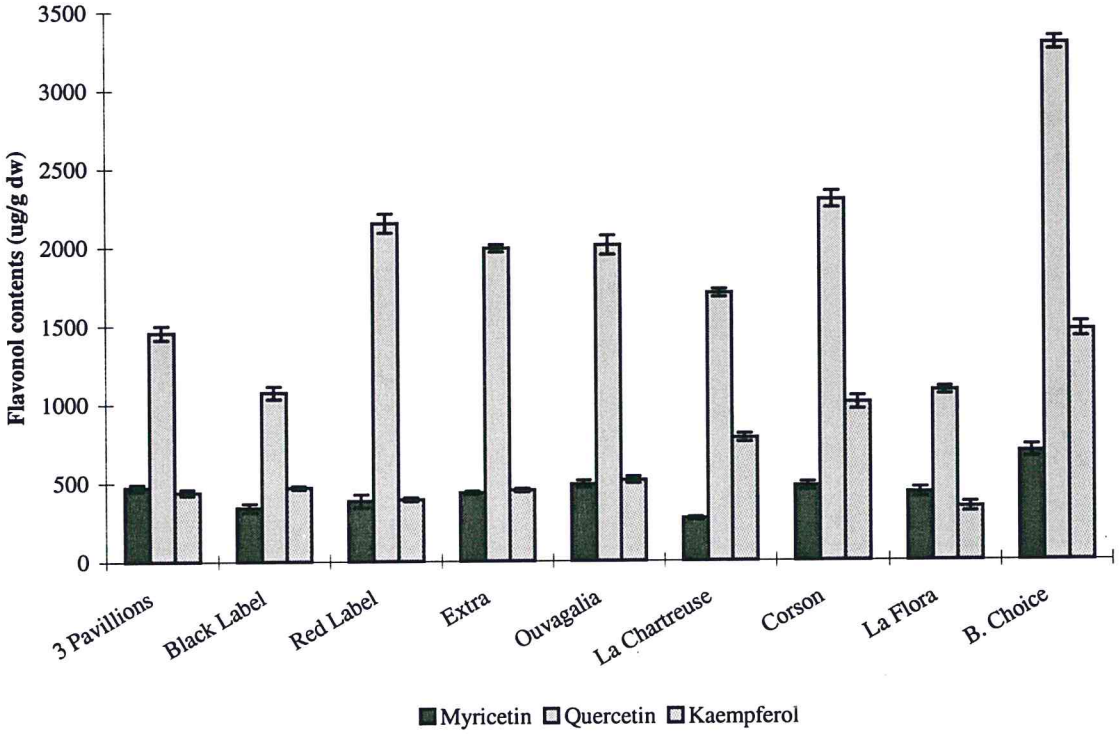
Values obtained for the total proanthocyanidin contents of the 9 infusates varied from 25 ± 2 to 74 ± 10 mg/g dry weight (**Figure 2**). Ouvagalia had the highest level of total proanthocyanidins while the lowest was measured in La Flora tea. Comparable levels were measured in infusates of Corson (39 ± 2 mg/g dry weight), Red Label (36 ± 7 mg/g dry weight), Extra (36 ± 7 mg/g dry weight), Black Label (35 ± 4 mg/g dry weight), and Chartreuse (32 ± 1 mg/g dry weight)

Figure 2: Total phenol, flavonoid and proanthocyanindin levels in tea infusates. Data expressed as mean values ± standard error (n = 3); [a]mg gallic acid/g dry weight; [b]mg quercetin/g dry weight; [c]mg cyanidin chloride/g dry weight.



HPLC screening of hydrolysed infusate extracts show that quercetin, myricetin and kaempferol are the main flavonol aglycones present. The concentrations measured (**Figure 3**) ranged from 1074 ± 40 to 3288 ± 42 μg/g dry weight, 339 ± 31 to 1467 ± 49 μg/g dry weight and 269 ± 10 to 693 ± 39 μg/g dry weight for quercetin, kaempferol and myricetin respectively. Buccaneer's Choice had the highest levels of flavonols while Black Label had lowest levels in quercetin and La Flora the poorest in kaempferol.

Figure 3: Flavonol aglycone contents of hydrolysed extracts of tea infusates (values expressed in μg/g dry weight)



Flavan-3-ol and gallic acid contents in the infusates of the black teas are shown in **Table 2.** (+)-C, (-)-EC, (-)-ECG, (-)-EGC and (-)-EGCG were the catechins characterized in the 9 tea brands. The major catechins (-)-EC), (-)-ECG, (-)-EGCG and the main phenolic acid, GA, were detected at the upper concentrations of 12601 ± 1035 μg/g dry weight (Red Label), 8265 ± 863 μg/g dry weight (Ouvagalia), 7284 ± 652 μg/g dry weight (Extra) and 10942 ± 902 μg/g dry weight (Corson) respectively. The infusion extracts had relatively low levels of (+)-catechin **(Table 2).** The calculation of a "catechin index" obtained by the summation of the amounts of individual catechins **(Table 2)** gave an indication of the catechin richness of the tea brands in the following order Ouvagalia > Red Label > Extra > Black Label > 3-Pavillions > Corson > Buccaneer's choice > Chartreuse > La flora. The concentrations of the dimeric procyanidins B1 and B2 ranged from 2464 ± 520 to 4993 ± 422 and from 1208 ± 99 to 3139 ± 861 μg/g dry weight respectively. Corson, Red Label and Ouvagalia teas contained the highest B1 and B2 combined procyanidin contents with relatively equivalent amounts (7609, 7506 and 7043

μg/g dry weight) followed by Chartreuse, Extra, Black Label, 3-Pavillions, La Flora and Buccaneer's Choice.

Table 2: Levels of catechins, dimeric procyanidins B1 and B2 and gallic acid from infusates of Mauritian black teas (μg/g dry weight)

Tea Brand	(+)-C	(-)-EC	(-)-ECG	(-)-EGC	(-)-EGCG	"Catechin index"	B1	B2	Gallic acid
3-Pavillions	1662 ± 102	9803 ± 253	3199 ± 276	1479 ± 213	4209 ± 823	20352	3117 ± 458	1642 ± 245	6863 ± 549
Black Label	1297 ± 92	9985 ± 382	3980 ± 423	968 ± 82	5082 ± 945	21312	2464 ± 520	3001 ± 562	7539 ± 864
Red Label	2052 ± 165	12601 ± 1035	7936 ± 521	1348 ± 93	4180 ± 801	28117	3245 ± 238	4261 ± 320	6972 ± 538
Extra	1635 ± 148	9749 ± 425	5124 ± 612	509 ± 42	7284 ± 652	24301	3001 ± 869	2523 ± 532	7102 ± 989
Ouvagalia	1671 ± 241	11917 ± 1253	8265 ± 863	1793 ± 49	7103 ± 746	30749	4020 ± 597	3023 ± 673	9899 ± 801
Chartreuse	970 ± 56	6253 ± 241	4362 ± 158	591 ± 23	5038 ± 891	17204	3556 ± 286	3139 ± 861	5503 ± 865
Corson	929 ± 62	8913 ± 129	3916 ± 359	303 ± 23	6095 ± 235	20156	4993 ± 422	2616 ± 531	10942 ± 1223
La Flora	1033 ± 73	5606 ± 391	3154 ± 147	903 ± 89	3916 ± 483	14612	2897 ± 382	1696 ± 298	7211 ± 487
Buccaneer's Choice	985 ± 76	8250 ± 241	2661 ± 186	1017 ± 378	5260 ± 257	18173	3059 ± 379	1208 ± 99	10614 ± 902

Methanol/acetone extracts

The total phenol contents, total flavonoid and total proanthocyanidin contents of acetone/methanol tea extracts are shown in **Figure 4.** Levels of the total phenols ranged from 39 ± 4 to 94 ± 11 mg/g dry weight, total flavonoids from 9 ± 2 to 29 ± 8 mg/g dry weight and total proanthocyanidins from 15 ± 1 to 39 ± 8 mg/g dry weight. Extra from Bois Chéri tea produced the highest total phenols, total flavonoids and total proanthocyanidins and La Flora had the lowest content in total phenols.

The average total flavonoid contents of the teas under solvent extraction were still low as compared to total phenols and total proanthocyanidins, this is consistent with what was observed in the infusates. Myricetin, kaempferol and quercetin were the predominant flavonol detected in the acetone/methanol extracts of the tea varieties studied. The highest levels of the flavonols were observed in Corson tea whilst the La Flora had the lowest flavonol aglycones **(Table 3).** The overall average flavonol contents of the organic extracts were relatively lower when compared to amounts obtained in the infusates **(Figure 3).**

Figure 4: Total phenol, flavonoid and proanthocyanindin levels in organic tea extracts. Data expressed as mean values ± standard error (n = 3); [a]mg gallic acid/g dry weight; [b]mg quercetin/g dry weight; [c]mg cyanidin chloride/g dry weight

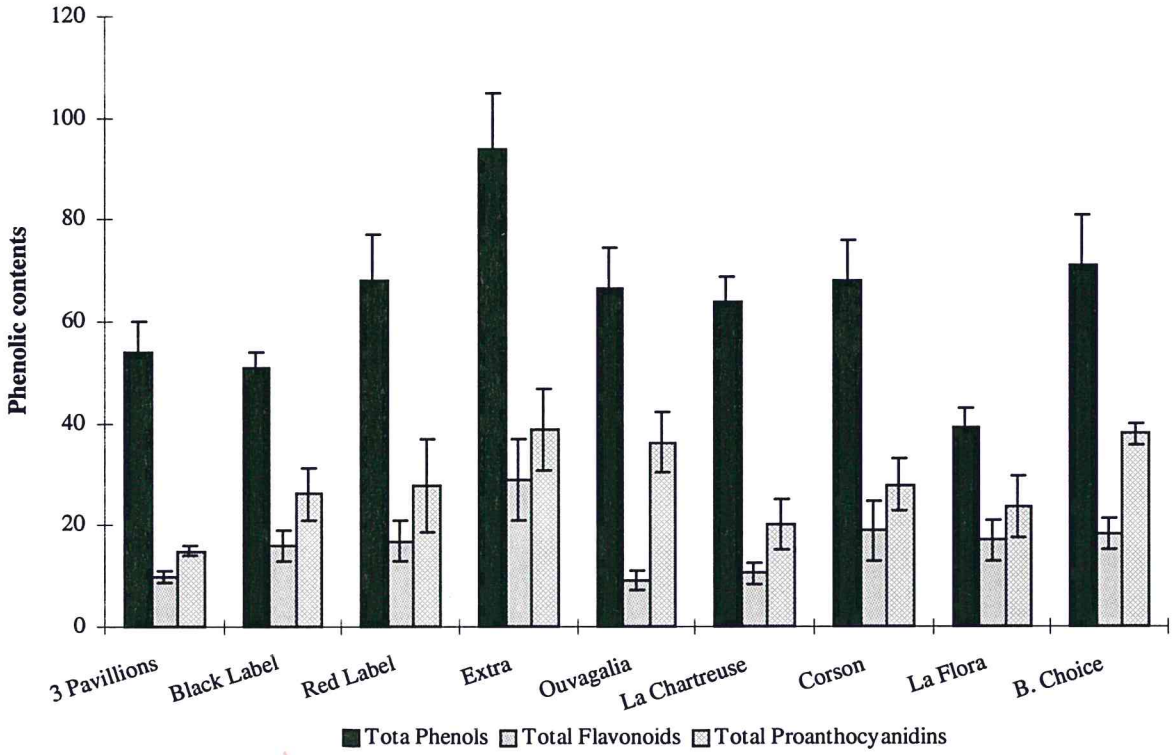


Table 3: Flavonol aglycones levels from hydrolysed organic tea extracts (values expressed in μg/g dry weight)

Tea	Brand	Myricetin	Quercetin	Kaempferol
Bois Chèri	3-Pavillion	185 ± 21	566 ± 86	246 ± 13
	Black Label	273 ± 35	581 ± 72	169 ± 20
	Red Label	231 ± 18	602 ± 70	334 ± 25
	Extra	269 ± 28	1997 ± 186	640 ± 69
	Ouvagalia	460 ± 58	948 ± 65	375 ± 19
Chartreuse	Chartreuse	264 ± 35	1621 ± 201	388 ± 56
Corson	Corson	793 ± 98	2039 ± 228	941 ± 82
La Flora	La Flora	65 ± 36	447 ± 56	239 ± 53
Buccaneer's Choice	Buccaneer's Choice	207 ± 56	658 ± 83	318 ± 29

HPLC data obtained for catechin and GA contents of the fermented teas when extracted with organic solvents are shown in **Table 4.** Significant levels of EC (5574 ± 358 to 12540 ± 1046 μg/g dry weight), GA (2047 ± 259 to 10174 ± 1283 μg/g dry weight) and (-)-EGC (894 ± 79 to 7728 ± 381 μg/g dry weight) were measured from the tea extracts. Moderate levels were obtained for (-)-EGCG (856 ± 54 to 4810 ± 285 μg/g dry weight) and (-)-ECG, which ranged from 1181 ± 102 to 3307 ± 426 μg/g dry weight. "Catechin index" shows that organic extracts of Extra and Corson contained highest catechin levels followed by Red Label, Ouvagalia and Buccaneer Choice with similar contents while 3-Pavillions, Black Label, Chartreuse and La Flora had relatively low values. The overall average catechin contents of the organic extracts were relatively lower when compared to amounts obtained in the infusates except for (-)- EGC and (-)-EC, the latter being slightly higher **(Table 2).**

Table 4: Levels of catechins, dimeric procyanidins B1 and B2 and gallic acid from organic extracts of Mauritian black teas (μg/g dry weight)

Tea Brands	(+)-C	(-)-EC	(-)-ECG	(-)-EGC	(-)-EGCG	"Catechin Index"	B1	B2	Gallic acid
3-Pavillion	1098 ± 189	9925 ± 523	2205 ± 356	1444 ± 112	1819 ± 85	16491	3367 ± 289	1337 ± 152	4843 ± 523
Black Label	1301 ± 145	9299 ± 469	1406 ± 56	894 ± 79	911 ± 62	13811	3080 ± 256	1431 ± 91	4126 ± 203
Red Label	1561 ± 86	11171 ± 1356	1872 ± 147	1904 ± 156	2716 ± 189	19224	7002 ± 520	2030 ± 324	3071 ± 486
Extra	949 ± 51	12540 ± 1046	1729 ± 89	7728 ± 381	4622 ± 382	27568	8213 ± 257	2178 ± 183	7546 ± 561
Ouvagalia	805 ± 59	8849 ± 543	1475 ± 43	6047 ± 459	1969 ± 73	19145	5541 ± 341	2007 ± 56	10174 ± 1283
Chartreuse	1375 ± 136	6495 ± 425	1204 ± 57	896 ± 97	2018 ± 56	11988	3650 ± 525	2287 ± 256	2047 ± 259
Corson	1907 ± 212	10425 ± 1284	3307 ± 426	3145 ± 259	4810 ± 285	23594	9720 ± 758	2610 ± 213	2549 ± 387
La Flora	648 ± 78	5574 ± 358	1181 ± 102	2208 ± 301	1010 ± 83	10621	2902 ± 364	962 ± 86	1738 ± 159
Buccaneer's Choice	2136 ± 452	9525 ± 879	2824 ± 258	3805 ± 419	856 ± 54	19146	5329 ± 589	1041 ± 72	5128 ± 295

Procyanidin B1 was present in significant levels in the organic extracts with values ranging from 2902 ± 364 to 9720 ± 758 μg/g dry weight whereas procyanidin B2 varied between 962 ± 86 to 2610 ± 213 μg/g dry weight. The level of procyanidin dimer B2 **(Table 4)** in all organic tea extracts were lower than the values obtained with infusates **(Table 2).**

Fresh tea leaf extracts

The infusates of fresh tea were found to produce 184 ± 36 mg/g dry weight of total phenols, 34 ± 5 mg/g dry weight total flavonoids and 64 ± 11 mg/g dry weight of total proanthocyanidins. However, higher levels of total flavonoids and total

proanthocyanidins were observed in fresh tea leaves when extracted with organic solvents **(Table 5).**

Table 5: Phenolic levels of infusates and organic extracts of fresh tea leaves (data expressed as mean values ± standard error (n = 3); [a]mg gallic acid/g dry weight; [b]mg quercetin/g dry weight; [c]mg cyanidin chloride/g dry weight, catechins, flavonol aglycones, procyanidin B1 and B2 dimers and gallic acid are expressed in μg/g dry weight)

Bioactive constituents	Extraction procedure	
	Infusion type	Solvent type
Total Phenols[a]	184 ± 36	154 ± 24
Total flavonoids[b]	34 ± 5	44 ± 9
Myricetin	969 ± 101	1593 ± 231
Quercetin	1504 ± 225	3386 ± 365
Kaempferol	569 ± 61	1593 ± 286
(+)-Catechin	2640 ± 187	1033 ± 121
(-)-EC	17021 ± 1456	15937 ± 1345
(-)-ECG	636 ± 85	722 ± 74
(-)-EGC	15065 ± 173	12006 ± 128
(-)-EGCG	25383 ± 1983	26631 ± 2054
Total proanthocyanidins[c]	64 ± 11	90 ± 16
Procyanidin dimer B1	3236 ± 452	5033 ± 568
Procyanidin dimer B2	3077 ± 275	6593 ± 312
Gallic acid	6761 ± 1258	4525 ± 356

Hydrolyzed extracts contain mostly quercetin, kaempferol and myricetin aglycones, which were similar qualitatively to the black tea aglycones. Quercetin still predominated with 1504 ± 225 μg/g dry weight. Myricetin content was 969 ± 101 μg/g dry weight and kaempferol level was 569 ± 61 μg/g dry weight. Again, the concentration of the three flavonols were higher in the solvent extract with quercetin amounting to 3386 ± 365 μg/g dry weight, myricetin 1593 ± 231 μg/g dry weight and kaempferol 1593 ± 286 μg/g dry weight **(Table 5)**.

The analysis of the flavan-3-ols contents showed important levels of (-)-EC, (-)-EGC and (-)-EGCG in the tea extracts **(Table 5)** for both systems of extraction. In addition 6761 ± 1258 μg/g dry weight of GA was obtained in boiled water extract with only 4525 ± 356 μg/g dry weight when extracted with organic solvents. However, the procyanidin dimers B1 and B2 were more abundant in the solvent extract **(Table 5)**.

The phenolic contents of fresh leaf extracts were compared with the average content of Bois Chéri black teas, (3-Pavillions, Black Label, Red Label, Extra and Ouvagalia). The respective total phenol, total flavonoid and total proanthocyanidin contents of fresh tea infusates were 53%, 45% and 35% higher than the concentrations in the Bois Chéri brands. Higher levels of myricetin, catechins and dimeric proanthocyanidins were also observed in fresh tea infusates **(Table 6)**.

Total phenols, total flavonoids and total proanthocyanidins obtained in fresh leaf organic extracts were 57%, 63% and 68% higher than the respective average phenolic levels of Bois Chéri brands. Fresh leaf organic extracts also contained higher amounts of flavonol aglycones, (-)-EC, (-)-EGC, (-)-EGCG and procyanidin dimer B2 **(Table 6)**.

Table 6: Average phenolic levels determined in Bois Chéri black tea brands (3-Pavillion, Black Label, Red Label, Extra and Ouvagalia) compared with fresh tea leaf infusates and methanol/acetone extracts ([a] mg gallic acid/g dry weight, [b] mg quercetin/g dry weight, [c]mg cyanidin chloride/g dry weight and flavonol aglycones, catechins, procyanidin B1 and B2 dimers and gallic acid are expressed in μg/g dry weight)

	Infusates		Methanol/acetone extracts	
	Average Bois Chéri black tea content	**Fresh tea Leaves**	**Average Bois Chéri black tea content**	**Fresh tea leaves**
Total Phenols[a]	86	184	67	154
Total flavonoids[b]	19	34	16	44
Myricetin	505	969	283	1593
Quercetin	2132	1504	939	3386
Kaempferol	755	569	353	1593
(+)-Catechin	1663	2640	1143	1033
(-)-EC	10811	17021	10357	15937
(-)-ECG	5701	636	1737	722
(-)-EGC	1219	15065	3604	12006
(-)-EGCG	5571	25383	2207	26631
Total Proanthocyanidins[c]	42	64	29	90
Procyanidin dimer B1	3169	3236	5441	5033
Procyanidin dimer B2	2890	3077	1797	6593
Gallic acid	7675	6761	5952	4525

Antioxidant capacities of teas

The antioxidant capacities as assessed by the TEAC and FRAP on infusates are shown in **Table 7A**. The TEAC values ranged from 424 ± 38 to 1147 ± 58 μmol/g dry weight and FRAP values from 357 ± 21 to 927 ± 51 μmol/g dry weight. Ouvagalia from Bois Chéri

tea exhibited highest antioxidant activities in both assays while La Flora tea showing weak free radical scavenging/reducing potentials. Both TEAC and FRAP assays show similar trend in antioxidant potentials (r = 0.97) with Chartreuse as the exception. Chartreuse was an effective free radical scavenger (TEAC = 535 ± 21 μmol/g dry weight) but had relatively poor reducing potency (FRAP = 376 ± 40 μmol/g dry weight). The TEAC and FRAP values of the tea extracted with organic solvents are shown in **Table 7B**. The TEAC values ranged from 335 ± 16 to 862 ± 31 μmol/g dry weight while a distribution from 230 ± 28 to 728 ± 46 μmol/g dry weight was obtained for FRAP values. Extra from Bois Chéri tea exhibited highest antioxidant activities while La Flora showed poor free radical scavenging and reducing potentials. Both the TEAC and FRAP values show identical trend (r = 0.93) in antioxidant activities.

Tables 7A and 7B: Antioxidant activities as assessed by the TEAC and FRAP of infusates (A) and organic extracts (B) of Mauritian teas Data expressed as mean values ± standard error (n = 3); [d]μmol Trolox/g dry weight; [e]μmol Fe^{2+}/g dry weight.

(A)

Tea	Brands	[d]TEAC	[e]FRAP
Bois Chéri	3-Pavillions	423 ± 31	428 ± 32
	Black Label	677 ± 72	541 ± 38
	Red Label	667 ± 81	554 ± 22
	Extra	655 ± 93	580 ± 32
	Ouvagalia	1147 ± 58	927 ± 51
Chartreuse	Chartreuse	535± 21	376 ± 40
Corson	Corson	540 ± 85	492 ± 25
La Flora	La Flora	424 ± 38	357 ± 21
Buccaneer's Choice	Buccaneer's Choice	854 ± 102	722 ± 45

(B)

Tea	Brand	[d]TEAC	[e]FRAP
Bois Chéri	3-Pavillions	335 ± 16	350 ± 32
	Black Label	393 ± 29	383 ± 29
	Red Label	595 ± 42	538 ± 41
	Extra	862 ± 31	728 ± 46
	Ouvagalia	661 ± 75	445 ± 31
Chartreuse	Chartreuse	592 ± 76	444 ± 36
Corson	Corson	517 ± 33	434 ± 52
La Flora	La Flora	283 ± 47	230 ± 28
Buccaneer's Choice	Buccaneer's Choice	660 ± 97	593 ± 26

There was a strong correlation between antioxidant activities and total proanthocyanidin content (TEAC: r = 0.96; FRAP: r = 0.95) and with total phenol contents (TEAC:

r = 0.90; FRAP: r = 0.92) (Table 8). The flavonoid contents weakly influenced the antioxidant potencies of the tea extracts under infusion extraction (TEAC: r = 0.19; FRAP: r = 0.33). Regression correlation coefficients also show important contribution of the contents in (-)-EGCG (TEAC: r = 0.61; FRAP: r = 0.63), (-)-ECG (TEAC: r = 0.59; FRAP: r = 0.55), (-)-EC (TEAC: r = 0.54: FRAP: r = 0.61) and GA (TEAC: r = 0.53; FRAP: r = 0.62). (+)-C and procyanidin dimers B1 and B2 seemed to weakly influence the antioxidant capacities of the tea infusates **(Table 8)**. The TEAC and FRAP values of the organic extracts are also strongly correlated with the total phenol contents (TEAC: r = 0.95 and FRAP: r = 0.96). The total proanthocyanidin and total flavonoid contents had moderate contribution: TEAC: r = 0.77 and FRAP: r = 0.71; TEAC: r = 0.48; FRAP: r = 0.62 respectively. These observations were different with the tea infusates. In rationalizing the antioxidant potential of the organic extracts in terms of individual phenolic compounds **(Table 8)**, a strong influence of (-)-EGC (TEAC: r = 0.76; FRAP: r = 0.65) followed by contents in procyanidin dimer B1 (TEAC: r = 0.62; FRAP: r = 0.62), (-)-EC (TEAC: r = 0.56; FRAP: r = 0.74) and GA (TEAC: r = 0.56; FRAP: r = 0.44) was observed. Procyanidin dimer B2, (-)-EGCG correlated moderately with TEAC values (r = 0.51, r = 0.51 and r = 0.44 respectively) and FRAP values (r = 0.38, r = 0.50 and r = 0.38 respectively). Weak correlation coefficients were obtained for (+)-C, and (-)-ECG with both assays **(Table 8)**.

Table 8: Correlation coefficients between TEAC/FRAP and phenolic contents of the infusates and organic extracts of Mauritian teas evaluated by the linear regression analysis.

Polyphenols	Tea infusates		Tea organic extracts	
	TEAC	FRAP	TEAC	FRAP
Total Phenols	0.90	0.92	0.95	0.96
Total Flavonoids	0.19	0.33	0.48	0.62
(+)-Catechin	0.25	0.31	0.19	0.35
(-)-EC	0.54	0.61	0.56	0.74
(-)-ECG	0.59	0.55	0.12	0.26
(-)-EGC	0.49	0.52	0.76	0.65
(-)-EGCG	0.61	0.63	0.51	0.50
Total Proanthocyanidins	0.96	0.95	0.77	0.71
Procyanidin dimer B1	0.14	0.17	0.62	0.62
Procyanidin dimer B2	0.19	0.09	0.51	0.38
Gallic acid	0.53	0.62	0.56	0.44

Infusate of the fresh tea leaves exhibited remarkable free radical scavenging activities higher than those measured for infusates and organic extracts of Bois Chéri commercial black tea preparations in both assaying systems (TEAC = 1637 ± 123 μmol/g dry weight, FRAP = 1238 μmol/g dry weight). These values are 56% (TEAC) and 51 % (FRAP)

higher than the average antioxidant capacities measured for Bois Chéri black tea brands. The organic fresh leaf extract however produced relatively lower activities with TEAC = 1211 ± 182 μmol/g dry weight and FRAP value as 996 ± 85 μmol/g dry weight. However, these values still remained greater than observed with Bois Chéri black teas. TEAC and FRAP were respectively 53 % and 51% higher in organic leaf extracts. The TEAC and FRAP values for the infusates and organic extracts of fresh tea leaves were expressed as mean values ± standard error (n = 3) in units of μmol Trolox/g dry weight and μmol Fe^{2+}/g dry weight respectively.

DISCUSSION

Tea is a potential rich dietary source of antioxidant power on the basis that their active components *in vitro*, have demonstrated radical trapping antioxidant properties. The constituents of green and black teas have been the subjects of intensive investigations for a long time (Hoefler and Coggon, 1976; Treutter, 1989; Lin et al., 1998; Khokhar & Magnusdottir, 2002). Recent trends partially in response to claims of health benefits associated with the beverage, show an increased preference for fruit and herbal teas in continental Europe and to a lesser degree in the United Kingdom; however, black tea with added milk (white tea) remains by far the most common form consumed in the United Kingdom (The Tea Council, 2001) and in Mauritius.

Brewing conditions (e.g. the temperature of the extraction water, the ratio of the leaf to water, the structure of the leaf, the period of extraction, and the nature and extent of any agitation, stirring, or squeezing from teabags) contributes to the nature and amount of the flavonoids extracted into the tea liquor. There is a lack of comparative data on individual teas, commonly consumed in various parts of the world. We studied the potential influences of tea preparation methods on the antioxidant properties of the teas *in vitro* and determined their polyphenolic bioactive components. The results show a highest total phenol content of 107 ± 22 mg/g dry weight, total flavonoid content of 26 ± 3 mg/g dry weight and total proanthocyanidin content of 74 ± 10 mg/g dry weight in the black tea infusate. HPLC quantification of the individual compound revealed remarkably very high levels of (-)-EC, (-)-ECG, (-)-EGCG and gallic acid from the tea infusates. Highest levels of (-)-EC was measured in Red Label (12.6 mg/g dry weight), of (-)-EGCG in Extra (7.3 mg/g dry weight), of (-)-ECG in Ouvagalia (8.3 mg/g dry weight) all from Bois Chéri tea while maximum GA level amounted to 10.9 mg/g dry weight in Corson tea. The concentrations of procyanidin B1 and B2 dimers approached levels of 5.0 mg/g dry weight in Corson brand and 4.3 mg/g dry weight in Red Label of Bois Chéri tea brand. The levels of (+)-C and (-)-EGC in the tea infusates were low. Indeed analysis of hydrolysed methanolic extracts indicated that quercetin was the dominant flavonol with maximum level of 3.3 mg/g dry weight produced by Buccaneers' Choice tea and traces of myricetin and kaempferol aglycones.

Linear regression analyses produced high correlation coefficient with contents in total proanthocyanidins (TEAC r = 0.96 and FRAP r = 0.95) and total phenols (TEAC r = 0.90 and FRAP r = 0.92). Total flavonoid content had a weak influence on antioxidant potentials of the tea infusates (TEAC r = 0.19 and FRAP r = 0.33). The infusate of 3-Pavillion from Bois Chéri tea had significant total flavonoids content (20 mg/g dry weight) but had low TEAC and FRAP values (Table 8). The greatest contribution of phenolic compounds to the antioxidant activities of the tea infusates came from (-)-EGCG, (-)-ECG, (-)-EC and GA. Levels of (-)-EGC, quercetin, (+)-C, myricetin, kaempferol, procyanidin B1 and B2 had moderate influence on the antioxidant activity of the Mauritian tea infusates however.

Organic extracts of the black teas contained lower levels of total phenols, total proanthocyanidins and total flavonoids compared with the corresponding tea infusates. As a consequence, the antioxidant activities (in the context of the TEAC and FRAP values) of the organic extracts were low (Table 6). Antioxidant activities in the organic extracts of black teas were strongly correlated with total phenol contents (TEAC: r = 0.95; FRAP: r = 0.96) but not with total proanthocyaninds and total flavonoid. Interestingly, The contribution of flavonoid from organic extracts was greater than that from tea infusates, indicating the effectiveness of use of organic solvents in extracting flavonoid compounds from plant materials. The organic extracts of Extra from Bois Chéri tea exhibited strongest antioxidant activity and had the highest levels of total phenol, total proanthocyanidin and total flavonoid. The best correlation for antioxidant activity was obtained with (-)-EGC followed by procyanidin dimer B1, (-)-EC, and GA.

Fresh tea leaves had high levels in total phenols, total flavonoids, total proanthocyanidin and exhibited greater antioxidant potentials when compared with black teas. Fresh tea leaf infusates had the highest total phenol content (184 mg/g dry weight) and the organic extract of the same exhibited the highest total flavonoid and total proanthocyanidin contents. Kallithraka et al., (1995) have suggested the use of pure methanol for extracting grape seed catechins and procyanidin oligomers (Wang et al., 2000). The procyanidin dimer B1 and B2 are reported here to be higher in the organic extracts of the Mauritian fresh tea-leaves

Literature data on the comparative studies of tea polyphenolic contents using different solvent systems are limited. Hot water extraction, was the best solvent for all catechins, an observation consistent with that of Khokkar and Magnusdottir (2002). Among the range of temperatures used greatest extraction was achieved at 100° C for 5-10 minutes of infusion. Interestingly, Langley-Evans (2000) also observed no apparent difference in antioxidant potential of tea leaves infused with and without bags at 90 °C but a decrease of 49 % was observed when infused at 70 °C.

There is convincing evidence in support of our data attesting that fresh tea leaves and green tea produced higher levels of polyphenolics as compared with black tea (Serafini et al., 1996; Khokhar and Magnusdottir, 2001). The fresh tea leaves cultivated in Mauritius produced remarkably very high levels of total phenols, total flavonoids, total proanthocyanidins, and in particular high levels of (-)-EC, (-)-EGC, (-)-EGCG and GA.

The observed total phenol levels, which ranged from 62 to 107 mg/g dry weight in the black teas are consistent with the average total phenol levels of 103.0 ± 22 mg/dry weight values reported in black teas commonly consumed in UK (Khokhar and Magnusdottir 2001). The levels of total phenols in tea leaves are affected by different agronomic conditions at manufacture, leaf age, and storage during and after transport, as well as the degree of fermentation. GA remains the most important phenolic acid in tea. The amount of GA for example, usually increases during fermentation owing to its liberation from catechin gallates (Lin et al., 1998), a point reflected by the high levels of GA in some of the Mauritian black teas reported here.

The antioxidant activities of fresh tea-leaves are reflected by the high TEAC and FRAP values which were largely accounted for by the high contents of (-)-EC, (-)-EGCG and (-)-EGC, GA and procyanidin dimers B1 and B2. The calculated FRAP values of the Mauritian black teas are consistent with literature values (Benzie and Szeto 1999). However, Langley-Evans (2000) reported observed FRAP value of 683 ± 11 μmol/g tea for black bagged tea without addition of milk and that green tea infusates had over twice the antioxidant potential of black teas.

Tea antioxidants with a greater number of phenolic hydroxyl groups have greater antioxidant power, i.e. (-)-EGCG 8 groups) > (-)-ECG (7 groups) > GC (6 groups) > (-)-EGC (6 groups) > (-)-EC (5 groups) (Matsuzaki and Hara, 1985; Wiseman et al., 1997; Rice-Evans and Miller, 1998; Lien et al., 1998) which is consistent with observed antioxidant activity correlation inthis study: (-)-EGCG > (-)-ECG > (-)-EC = GA > (-)-EGC in the tea infusate. Organic extracts of the black teas gave the antioxidant correlation of (-)-EGC > procyanidin dimer B1 > (-)-EC = GA > (-)-EGCG > procyanidin dimer B2. However the antioxidant capacities have a different order depending on the mode with which the antioxidant index is determine. Gardner et al., (1998) suggested the antioxidant activity profile: (-)-EGCG > (-)-ECG > (-)-EC > (-)-EGC > (+)-C > GA based on the use of the Fremy's radical (potassium nitrosodisulphonate) as the oxidant in an aqueous medium. However, use of galvanoxyl as oxidizing agent in ethanol produced a different order: (-)-EGCG > (-)-ECG > GA > (-)-EC = (+)-C > (-)-EGC. Thus ranking of antioxidant activity is system-dependent. Indeed the area of *in vitro* antioxidant characterization continue to receive wide attention such that it is now being suggested that use of a mix of methods would present a logical approach in seeking data to support antioxidant potencies (Aruoma 2003, Schlesier et al 2002).

A longitudinal study addressing the relation between tea intake and the risk of first incident of myocardial infarction in a population-based cohort of men and women aged 55 and above has reported that an "increased intake of tea and flavonoids may contribute to the primary prevention of ischemic heart disease" (Geleijnse et al., 2002). The potential for all types of Mauritian teas to contribute significantly to the dietary intake of antioxidant power is high. Assuming average tea consumption in Mauritius to be 3 cups (1.5% w/v) per day, the calculated total phenols intake from the tea infusates will be approximately 0.64 g/day, which is very close to the 1 g intake from the USA diet (Kühnau, 1976). Thus Mauritian black teas and fresh tea leaves represent excellent sources of polyphenolic compounds and they exhibit important antioxidant activities. The

higher levels of polyphenolic compound and greater antioxidant activities in fresh tea leaves indicate possible degradation of antioxidant constituents during tea processing. Further studies are being undertaken to determine absorptive and metabolic profiles of these teas as well as to evaluate their neuroprotective benefits (Aruoma, 2002).

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4. ASSESSMENT OF ANTIOXIDANT AND PROOXIDANT CAPACITIES OF MAURITIAN TEAS USING HOCl, HYDROXYL SCAVENGING AND Cu-PHENANTHROLINE ASSAYS

Our results on Mauritian teas indicate important antioxidant efficacies of the extracts (assayed by TEAC and FRAP methods) associated with high levels of phenolics. We report here additional antioxidant data confirming previously obtained results, thereby setting the stage for further studies on the prophylactic potentiality of tea infusates. Antioxidant activities were therefore assessed using the hypochlorous scavenging and hydroxyl scavenging assays. Furthermore the prooxidant activities of the tea infusates were also determined using the copper-phenanthroline assay.

1. Hypochlorous Acid Scavenging Assay

The assay was adapted from Weiss et al., (1982) and is based on the ability of the tea infusates to scavenge the hypochlorous acid radical thereby preventing the oxidation of the β-amino acid taurine. For this assay, 600 μM HOCl was prepared immediately before use by adjusting a solution of NaOCl, pH 7.4, to an absorbance of 0.210 at 292 nm assuming an extinction coefficient of 350 $M^{-1}cm^{-1}$.The reaction mixture of the assay contained 100 μl taurine (150 mM), 100 μl diluted extract, 100 μl HOCl made to 1 ml with phosphate buffer saline. Reaction of the chlorinating species HOCl with taurine results in a stable taurine chloramines complex as follows:

$$HOCl + H_2N\text{-}CH_2CH_2SO_3H \longrightarrow ClNH\text{-}CH_2CH_2SO_3H + H_2O$$

At the end of an incubation period of 10 mins at room temperature in screw-cap tubes, formation of taurine chloramines was assayed by addition of 10 μl KI (2M) and absorbance was read at 350 nm. A yellow coloration was developed due to oxidation of I^- ions to I_2 by the taurine complex. Analyses were made in triplicate and results expressed in IC_{50} (g dry mass/L).

Hypochlorous acid is a strong reactive oxygen species produced in organisms by oxidation of Cl^- ions at sites of inflammation by the neutrophil enzyme myeloperoxidase. In this assay the HOCl oxidizes taurine to taurine chloramines which is a stable oxidant complex. A HOCl scavenger inhibits the oxidation of taurine by this species and resulted in less oxidized I_2. Mauritian black tea infusates exhibited protective activity as shown in Table 1. The activity was in the following decreasing order, as indicated by the increasing IC_{50} value, for the black tea: Ouvagalia > Extra = Buccaneer's Choice > Red Label = Corson > Black Label > La Flora > 3-Pavillions > Chartreuse. Fresh tea leaves demonstrated higher HOCl scavenging capacity with a lowest IC_{50} value of 0.18 g dry mass/L.

Linear regression analysis (Table 2) shows strong negative correlation with total phenols (r = -0.98), EGCG (r = -0.93), EGC (r = -0.90), total flavonoids (r = -0.89), myricetin (r = - 0.89) and EC (r = -0.89) and IC_{50} values obtained for HOCl scavenging capacities of tea infusates.

Table 1: Hypochlorous acid scavenging capacities of Mauritian black tea infusates

BLACK TEA	Brands	Conc (g/L)	Abs (350 nm)	% Taurine chloramine	% HOCl Scavenging by extracts	IC50 (g/L)
Bois Chéri tea	Red Label	0.75	0.12	7.57	92.43	0.39
		0.38	0.78	47.99	52.01	
		0.19	0.99	61.11	38.89	
		0.13	1.40	86.50	13.50	
	Black Label	0.83	0.23	13.25	86.75	0.42
		0.42	0.62	35.53	64.47	
		0.21	1.03	59.22	40.78	
		0.14	1.30	74.84	25.16	
	3-Pavillions	0.76	0.28	16.14	83.86	0.44
		0.38	0.94	53.93	46.07	
		0.19	1.25	71.72	28.28	
		0.13	1.48	85.17	14.83	
	Extra	0.75	0.16	9.08	90.92	0.38
		0.37	0.80	45.83	54.17	
		0.19	1.10	63.12	36.88	
		0.12	1.35	77.64	22.36	
	Ouvagalia	0.76	0.10	6.15	93.85	**0.36**
		0.38	0.53	32.87	67.13	
		0.19	1.01	62.18	37.82	
		0.13	1.29	79.65	20.35	
Chartreuse tea	-	0.75	0.36	22.05	77.95	0.48
		0.38	1.01	62.16	37.84	
		0.19	1.26	77.67	22.33	
		0.13	1.35	83.39	16.61	
La Flora tea	-	0.75	0.30	17.35	82.65	0.43
		0.38	1.03	59.41	40.59	
		0.19	0.95	54.77	45.23	
		0.13	1.47	84.69	15.31	
Corson tea	-	0.56	0.50	28.75	71.25	0.39
		0.28	1.12	64.43	35.57	
		0.14	1.43	81.99	18.01	
		0.09	1.41	80.99	19.01	
Buccaneer's Choice	-	0.75	0.18	10.61	89.39	0.38
		0.37	0.75	42.86	57.14	
		0.19	0.96	55.21	44.79	
		0.12	1.34	77.05	22.95	
FRESH TEA LEAVES	-	0.27	0.41	25.57	74.43	**0.18**
		0.13	1.05	64.65	35.35	
		0.07	1.34	82.75	17.25	
		0.04	1.41	87.26	12.74	

Table 2: Correlation coefficients between phenolic levels, HOCl scavenging capacities and hydroxyl scavenging capacities of Mauritian black tea infusates

Polyphenols	HOCl Scavenging capacities (IC50)	Hydroxyl Scavenging capacities (IC50)
(+)-Catechin	-0.76	-0.49
(-)-EC	-0.86	-0.66
(-)-EGC	-0.90	-0.50
(-)-EGCG	-0.93	-0.52
(-)-ECG	0.23	-0.09
Procyanidin dimer B1	-0.05	0.03
Procyanidin dimer B2	-0.19	-0.07
Gallic Acid	-0.11	-0.49
Myricetin	-0.89	-0.74
Quercetin	-0.07	-0.41
Kaempferol	-0.008	-0.19
Total Phenols	-0.98	-0.73
Total Flavonoids	-0.89	-0.65
Total Proanthocyanidins	-0.71	-0.81

2: Hydroxyl Scavenging Assay (Deoxyribose assay)

The hydroxyl scavenging propencities of the tea infusates were investigated using the deoxyribose assay (Halliwell et al., 1987, Aruoma, 1991). The reaction mixture contained in a final 1 ml volume of the following reagents: EDTA-Na (150 μM), $FeCl_3$ (100 μM), vitamin C (100 μM), H_2O_2 (1 mM), phosphate buffer (20 mM) at pH 7.4, deoxyribose sugar (50 mM) and diluted extracts. After incubation at 37° C for 1 hr, 1 ml of HCl (25 %, v/v) and 1 ml TBA (1 %, w/v) were added, and the mixture was heated in a water bath at 80° C for 20 mins. The absorbance of the pink coloration developed was measured at 532 nm and results expressed as IC 50 g dry mass/L from three determinations.

In this assay the hydroxyl radical is generated in the following reactions:

$$Fe^{2+}\text{-EDTA} + O_2 \leftrightarrow Fe^{3+}\text{-EDTA} + O_2\text{-}$$
$$2O_2^{\cdot} + 2H^+ \longrightarrow H_2O_2 + O_2$$
$$Fe^{2+}\text{-EDTA} + H_2O_2 \longrightarrow OH^- + OH^{\cdot} + Fe^{3+}\text{-EDTA}$$

The sugar deoxyribose is degraded on exposure to hydroxyl radicals generated by the above Fenton reactions. When the resulting complex mixture of products is heated under acid conditions, malondialdehyde (MDA) is formed and is detected by its ability to react with thiobarbituirc acid (TBA) to form a pink chromogen.

$$OH^{\cdot} + \text{deoxyribose} \longrightarrow \text{fragments} \xrightarrow{\text{heat with TBA plus acid}} \text{MDA}$$
$$2\text{TBA} + \text{MDA} \longrightarrow \text{Chromogen}$$

Any other molecule added to the reaction mixture capable of reacting with $OH^{\cdot}$ should compete with deoxyribose for $OH^{\cdot}$ to an extent depending on the concentration relative to deoxyribose. The tea infusates tested appeared to be a scavenger of the hydroxyl radical generated in the reaction. The reaction was dose dependant and capacities of the infusates decreases in the following order: Ouvagalia > Buccaneer's Choice > Red Label > Extra > Corson > La Flora > 3-Pavillions > Chartreuse. Black show probable prooxidant activity as a decreasing scavenging capacity was observed with increasing concentration (Table 3). This is possible since some compounds are capable of redox cycling the metal ion required for hydroxyl generation, thus increasing the radical production, exhibiting proxidant activity. Fresh tea leaves demonstrated highest protection against hydroxyl attack on deoxyribose radical as a lowest IC_{50} value of 0.65 g dry weight/L was measured for hydroxyl scavenging capacity.

Linear regression analysis (Table 2) shows strong negative correlation with total proanthocyanidins (r = -0.81), myricetin (r = -0.74), total phenols (r = -0.73) and EC (r = -0.66) and IC_{50} values obtained for $OH^{\cdot}$ scavenging capacities of tea infusates.

3: The Copper-Phenanthroline assay (Prooxidant assays)

The copper-phenantholine assay, adapted from Gutteridge and Halliwell, 1982 and Aruoma et al., 1992, measures damage caused to DNA base by hydroxyl radicals. The reaction mixture contained in a final volume of 1.2 ml, the following reagents in order of addition indicated: 100 µl 1, 10-phenanthroline (1.8 mM stock solution made up in water having initially dissolved he crystals in 50 µl ethanol), 480 µl copper (II) chloride (250 µM), 300 ul DNA (1.68mg/ml) and 120 µl KH2PO4-KOH buffer at pH 7.4 (100 mM). Extracts (100 µl) were added to initiate the reaction. After an incubation period of 1 hr at 37° C, reaction was stopped by addition of 100 µl EDTA (0.1 M). DNA damage was assessed by adding 1 ml HCl (25 % v/v) followed by 1 ml TBA (1%, w/v) incubated at 80° C for 20 minutes and the pink chromogen so-formed was read at 532 nm. Results were expressed as % DNA damage and hydroxyl scavenging capacities from three determinations.

Tea infusates show slight prooxidant activities against DNA damage by hydroxyl radicals in a dose dependant system. It was observed that on average prooxidant activity of Mauritian black tea infusates starts at 9 g dry mass/L (where DNA damage > 30 %) and maximum activity is reached at nearly 30g/L, which equates to about 4 cups of tea/day (assuming a serving is 2g/250 ml). However, there still remains the issue of bioavailability. Moreover, as some of the assays are conducted in non-physiological pH values, it is difficult to extrapolate the results to physiological environment. The proof of bioefficacy must emanate from application of reliable in vivo models where markers of baseline oxidative damage are examined from the standpoint of how they are affected by changes in diet or by antioxidant supplements.

Table 3: Hydroxyl radical scavenging capacities of Mauritian black tea infusates

BLACK TEA	Brands	Conc (g/L)	Abs (532 nm)	% Deoxyribose Damage	% $OH^{\cdot}$ Scavenging	IC50 (g/L)
Bois Chéri Tea	Red Label	0.75	0.52	68.75	31.25	
		0.38	0.56	74.47	25.53	1.01
		0.19	0.59	83.59	16.41	
		0.13	0.61	90.38	9.62	
	Black Label	0.83	0.46	70.50	30.50	-
		0.42	0.48	70.34	29.66	
		0.21	0.45	65.92	34.08	
		0.14	0.46	66.46	33.54	
	3-Pavillions	0.76	0.56	72.67	27.33	
		0.38	0.58	87.34	12.66	
		0.28	0.60	92.44	7.56	1.47
		0.19	0.64	95.86	4.14	
	Ouvagalia	0.78	0.47	47.67	52.33	
		0.38	0.49	71.21	28.79	**0.70**
		0.19	0.55	80.39	19.61	
		0.13	0.57	83.11	16.89	
	Extra	0.75	0.48	71.68	28.32	
		0.37	0.53	78.96	21.04	
		0.19	0.59	88.93	11.07	1.18
		0.13	0.61	94.40	5.60	
Chartreuse Tea	-	0.75	0.50	76.88	23.12	
		0.38	0.52	89.42	10.58	1.72
		0.28	0.56	93.42	6.58	
		0.19	0.61	96.11	3.89	
La Flora Tea	-	0.75	0.49	70.31	29.69	
		0.38	0.52	83.74	16.26	1.26
		0.28	0.56	89.80	10.20	
		0.19	0.59	92.82	7.18	
Corson Tea	-	0.56	0.47	80.16	19.84	1.24
		0.28	0.54	84.30	15.70	
		0.21	0.55	88.77	11.23	
		0.14	0.59	96.21	3.79	
Buccaneer's Choice	-	0.74	0.35	51.45	48.55	0.73
		0.37	0.51	74.51	25.49	
		0.19	0.56	81.50	18.50	
		0.12	0.58	84.66	15.34	
FRESH TEA LEAVES	-	0.79	0.53	35.48	64.52	
		0.53	0.58	64.74	35.26	**0.65**
		0.27	0.64	78.39	21.61	
		0.13	0.67	89.01	10.99	

Table 4: Percentage DNA damage and Hydroxyl scavenging capacities as assessed by the copper-phenanthroline system of the Mauritian black tea infusates

BLACK TEA	Brands	Conc (g/L)	Abs (532 nm)	% DNA damage	% OH Scavenging	Conc (g/L) at maximum DNA damage	Corresponding g/day	Equivalent Number of cups/day
Bois Chèri tea	Red Label	37.52	0.19	59.90	40.10	30.42	7.61	4
		18.76	0.15	48.34	51.66			
		12.51	0.14	45.56	54.44			
		9.38	0.12	33.99	66.01			
	Black Label	41.54	0.11	48.13	51.88	36.75	9.19	5
		20.77	0.09	36.64	63.36			
		13.85	0.08	33.86	66.14			
		10.38	0.07	22.43	77.57			
	Ouvagalia	38.10	0.12	62.73	37.27	32.15	8.04	4
		19.05	0.10	53.02	46.98			
		12.70	0.09	41.49	58.51			
		9.52	0.08	33.43	66.57			
	Extra	37.38	0.10	44.85	55.15	31.65	7.91	4
		18.69	0.09	40.93	59.07			
		12.46	0.08	29.36	70.64			
		9.35	0.07	20.95	79.05			
	3-Pavillion	38.10	0.15	49.68	50.32	29.04	7.26	4
		19.05	0.14	44.79	55.21			
		12.70	0.13	38.68	61.32			
		9.52	0.12	32.46	67.54			
Chartreuse tea	-	37.74	0.12	47.52	52.48	29.13	7.28	4
		18.87	0.10	41.23	58.77			
		12.58	0.09	37.12	62.88			
		9.43	0.07	30.29	69.71			
La Flora tea	-	37.74	0.16	50.89	49.11	28.56	7.14	4
		18.87	0.15	45.32	54.68			
		12.58	0.13	41.97	58.03			
		9.43	0.11	32.51	67.49			
Corson tea	-	28.01	0.16	64.53	35.47	29.87	7.47	4
		14.01	0.13	45.60	54.40			
		9.34	0.10	33.10	66.90			
		7.00	0.09	28.93	71.07			
Buccaneer's Choice	-	37.17	0.12	62.61	37.39	30.03	7.51	4
		18.59	0.11	56.30	43.70			
		12.39	0.10	46.45	53.55			
		9.29	0.09	31.96	68.04			
FRESH TEA LEAVES	-	13.35	0.12	33.99	66.01	19.94	4.985	2
		6.68	0.10	22.33	77.67			
		4.45	0.09	14.34	85.66			
		3.34	0.08	8.63	91.37			

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