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Original article

Evaluation of phenolic-linked anti-hyperglycemic properties of tropical Brazilian fruits for potential management of early stages Type 2 diabetes

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Summary

Introduction – **Tropical colored fruits rich in dietary phenolics and high antioxidant capacity have diverse human health benefits and can be targeted for improving consumer health, especially to counter chronic oxidative stress-linked non-communicable diseases (NCDs). The aim of this study was to evaluate phenolic bioactives and antioxidant-linked functionalities of several underutilized tropical colored fruits from Brazil for their potential relevance in diet-linked hyperglycemia control of early stages Type 2 diabetes.** *Materials and methods* – **Aqueous and ethanolic extracts from peel, pulp, and seeds of seven important colored tropical fruits from North East Brazil were evaluated for total soluble phenolic content, total antioxidant activity and glycemic control relevant α-amylase, and α-glucosidase inhibitory activity using *in vitro* assays.** *Results and discussion* – **Among all fruits, aqueous extracts of jaboticaba (*Myrciaria cauliflora*) had higher total soluble phenolic content, higher α-amylase and α-glucosidase inhibitory activities. Similarly, significant phenolic antioxidant-linked functionalities were also observed in all other evaluated fruits and showed potential to incorporate them either as a whole fruit or as functional ingredients as part of overall dietary support against early stages hyperglycemia linked to Type 2 diabetes and associated complications.** *Conclusion* – **This *in vitro* study provides significant insights and scientific rationale for potential utilization of these colored tropical fruits as ingredients for designing dietary support against hyperglycemia-linked early stages of Type 2 diabetes.**

Keywords

Jaboticaba, *Myrciaria cauliflora*, antioxidant activity, bioactive compounds, enzyme inhibitors, fruit quality, health and nutrition

Résumé

Évaluation des propriétés anti-hyperglycémiques liées aux composés phénoliques des fruits tropicaux brésiliens pour la gestion potentielle des stades précoces de diabète de Type 2.

Significance of this study

What is already known on this subject?

- Tropical colored fruits are rich in phenolic bioactives and dietary antioxidants.

What are the new findings?

- Significant phenolic antioxidant-linked anti-hyperglycemic properties were observed in tropical Brazilian fruits.

What is the expected impact on horticulture?

- Colored tropical fruits can be targeted to develop functional food ingredients for health, specifically to manage early stages of Type 2 diabetes.

Introduction – **Les fruits tropicaux colorés, riches en composés phénoliques alimentaires et à forte capacité anti-oxydante, présentent divers bénéfices pour la santé humaine et peuvent être ciblés pour améliorer la santé du consommateur, en particulier pour lutter contre les maladies non transmissibles liées au stress oxydant chronique. Le but de cette étude était d'évaluer la pertinence potentielle des composés phénoliques bioactifs et les fonctionnalités liées aux anti-oxydants de plusieurs fruits tropicaux colorés sous-utilisés du Brésil, dans le contrôle de l'hyperglycémie par régime alimentaire des stades précoces du diabète de Type 2.** *Matériels et méthodes* – **Les extraits aqueux et éthanoliques des pelures, pulpes et graines de sept fruits tropicaux colorés importants du nord-est du Brésil ont été analysés pour déterminer leur teneur totale en phénols solubles, leur activité anti-oxydante totale et leur activité inhibitrice de l'α-amylase et de l'α-glucosidase, en utilisant des tests *in vitro*.** *Résultats et discussion* – **Parmi tous les fruits, les extraits aqueux de jaboticaba (*Myrciaria cauliflora*) ont présenté une teneur en composés phénoliques solubles totaux supérieure, des activités inhibitrices de l'α-amylase et de l'α-glucosidase plus élevées. D'intéressantes fonctionnalités phénoliques liées aux anti-oxydants ont également été observées pour tous les autres fruits évalués et ont mis en valeur leur potentiel, soit comme fruit entier, soit comme ingrédients fonctionnels dans le cadre du soutien alimentaire global**

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contre l'hyperglycémie aux stades précoces du diabète de Type 2 et les complications associées. *Conclusion* **– Cette étude *in vitro* fournit des informations significatives et une justification scientifique pour l'utilisation potentielle des fruits tropicaux colorés en tant qu'ingrédients pour la conception d'un support alimentaire contre l'hyperglycémie aux stades précoces du diabète de Type 2.**

Mots-clés
Jaboticaba, *Myrciaria cauliflora*, activité anti-oxydante, composés bioactifs, inhibiteurs d'enzyme, qualité du fruit, santé et nutrition

Introduction

Diabetes mellitus is a complex metabolic disorder resulting either from insulin insufficiency or insulin dysfunction (Green *et al.*, 2003). Type 2 diabetes mellitus (T2DM) is the most common form of diabetes (90%) and remains as one of the most important health risks to the wider global population both in developed and in developing countries (WHO, 2016). Further this non-communicable chronic disease (NCD) imposes a significant economic and social burden to the population worldwide (Shaw *et al.*, 2010). The health risks of T2DM is multifactorial and leads to several long term macro- and micro-vascular complications such as substantial morbidity, mortality, and organ failures (Shaw *et al.*, 2010). Thus to manage the epidemic of T2DM and its associated health risks, an integrated system of science based solutions combining diet, lifestyle, and pharmacological strategies is essential. One common factor involved in the pathogenesis of T2DM and its associated health risks is the chronic state of cellular oxidative stress and the partial or complete breakdown of cellular redox balance (Wild *et al.*, 2004). Such chronic oxidative stress not only just complicates further insulin production and function but also leads to the breakdown of other metabolic regulations. The oxidative stress-induced T2DM is usually accompanied by increased production of free radicals or impaired antioxidant defense systems. Implication of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen free-radical generation but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired glutathione metabolism, alteration in antioxidant enzymes, lipid peroxides formation, and decreased ascorbic acid levels (Grattagliano *et al.*, 2008). So for the long-term and effective management of T2DM including early developmental stages of this disease, it is important to develop different strategies involving antioxidant defense systems and maintenance of glucose and redox homeostasis at cellular level and plant foods can be part of the solution.

Plant-based diet rich in natural antioxidants and other bioactives with specific function to improve glucose metabolism potentially provide safe and cost-effective solutions to the dietary management of T2DM. Among plant-based foods, fruits are enriched with diverse bioactive molecules and when consumed as diet provide specific protection against chronic oxidative stress-associated diseases such as T2DM (Bisbal *et al.*, 2010). Many natural substances present in fruits such as triterpenes, flavonoids, coumarins, saponins, alkaloids, and polysaccharides indicated anti-diabetic potential and have been reported as beneficial targets for dietary management of T2DM (Vikrant *et al.*, 2001). As the cost of pharmacological treatments is rising rapidly and becoming less affordable to a wider population globally, the interest in edible and bioactive enriched colored tropical fruits for the dietary management of NCDs including T2DM has potential (Shaw *et al.*, 2010). Various extracts obtained from several fruits were reported to possess anti-diabetic properties (Prince *et al.*, 2003).

A good strategy for dietary management of T2DM is the inhibition of enzymes that hydrolyze dietary polysaccharides in the gut. These can significantly reduce the rise of blood sugar levels (glycemic index) after a meal by reducing the absorption of monosaccharides by the entrocytes of the small intestine (McDougall and Stewart, 2005). Enzymes that hydrolyze dietary polysaccharides and modulate gut absorption are pancreatic α-amylase and intestinal α-glucosidase (McDougall *et al.*, 2005). It has been widely reported that digestive enzymes, such as α-amylase and α-glucosidase, can be inhibited by phenolic bioactives from plant-based foods (Hanhineva *et al.*, 2010; McDougall *et al.*, 2005). The inhibition of these digestive enzymes by dietary phenolics offers an exciting mechanism for delivering some health benefits in early stages of the disease attributed to a diet rich in fruits and vegetables and especially its potential role to control hyperglycemia-linked progression of T2DM (Sarkar and Shetty, 2014). However, a common side effect of these enzyme inhibitory pharmacological drugs like acarbose and related drugs is the excessive inhibition of pancreatic α-amylase, which can result in abdominal distention, flatulence, and diarrhea (Holman *et al.*, 1999). Phenolic bioactives present in colored fruits have shown high inhibitory potential against α-glucosidases with potential of minimal side effects from lowered α-amylase inhibitory activity (Kwon *et al.*, 2006; McDougall *et al.*, 2005). The antioxidant capacity of these phenolic bioactives also provide additional protection against chronic oxidative stress associated with T2DM (Sarkar and Shetty, 2014).

Based on this above scientific rationale, the major aim of this study was to determine phenolic antioxidant-linked functionalities of seven different red and black colored Brazilian fruits of NE tropical region such as jambolan (*Syzygium cumini*), kaki (*Diospyros kaki* L.), jaboticaba (*Myrciaria cauliflora*), red grape (*Vitis vinifera* L.), black grape (*Euterpe edulis*), plum (*Prunus domestica*), Surinam cherry (*Eugenia uniflora* L.) for their potential relevance and benefits in targeting the dietary management of early stages T2DM. Previous research with these fruits have shown their potential health benefits (Table 1), but there is a gap in understanding about the specific roles of different parts (pulp, peel and seeds) of these fruits for potentially improving antioxidant-linked glucose metabolism. Thus the objective of this study was to determine the phenolic bioactive-linked functionalities of different parts of these fruits targeting diet-based glycemic control in the management of early stages T2DM using *in vitro* assay models. The rapid screening of T2DM relevant dietary benefits of targeted tropical fruits through *in vitro* assay models provides the biochemical rationale to include either whole fruits or specific parts (pulp, peel, and seed) of these fruits as dietary ingredients to support further animal, epidemiological or clinical studies against hyperglycemia linked to early stages of T2DM and associated complications.

Table 1. Details of the tropical Brazilian fruits in study, their origins, main production areas, and the reported health benefits.

Common names	Scientific names	Botanical families	Origins and major regions of production	Health benefits
Jambolan plum, Java plum, Jambul	*Syzygium cumini*	Myrtaceae	India and Northeast of Brazil	Antidiabetic effects (Gordon *et al.*, 2011)
Persimmon	*Diospyros kaki*	Ebenaceae	China, Japan and South of Brazil	Protective effect against cardiovascular disease; decreased atherosclerotic lesions (Gorinstein *et al.*, 2011)
Plum	*Prunus domestica*	Rosaceae	China and Asia	Reduces the levels of total cholesterol and its LDL fraction, blood serum triglycerol and homocystein concentrations; protective action on blood vessels (Arion *et al.*, 2014)
Red Grape	*Vinis vinifera* L.	Vitaceae	Mediterranean region and North of Asia	Anti-inflammation activities, cardioprotective and antimicrobial activities (Xia *et al.*, 2010)
Black Grape	*Vitis labrusca* L.	Vitaceae	Chile, North of America and Andes	Anti-cancer; anti-inflammatory activity, antiulcerative, antiarthritic, anti-viral, prevent skin aging, scavenge free radicals and inhibit UV-radiation (Lakshmi *et al.*, 2013)
Jabuticaba, Jaboticaba	*Plinia trunciflora*	Myrtaceae	Northeast and Southeast of Brazil	Anti-inflammatory, against asthma and anti-diarrhea (Reynertson *et al.*, 2006)
Surinam cherry, Brazilian cherry	*Eugenia uniflora* L.	Myrtaceae	Atlantic forest of Brazil, South America, Africa and Central America	Anti-diarrheic, diuretic, anti-rheumatic, anti-febrile and anti-diabetic (Oliveira *et al.*, 2006)

Materials and methods

Materials

Seven fruits used in this study were purchased from Central Market in the city of Aracaju, State of Sergipe in Northeastern Brazil. The jambolan, persimmon, jaboticaba, red grape, black grape, plum, Surinam cherry fruits used for this study were at the ripened stage of maturation. The porcine pancreatic α-amylase (EC 3.2.1.1), α-glucosidase (EC 3.2.1.20), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO).

Pulp, peel, and seed dehydration

Each part of the fruits such as pulp, peel, and seeds were dried in a lyophilizer (Make Christ, model A-1-4 LSC). The pulp portion of fruits were sliced into pieces of about 5 mm thickness and placed in aluminum trays while the peels and seeds were separated manually and macerated, and then were frozen in ultrafreezer (-80 °C for 24 h). After freezing, the samples were placed in the lyophilizer for drying. The freezing was carried out under a total pressure of 1.3×10^{-1} mbar and at a temperature of -30 °C in a drying chamber. When the samples were dried completely, they were removed from the lyophilizer, packed in foil bags under vacuum, and kept under refrigeration for the biochemical analysis.

Ethanolic and aqueous extracts

Ten gram samples of dehydrated pulp, peel, and seeds were weighed and homogenized in 100 mL of 12% ethanol. These were maintained under stirring for 20 min at a temperature of 75 °C while for aqueous extraction 10 g of each sample was homogenized in 100 mL distilled water and maintained under stirring for 30 min at 60 ± 5 °C. The aqueous and ethanolic extracts were centrifuged at 8,500 rpm for 30 min in the centrifuge tubes of 30 mL. The supernatant was separated and subjected to a further centrifugation (8,500 rpm for 15 min) and this time, 2.0 mL Eppendorf tubes were used in order to remove small particles still present in the extracts. The final extracts were stored under refrigeration (4 °C) for biochemical analysis.

Total soluble phenolic content

The total soluble phenolic content of the targeted seven different fruits was determined by using a method modified by Shetty *et al.* (1995). Briefly, 0.5 mL of extracts was taken in a test tube and mixed with 0.5 mL of 95% ethanol and 5 mL distilled water. Each sample was mixed after adding 0.5 mL of 50% (v/v) Folin-Ciocalteu reagent. After 5 min, 1 mL of 5% Na_2CO_3 was added to the reaction mixture and vortexed before incubation in dark for 1 h. Using a spectrophotometer (Genesys UV-visible, Milton Roy, Inc., Rochester, NY) the absorbance was read at 725 nm. Standard curve was prepared using different concentrations (25–300 µL) of gallic acid solution. The results were expressed as micrograms of gallic acid equivalent (GAE) per gram of pulp, peel, and seed on dry weight (DW) basis.

Total antioxidant activity

The total antioxidant activity was determined by the DPPH radical (2,2–Diphenyl-1-picrilhydrazil) scavenging method modified from Kwon *et al.* (2006). A 250 µL aliquot of the sample extract was mixed with 1.25 mL DPPH. The absorbance was read at 517 nm using a spectrophotometer (Genesys UV-visible, Milton Roy, Inc.) after 5 min. The readings were compared with the controls, containing 95% ethanol instead of the sample extract. The percentage inhibition was calculated by Equation 1:

$$(\%)\ \text{Inhibition} = \frac{(\Delta Abscontrol - \Delta Absextract)}{\Delta Abscontrol} \times 100 \qquad \text{[Eq. 1]}$$

where $Abs_{control}$ is the control absorbance and $Abs_{extract}$ is the extract absorbance.

Alpha-amylase inhibition assay

The α-amylase inhibitory activity of different parts (pulp, peel, seeds) of fruits was determined according to an assay described in the Worthington Enzyme Manual (1993) and used widely (Kwon *et al.*, 2006). Porcine pancreatic α-amylase (1 U liberates 1.0 mg maltose from starch in 3 min at pH 6.9 at 20 °C) was purchased from Sigma Chemical Co. A total of 500 µL of sample extract and 500 µL of 0.02 M

sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase solution (0.5 mg mL^{-1}) were incubated at 25 °C for 10 min. After pre-incubation, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube for 10 min. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 10 min and cooled to room temperature. The reaction mixture was diluted after addition of 15 mL distilled water and absorbance was measured at 540 nm using a spectrophotometer (Genesys UV-visible, Milton Roy, Inc.). The readings were compared with the control containing buffer instead of the sample extract. The effect of different doses (without dilution, half, and one-fifth dilution) was evaluated by measuring the readings for each of the dilution. The α-amylase inhibitory activity was expressed as percentage inhibition and was calculated according to Equation 2:

$$\% \text{ Inhibition} = \frac{[Abscontrol - (Absextract - Absblank)]}{Abscontrol} \times 100 \quad \text{[Eq. 2]}$$

where, $Abs_{control}$ is the control absorbance, Abs_{blank} is the blank absorbance and $Abs_{extract}$ *is* the extract absorbance.

Alpha-glucosidase inhibition assay

A mixture containing 50 µL of the extract, 50 µL of 0.1 M potassium phosphate (pH 6.9) buffer solution and 100 µL of α-glucosidase (1 U mL^{-1}) were incubated in polystyrene microplates with 96 wells (Molecular Device, CA) at 25 °C for 10 min. After pre-incubation, 50 µL of solution of (5 mM) *p*-nitrophenyl-α-D-glucopyranoside was added, and then the first baseline absorbance reading was taken, followed by another reading after 5 min incubation. The absorbance before and after incubation was measured at 405 nm in a microplate spectrophotometer (Thermoplate Reader, Molecular Device, USA) and compared with the control in which 50 µL of the extract was replaced with 50 µL potassium phosphate buffer (0.1 M pH 6.9). The effect of different doses (10, 25 and 50 µL) was evaluated by measuring the readings for each of the dilution. The inhibitory activity of α-glucosidase was expressed in percentage of inhibition and calculated according to Equation 3:

$$(\%) \text{ Inhibition} = \frac{(\Delta Abscontrol - \Delta Absextract)}{\Delta Abscontrol} \times 100 \quad \text{[Eq. 3]}$$

where, $\Delta Abs_{control}$ is the difference in absorbance reading measured at 0 and 5 min in control samples, and the $\Delta Abs_{extract}$ is the difference in absorbance reading measured at 0 and 5 min in extracts.

Statistical analysis

All analyses were performed in triplicate, except the inhibition assay of α-glucosidase and α-amylase, which were determined in octoplicate (8×). All results are presented as mean ± standard deviation values. For comparison of means, analysis of variance (ANOVA) and Tukey's test were performed using the software Assistat 7.7 beta version. The linear correlations between different variables were determined by using the Origin 8.0 software. The level of significance for the difference between means was 5% ($P < 0.05$).

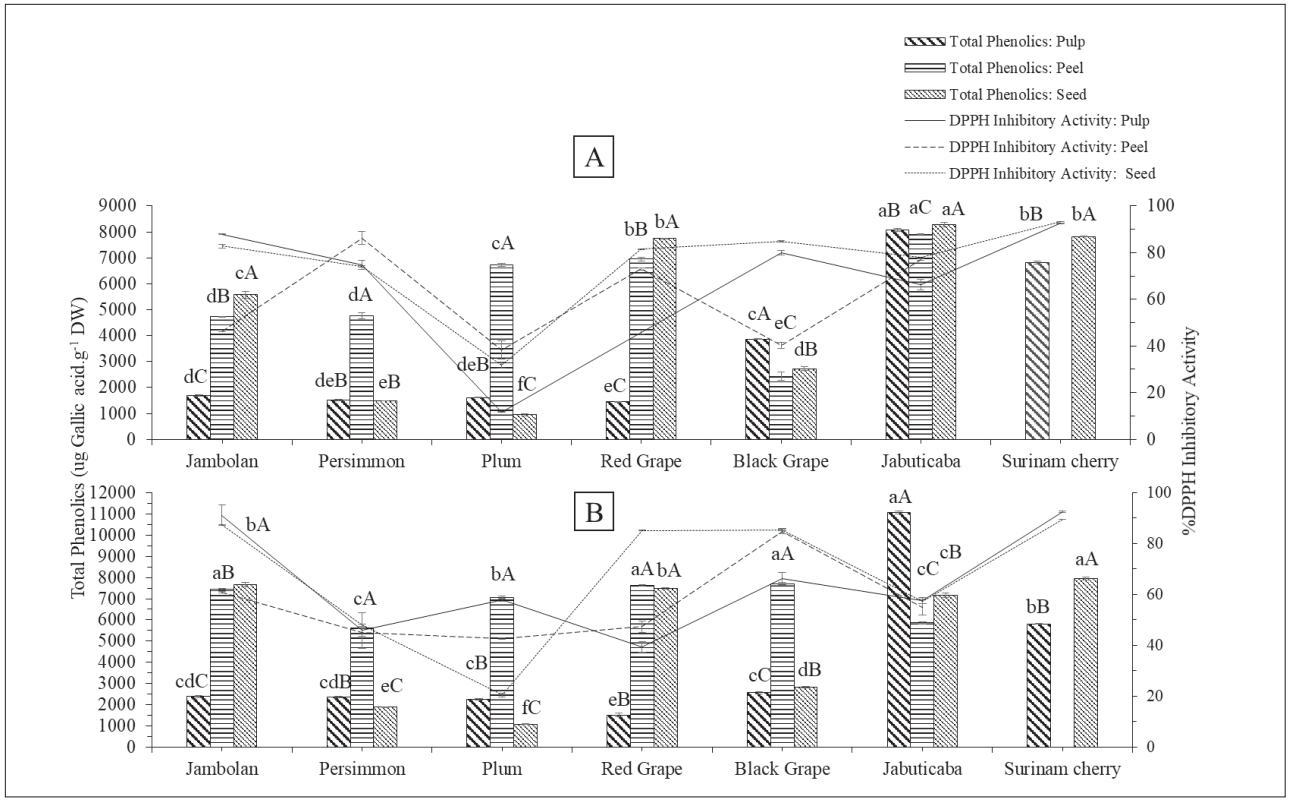

Figure 1. Total soluble phenolic content and total antioxidant activity (DPPH radical scavenging inhibition) of the pulp, peel and seeds of red and black colored fruits. A: Extracts using 12% ethanol, B: Extracts using water (60 ± 5°C by 30 min).
* Mean values with different small-case letters are significantly different ($P < 0.05$). Correlations are between total phenolic compounds content in all fruit pulps.
** Mean values with different capital letters are not significantly different ($P < 0.05$). Correlations are between total phenolic compounds of the pulp, peel and seed of each fruit (separately).

Results

Total soluble phenolic content and total antioxidant activity

Total soluble phenolic content present in different parts of these 7 fruits were determined. Overall, high total soluble phenolic content was observed in all analyzed fruits (Figure 1A). Among all fruits, jaboticaba had very high total soluble phenolic content in aqueous extracts of pulp, peel, and seeds (11,064.4, 5,859.5 and 7,167.3 µg GAE g^{-1} DW, respectively) followed by ethanolic extracts of the same fruit (8,075.1, 7,911.1 and 8,268.3 µg GAE g^{-1} DW, respectively). There were significant differences ($P<0.05$) in the total soluble phenolic content between pulp, peel, and seeds of each analyzed fruit. It is noteworthy that the peel of persimmon contained significantly higher concentration of soluble phenolic in both extracts (aqueous and ethanolic) when compared with pulp and seed extracts of the same fruit. Similarly, peels of jambolan, plum, and red grape also showed significantly higher total soluble phenolic content ($P<0.05$) when compared to their pulp extracts. Whereas black grapes and jaboticaba pulp had significantly higher total soluble phenolic content when compared to the peel extracts ($P<0.05$). The total soluble phenolic content was significantly ($P<0.05$) higher in jambolan, red grape, and Surinam cherry, respectively (7,665.8, 7,478.2 and 7,953.7 µg GAE g^{-1} DW) in seed extracts than their pulp extracts (2,381.3, 1,512.9 and 5,815.30 µg GAE g^{-1} DW).

Total antioxidant activity of all fruits measured by DPPH radical inhibition assay showed positive correlations with total soluble phenolic content in aqueous and ethanolic extracts of peel and seeds (0.86 and 0.70 in aqueous extracts, and 0.75 and 0.63 in ethanolic extracts, respectively) (Table 4). The total antioxidant activity of all fruits varied from 12 to 93% (Figure 1B), and among all fruits, Surinam cherry pulp and seed extracts showed the highest total antioxidant activity, respectively (92 and 90% in ethanolic extracts, 92 and 89% in aqueous extracts), followed by jambolan (88 and 83% in ethanolic extracts, 90 and 87% in aqueous extracts), and black grape (80 and 85% in ethanolic extracts, 66 and 86% in aqueous extracts). Except plum and persimmon, seed extracts of all other fruits showed higher antioxidant activity when compared with peel and pulp extracts of the same fruit.

Table 4. Pearson correlation coefficients (r) between the total phenolic content, antioxidant activity (DPPH), and α-amylase and α-glucosidase enzyme inhibitory activity of jambolan, persimmon, plum, red grape, black grape, jaboticaba, and Surinam cherry fruits.

Ethanolic extracts (12%)				
Fruit parts	Variables	DPPH	α-Amylase	α-Glucosidase
Pulp	Total phenolics	0.3826	-0.1168	-0.2007
	DPPH		-0.5977	-0.2512
	α-Amylase			-0.4698
	α-Glucosidase			
Peel	Total phenolics	0.7491	0.7251	0.8323*
	DPPH		0.4005	0.7097
	α-Amylase			0.6000
	α-Glucosidase			
Seed	Total phenolics	0.6259	0.1773	0.7561*
	DPPH		0.8040*	0.8206*
	α-Amylase			0.6026
	α-Glucosidase			
Aqueous extracts (60 ± 5 °C by 30 min)				
Fruit parts	Variables	DPPH	α-Amylase	α-Glucosidase
Pulp	Total phenolics	0.1456	0.6905	0.3242
	DPPH		-0.4973	0.2490
	α-Amylase			-0.0309
	α-Glucosidase			
Peel	Total phenolics	0.8658*	0.8494*	0.6727
	DPPH		0.6372	0.5104
	α-Amylase			0.9099**
	α-Glucosidase			
Seed	Total phenolics	0.7091	0.4165	0.7953*
	DPPH		0.7962*	0.7493
	α-Amylase			0.8123*
	α-Glucosidase			

In general data significance is at 5% level of probability ($P<0.05$).
* Significance between 1 and 5% level of probability ($0.01<P<0.05$).
** Significance at 1% level of probability ($P\leq0.01$).

Alpha-amylase enzyme inhibitory activity

Extracts of peel, pulp and seeds of all fruits showed low to high α-amylase inhibitory activity, with values ranging from 3% to 80% inhibition in undiluted sample. Like total soluble phenolic content, higher α-amylase inhibitory activity was also found in undiluted jaboticaba pulp and peel extracts (94 and 86%, in ethanolic extracts, 91 and 97%, in aqueous extracts) (Table 2). On the contrary jaboticaba seeds had lower α-amylase inhibitory activity (43 and 31% in aqueous and ethanolic extracts, respectively) when compared to seed extracts of other fruits. Overall ethanolic extracts had higher α-amylase inhibitory activity when compared to aqueous extracts. Significant dose responses for α-amylase inhibitory activity were observed in peel, pulp, and seed extracts of all fruits.

The aqueous peel extracts of fruits such as jambolan (91%), plum (81%), red grape (93%), and jaboticaba (97%) had significantly ($P<0.05$) higher inhibition of α-amylase than the pulp extracts of these fruits (42, 53, 70, and 91%, respectively) with average standard deviation of less than 1% in each case. The results also showed positive correlations between α-amylase inhibition and the total soluble phenolic content ($P<0.05$) with high value of Pearson correlation coefficient ($r=0.85$) ($P<0.05$) in aqueous peel extracts (Table 4). Similarly, when comparing the data of aqueous and ethanolic pulp extracts, a positive correlation was found between the α-amylase inhibitory activity and the antioxidant activity (DPPH radical scavening based assay). Moreover, it is important to note that there is also a significant correlation ($P<0.05$) between α-amylase inhibitory activity and the antioxidant activity (DPPH) in ethanolic extracts ($r=0.80$) and aqueous extracts ($r=0.79$) of seeds.

Alpha-glucosidase enzyme inhibitory activity

The results showed that α-glucosidase enzyme inhibitory activity is proportional with doses. Among all fruits of this study, undiluted sample of jambolan had the highest α-glucosidase inhibitory activity (94–99%), but no significant ($P<0.05$) difference was observed between pulp and seeds extracts (Table 3). Undiluted aqueous extracts of red grape seeds had higher α-glucosidase inhibitory activity (99%),

Table 2. Alpha-amylase inhibitory activity in aqueous and ethanolic extracts of pulp, peel, and seeds of seven red and black colored fruits.

Ethanolic extracts (12%) of different doses									
Fruit	Undiluted			Half dilution			One-fifth dilution		
	Pulp	Peel	Seeds	Pulp	Peel	Seeds	Pulp	Peel	Seeds
Jambolan	44.65±0.70^{dD}	73.14±0.57^{eB}	84.45±0.82^{bA}	25.88±0.91^{dF}	36.17±0.89^{eE}	52.10±0.60^{dC}	16.19±0.38^{fG}	12.69±0.27^{dH}	12.74±0.14^{dH}
Persimmon	88.02±0.43^{cA}	29.55±0.70^{fF}	81.83±0.43^{cB}	55.02±0.28^{cD}	12.30±0.46^{fG}	61.15±0.26^{cC}	35.48±0.83^{bE}	*NI	35.83±0.56^{cE}
Plum	90.81±0.1^{9bA}	91.46±0.59^{aA}	2.91±0.25^{fE}	76.20±0.46^{bB}	76.77±0.51^{aB}	*NI	33.47±0.42^{cC}	19.76±1.23^{bD}	*NI
Red Grape	88.42±0.53^{cA}	88.59±0.14^{bA}	83.35±0.60^{bB}	76.24±0.34^{bC}	69.43±0.76^{bD}	75.50±0.57^{aC}	26.08±0.62^{eG}	31.51±0.58^{aF}	48.65±0.30^{aE}
Black Grape	89.97±0.14^{bA}	84.83±0.23dC	87.17±0.23^{aB}	78.26±0.40^{aD}	48.89±0.20^{dF}	68.01±0.12^{bE}	45.43±0.41^{aG}	18.31±0.52^{cI}	43.36±0.58^{bH}
Jaboticaba	93.77±0.11^{aA}	86.05±0.07^{cB}	30.93±0.16^{eF}	76.17±0.61^{bC}	65.78±0.47^{cD}	22.06±1.06^{fG}	28.15±0.11^{dF}	18.29±0.26^{cH}	3.44±0.33^{eI}
Surinam cherry	42.78±0.35^{eB}	–	72.15±0.17^{dA}	21.06±0.47^{eC}	–	42.99±0.36^{eB}	12.95±0.72^{gD}	–	12.06±0.19^{dD}
Aqueous extracts (60 ± 5 °C by 30 min) of different doses									
Fruit	Undiluted			Half dilution			One-fifth dilution		
	Pulp	Peel	Seeds	Pulp	Peel	Seeds	Pulp	Peel	Seeds
Jambolan	41.66±0.76^{fC}	89.90±0.83^{cA}	88.87±0.89^{aA}	25.67±066^{eF}	43.75±0.62^{aC}	59.23±0.68^{aB}	20.79±084^{eG}	37.34±0.49^{cD}	32.86±0.44^{aE}
Persimmon	71.83±0.94^{bB}	52.18±0.36^{fC}	78.47±0.43^{bA}	44.31±0.34^{bD}	29.22±0.58^{fF}	42.35±0.62^{bE}	22.03±0.15^{deG}	10.99±0.88^{fH}	21.86±0.52^{cG}
Plum	53.10±0.58^{eB}	81.07±0.28^{dA}	23.98±0.49^{fG}	33.23±0.46^{dD}	44.41±0.44^{aC}	16.36±0.37^{eH}	29.27±0.33^{bE}	25.86±0.25^{dF}	10.19±0.44el
Red Grape	69.92±0.25^{cD}	93.22±0.36^{bA}	78.25±0.16^{bB}	43.21±0.33^{bcF}	74.07±0.21^{cC}	41.57±0.12^{bG}	21.24±0.73^{eH}	59.20±0.34^{aE}	19.98±0.20^{dH}
Black Grape	65.71±0.29^{dB}	58.54±0.21^{eC}	72.54±0.58^{dA}	42.02±0.06^{cD}	35.54±0.59^{eF}	40.14±0.31^{cE}	25.84±0.08^{cG}	20.87±0.19^{eI}	23.74±0.42^{bH}
Jaboticaba	90.85±0.13^{aB}	97.36±0.11^{aA}	42.79±1.60^{eG}	77.73±0.26^{aD}	79.53±1.22^{bC}	31.43±0.51^{dH}	52.39±0.46^{aE}	49.95±0.44^{bF}	10.82±0.04^{eI}
Surinam cherry	65.63±0.79^{dB}	–	74.54±0.19^{cA}	42.72±0.05^{cC}	–	39.05±0.26^{cD}	23.29±0.63^{dE}	–	10.09±0.11^{eF}

Means with different capital letters are significantly different ($P<0.05$) between different doses of extracts among each specific part (pulp, peel and seeds) of different fruits. In columns: Means with different small-case letters are significantly different ($P<0.05$) between different fruits among their specific part (pulp, peel or seeds).

Table 3. Alpha-glucosidase inhibitory activity in ethanolic and aqueous extracts of pulp, peel, and seeds of seven red and black colored fruits.

Ethanolic extracts (12%) of different doses									
Fruit	Undiluted			Half dilution			One-fifth dilution		
	Pulp	Peel	Seeds	Pulp	Peel	Seeds	Pulp	Peel	Seeds
Jambolan	99.84±0.08^{aA}	62.70±0.21^{dC}	99.49±0.28^{aA}	53.94±0.79^{eE}	27.54±0.17^{eG}	77.87±0.77^{bB}	31.28±0.08 ^{eF}	19.38±0.12^{eH}	59.53±0.09^{bD}
Persimmon	76.13± 0.31^{eB}	75.14±0.57^{cB}	86.95±0.25^{cA}	64.06±0.46^{dC}	59.88±0.13^{cD}	76.02±0.17^{bB}	44.27±0.49^{cG}	52.72±0.37^{bF}	56.77±0.06^{cE}
Plum	93.57±0.53^{bA}	83.42±0.13^{bB}	19.43±0.66^{eG}	74.73±0.26^{bC}	70.27±0.71^{bD}	1.64±0.06^{eH}	60.77±0.64^{bE}	55.01±0.68^{aF}	*NI
Red Grape	93.31±0.38^{bB}	44.28±0.82^{eF}	98.47±0.06^{aA}	80.53±0.49^{aC}	15.63±0.09^{fH}	77.32±0.15^{bD}	64.20±0.17^{aE}	9.97±0.45^{fI}	28.32±0.38^{eG}
Black Grape	44.31±0.40^{fB}	45.46±0.04^{eB}	54.46±0.37^{dA}	30.59±0.05^{fD}	33.35±0.09^{dC}	28.54±0.19^{dE}	18.66±0.33^{fG}	24.18±0.77^{dF}	12.36±0.25^{fH}
Jaboticaba	78.88±0.22^{dB}	95.88±0.83^{aA}	94.30±0.37^{bA}	66.56±0.10^{cD}	75.55±0.57^{aC}	67.88±1.34^{cD}	42.49±0.33^{cE}	43.45±0.30^{cE}	33.55±0.40^{dF}
Surinam cherry	91.20±0.86^{cB}	-	99.77±0.16^{aA}	75.51±1.93^{bC}	-	99.90±0.04^{aA}	38.78±0.54^{dD}	-	99.91±0.02^{aA}
Aqueous extracts (60±5°C by 30 min) of different doses									
Fruit	Undiluted			Half dilution			One-fifth dilution		
	Pulp	Peel	Seeds	Pulp	Peel	Seeds	Pulp	Peel	Seeds
Jambolan	99.03±0.18^{aA}	94.16±0.66^{bB}	99.61±0.04^{aA}	83.60±0.30^{aC}	52.92±0.22^{cE}	72.40±0.45^{bD}	53.55±0.24^{bE}	19.81±0.61^{cG}	48.44±0.09^{bF}
Persimmon	56.24±0.15^{eB}	52.75±0.09^{dC}	75.87±0.11^{bA}	38.56±0.02^{fD}	35.71±0.24^{dE}	52.34±0.15^{dC}	18.35±0.34^{eG}	18.44±0.28^{dG}	34.93±0.07^{dF}
Plum	63.57±0.25^{dA}	44.45±0.24^{eC}	11.65±0.25^{eF}	49.49±0.56^{eB}	17.75±0.31^{fE}	7.22±0.43^{gH}	29.53±0.14^{dD}	8.97±0.06^{eG}	2.22±0.16^{gI}
Red Grape	92.58±0.14^{bB}	93.06±0.25^{cB}	99.83±0.10^{aA}	76.37±0.09^{cD}	78.50±0.23^{bC}	70.20±0.06^{cE}	68.42±0.31^{aF}	48.32±0.29^{bG}	43.35±0.20^{cH}
Black Grape	22.51±0.18^{fD}	38.52±0.22^{fB}	44.48±0.26^{dA}	16.53±0.35^{gE}	28.59±0.28^{eC}	13.84±0.13^{fF}	7.72±0.22^{fG}	8.27±0.24^{fG}	4.70±0.31^{fH}
Jaboticaba	90.85±0.13^{cB}	99.61±0.04^{aA}	63.32±0.20^{cE}	74.14±0.03^{dD}	81.75±0.19^{aC}	39.57±0.38^{eG}	42.52±0.28^{cF}	63.41±0.24^{aE}	10.64±0.36^{eH}
Surinam cherry	90.63±0.43^{cB}	-	99.55±0.13^{aA}	78.62±0.12^{bC}	-	99.83±0.01^{aA}	29.19±0.39^{dD}	-	99.93±0.01^{aA}

Means with different capital letters are significantly different ($P<0.05$) between different doses of extracts among each specific part (pulp, peel and seeds) of different fruits. In columns: Means with different small-case letters are significantly different ($P<0.05$) between different fruits among their specific part (pulp, peel or seeds)

followed by Surinam cherries (99%), persimmon (76%), and black grapes (44%) and and the values of seed extracts were significantly ($P < 0.05$) higher than the pulp extracts of the same fruits (93, 91, 56, and 22% respectively). The α-glucosidase inhibitory activity in seed extracts of all these fruits showed similar trend as total soluble phenolic content and positive correlation between these two parameters was observed (r = 0.79) (Table 4). Similarly positive correlation between α-glucosidase and α-amylase inhibitory activities was observed in aqueous and ethanolic extracts of peel (r = 0.91 and r = 0.83), and seeds (r = 0.81 and r = 0.75), respectively (Table 4). In addition, jambolan seeds extracts also showed positive correlation between α-glucosidase inhibitory activity and DPPH antioxidant activity (r = 0.82).

Discussion

Phenolic compounds in fruits have high antioxidant potentials and when consumed as part of the overall diet it can support mitigating of oxidative stress-induced cellular damages (Hanhineva *et al.*, 2010). Colored tropical fruits with high phenolic content and high antioxidant capacity may have significant potential for specific consumer health benefits, especially to counter oxidative stress-associated chronic diseases (Anhê *et al.*, 2013; Gonçalves *et al.*, 2010; Halliwell, 1994). In this study, high total soluble phenolic content along with high antioxidant activity was found in all analyzed parts of fruit samples. Further, positive correlation between these two parameters was also observed, which indicated that the soluble phenolic content in these fruits has contributed significantly to the higher antioxidant activity. In a previous study with several fruits, Guo *et al.* (2003) determined antioxidant capacity based on FRAP method-based values and reported that in general the antioxidant activity was more in peel as compared to the pulp. In this study, most fruits showed similar trend with DPPH inhibition based assay but aqueous extracts of persimmon and plum, and ethanolic extracts of black grape and jambolan had different trend, as in some fruits and their extracts it followed similar trend while it was opposite in others. Gu *et al.* (2008) explained that tannins are primarily located within the vacuoles or surface wax of plants and the condensed tannins are the major antioxidants in peel and seed of the major dark colored fruits. In persimmon fruit, high phenolic content was found in its peel (4,751 μg GAE g^{-1} DW) and tannins may have contributed to this finding. However, not only just tannins but other soluble phenolics are also present in higher concentrations in peel (Li *et al*., 2011). This is also true for values obtained in this study for the red grapes, jambolan, plum, and persimmon fruits.

In a previous study, the concentration of the total phenolic content in red grapes varied from 54.4 to 974.2 mg GAE kg^{-1} in organic samples and 27.8 to 447.7 mg GAE kg^{-1} in conventional grapes (Mulero *et al.*, 2010). However, these values were lower than the values obtained in this study from pulp, peel, and seeds of grapes which varied from 1,447.6 to 7,739.1 mg GAE kg^{-1} DW in red grapes and 2,423.9 to 7,707.1 mg GAE kg^{-1} DW in black grapes. Determination of total soluble phenolic content on the basis of dry weight may have contributed to such higher values but other factors may also play significant roles. Hidalgo (2003) pointed out that the concentration of phenolic compounds of the grape peel vary widely between varieties and also with the maturity of the fruit. According to Reynertson *et al.* (2006) the jaboticaba fruit is rich in anthocyanins, phenolic acids, and flavonoids and it imparts antiradical, anti-inflammatory, and cytotoxic activity. Rufino *et al.* (2011) also reported total phenolic content of 440 mg GAE 100 g^{-1} FW in jaboticaba. However in this study, higher values of total soluble phenolic were found in ethanolic and aqueous extracts of jaboticaba pulp (8,075.1 and 11,064.4 μg GAE g^{-1} DW, respectively). Jaboticaba fruits with high total soluble phenolic content has significant potential to be utilized either as a whole fruit or as a functional ingredient for human health applications targeting diet-linked hyperglycemia and oxidation-linked complications associated with Type 2 diabetes.

According to Tomas-Barberan *et al.* (2001), most of phenolic compounds, especially anthocyanins and neochlorogenic acid, are concentrated in the peel of plums. Rufino *et al.* (2011) found modest values regarding polyphenol content in aqueous-organic extracts of jambolan. In this study, the total soluble phenolic content varied from 1,692.3 to 7,665.7 μg GAE g^{-1} DW in pulp, peel, and seeds. However, highest value was found in aqueous extracts of jambolan seeds. Thus, the extraction methods may play a decisive role in the estimation of the phenolic content and subsequent determination of the antioxidant activity. The jambolan fruit showed significantly higher antioxidant activity which might be due to the presence of soluble phenolics, tannins, and anthocyanins with higher antioxidant potentials. Except plum all other fruits in this study showed high antioxidant activity and positive correlation with soluble phenolic content. This positive correlation between total soluble phenolic content and total antioxidant activity in all these fruits have significant impacts, especially for improving fruit post-harvest preservation and potentially for human health applications targeting oxidation-linked chronic diseases.

Chronic oxidative stress is a major contributor to the pathogenesis of T2DM, and thus the dietary strategy to prevent and manage this disease needs to include plant-based foods rich in natural antioxidants. The present study showed a significant promise to incorporate these tropical fruits with high phenolic content and high antioxidant activity in designing overall dietary interventions to counter T2DM-induced redox imbalance. The benefits of high phenolics and high antioxidant in these fruits is not limited to counter T2DM only but also have potential to mitigate other macro- and micro-vascular complications associated with this disease. Chronic inflammation is common factor-linked to these macro- and micro-vascular complications and fruits with high antioxidant activity can help to reduce inflammatory state in human body (Oviedo and Beane, 2009). Like redox balance, maintaining glucose homeostasis is also critical for Type 2 diabetes patients. Phenolics present in these fruits also have significant role for improving glucose metabolism by inhibiting key enzymes such as α-glucosidase and α-amylase. Moderate to high α-glucosidase and α-amylase inhibition was found in all analyzed fruit samples in this study. Even after one-fifth dilution some fruits showed very high α-glucosidase inhibitory activity (Surinam cherry, red grape, and jambolan). Similar results were obtained with other Brazilian tropical fruit such as camu-camu (*Myrciaria dubia* Mc.Vaugh) (Fujita *et al.*, 2015).

Romero *et al.* (2008) have reported that the aqueous extracts of fruits showed lower α-amylase inhibition, while ethanol extracts showed greater α-glucosidase inhibition. In the present study, ethanolic extracts of pulp from most fruits showed higher α-amylase inhibitory activity compared to the aqueous pulp extracts. Certain phenolic compounds present in fruits appear to be in glycosylated form and thus the type of sugar and the glycosylation position of these compounds

may have contributed in the observed differences (Romero *et al.*, 2008). Kim *et al.* (2002) reported that glycosylated compounds can bind to α-glucosidase increasing the action of this enzyme, and resulting in high or low inhibitory effect on the α-glucosidase enzyme activity. According to Correia *et al.* (2004), the enzyme inhibition is a reflection of the specific properties of phenolic compounds and not due to the quantity of the phenolic present in the sample. The most significant outcome of this study was the positive correlation between total phenolic content, α-glucosidase inhibition, and α-amylase inhibition in peel and seed extracts of the analyzed fruits. These findings indicate that soluble phenolic compounds present in these fruits may have contributed to the inhibition of these enzymes and thus indicates a potential target to improve glucose metabolism as part of an overall diet-based support system. Earlier studies also reported about the significance of phenolic compounds from plant-based foods for inhibiting these critical enzymes and thus potentially maintaining glucose homeostasis (Hanhineva *et al.*, 2010). The present study also provides important information on variations in phenolic content, antioxidant activity, α-glucosidase, and α-amylase inhibitory activities in peel, pulp, and seed of the analyzed fruits. This will help to select and utilize specific parts of the fruit with higher phenolic bioactive profile for designing and developing new functional ingredients as part of food-based support systems along with drugs to manage early stages of T2DM and its associated complications.

Conclusion

Peel, pulp, and seed extracts of all analyzed fruits had high phenolic-antioxidant linked functionalities in the context of biochemical relevance for dietary support systems for the management of early stages T2DM using targeted *in vitro* assays. The rapid *in vitro* screening strategy provides scientific insights and biochemical rationale to select fruits and their parts with high phenolic bioactive content and associated antioxidant and anti-hyperglycemic properties for further animal model based or clinical studies. The doses and concentration of fruits extracts that has direct dietary relevance for T2DM associated health benefits can be determined from such extended animal model based studies. However, fruits and fruit extracts that exhibited 70–90% α-glucosidase and α-amylase inhibitory activities with *in vitro* assay models would potentially have significant dietary relevance and benefits even in *in vivo* or in clinical studies. Among all fruits, jaboticaba had very high total soluble phenolic content, moderate total antioxidant activity, high α-glucosidase, and high α-amylase inhibitory activities. High antioxidant capacity along with higher inhibition of key carbohydrate metabolism-linked enzymes using these fruits showed significant potential to utilize them either as a whole fruit or as functional ingredients in food formulations as support systems for dietary management of early stages of T2DM and its associated complications. These tropical fruits can potentially provide safe and cost-effective dietary solutions to a wider population, especially where prevalence of T2DM is increasing rapidly and the majority cannot afford the higher health care costs in poorer regions of NE Brazil. Most of these fruits grow well in tropical and subtropical climate of NE Brazil and if better access of these fruits with scientific relevance can be ensured, then it has potential to provide cost effective contribution in health care solutions. This can be achieved through better and effective dietary support for prevention and management of T2DM, especially in early pre-diabetes stages of the disease when hyperglycemia is elevated. Therefore, this study provides biochemical rationale and foundation and baseline data for further animal, clinical and epidemiological studies to validate and more comprehensively implement the findings.

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