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Antioxidant Activity of Spray-Dried Extracts of *Psidium guajava* Leaves

Maurette R. V. Fernandes[1], Luciana M. Kabeya[1], Cláudia R. F. Souza[1], Adna P. Massarioli[2], Severino M. Alencar[2] & Wanderley P. Oliveira[1]

[1]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Av. do Café s/n, Ribeirão Preto, SP, 14040-903, Brazil

[2]Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Av. Pádua Dias, 11, Piracicaba, SP, 13418-900, Brazil

Correspondence: Prof. Dr. Wanderley P. Oliveira, Laboratory of R&D on Pharmaceutical Processes, LAPROFAR, Faculdade de Ciências Farmacêuticas de Ribeirão Preto/USP, Av. do Café s/n, Bloco Q, 14040-903, Ribeirão Preto, SP, Brazil. E-mail: wpoliv@fcfrp.usp.br

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Abstract

Antioxidants from *Psidium guajava* leaves were extracted with 70% ethanol in water. The extractive solution was concentrated and submitted to spray drying in the presence of technological adjuvants at a proportion of 8 % wet base. Three distinct technological adjuvants were evaluated: β-cyclodextrin (βCD-80), maltodextrin DE10:Aerosil® (MA-80 - 7:1), and maltodextrin DE10:Encapsia®:Aerosil® (MDEA-80 - 5:2:1). The antioxidant activity of the concentrated extract and spray-dried powders was assessed by three antioxidant assays, namely: the 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging, ferric reducing antioxidant power (FRAP), and oxygen radical absorption capacity (ORAC). The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging capacity was determined previously. The spray-dried powders exhibited strong antioxidant activity (IC_{50} value = 7.96 to 9.76 μg/mL – DPPH method; 3,125.1 to 3,406.0 μM TE/g dry weight – ABTS method; 4,210 to 4,540 μM $FeSO_4$ E/g – FRAP method; 1,820-2,020 μM TE/g – ORAC method). The technological adjuvants did not significantly interfere with the antioxidant activity of the dried products, regardless the type of antioxidant assay used. The results here reported strongly evidenced that the concentrated and spray-dried extracts of *Psidium guajava* are rich sources of natural antioxidants with potential application in food, pharmaceutical, and cosmeceutical products.

Keywords: antioxidant activity, dried extract, polyphenols, *Psidium guajava*, spray-drying.

1. Introduction

Psidium guajava Linn. (Myrtaceae) is native from Central and South America and has been cultivated in various tropical and subtropical countries. The roots, bark, leaves, and immature fruits of *P. guajava* are used in many parts of the world for the treatment of wounds and a plethora of diseases, including diabetes, hypertension, and gastroenteritis. The ripe fruits are widely consumed by humans and employed by the food industry to produce juice, jelly, and candies (Gutiérrez, Mitchell, & Solis, 2008). Extracts of *P. guajava*, especially from mature leaves, are rich sources of phenolic compounds (Gutiérrez et al., 2008; Venkatachalam, Singh, & Marar, 2012).

Currently, there is considerable interest in the use of antioxidant compounds from natural sources to preserve and improve the shelf-life of food products, and to increase the stability of fats and meat products (Hygreeva, Pandey, & Radhakrishna, 2014). A raw herbal material, however, needs to be transformed into a standardized product suitable for industrial use through a multistage process that demands specific processing technologies, including extraction, concentration and drying of bioactive compounds. Standardized dried extracts have several advantages over unprocessed plant material and liquid forms, such as higher physicochemical and microbial stability, easiness in dosage, higher concentration of bioactive compounds, lower transport and storage costs, and capability to be transformed into several dosage forms, such as tablets, granules, and capsules (Oliveira & Petrovick, 2010).

Spray-drying is a technique commonly used in the pharmaceutical and food industry that is appropriate to dry

heat-sensitive components such as bioactive compounds present in plant extracts, enzymes, and other pharmaceuticals. Technological carriers such as gums, semi-synthetic cellulose derivatives, and synthetic polymers are commonly added to the drying mixture to protect these components from degradation, optimize the drying performance, and improve the physicochemical properties of the products (Sollohub & Cal, 2010; Cortés-Rojas & Oliveira, 2012).

Hydroalcoholic extracts from *P. guajava* leaves are rich in phenolic compounds, flavonoids, and tannins (Venkatachalam et al., 2012; Fernandes et al., 2014a) and may probably act as natural antioxidants for food and pharmaceutical products. Crude and spray-dried extracts from *P. guajava* leaves (PG-SD) exhibit antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Fernandes, Dias, Carvalho, Souza, & Oliveira, 2014b), inhibit the production of oxidant species by human neutrophils (Fernandes et al., 2014a), and scavenge hydrogen peroxide, nitric oxide, and superoxide anion radical (Venkatachalam et al., 2012). The biological activity of the PG-SD extracts resembled that of the initial concentrated extract (Fernandes et al., 2014a).

This study reports the antioxidant activity of crude and spray-dried extracts of *P. guajava* leaves assessed by three distinct *in vitro* antioxidant methods – 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging, ferric reducing antioxidant power (FRAP), and oxygen radical absorption capacity (ORAC) – and compared with the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity determined previously (Fernandes et al., 2014b). Analysis of the correlation between the antioxidant capacity and the phenolic and flavonoid content of the crude and dried extracts is also presented.

2. Materials and Methods

2.1 Chemicals

2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), fluorescein sodium salt, gallic acid, and Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol, $AlCl_3$, phosphomolybdic acid, and sodium tungstate were acquired from Vetec Química Fina (Rio de Janeiro, Brazil). The technological adjuvants colloidal silicon dioxide (Aerosil® 200; Evonik Degussa, Hanau, Germany), maltodextrin (MOR-REX 1910; Corn Products of Brazil), β-cyclodextrin (Roquette; Lestrem, France), and gum arabic (Encapsia®; NEXIRA Brazil, São Paulo, SP, Brazil) were obtained from different suppliers.

2.2 Plant Material

The leaves of *Psidium guajava* were collected in 21/02/2011 at "Casa da Goiaba", a farm and food industry located in Lavras, Minas Gerais State, Brazil (21°13'32.9" S, 44°59'06.05" W at an altitude of 883 m). The plant was identified by Prof. Dr. Marcelo Polo and a voucher specimen was deposited at the herbarium of the Federal University of Alfenas (Alfenas, MG, Brazil) under the code UALF-01505.

2.3 Preparation and Spray-drying of the P. guajava Extract

The hydroalcoholic extract of *P. guajava* leaves was prepared and dried as reported previously (Fernandes et al., 2014b). Briefly, *P. guajava* leaves were dried in a circulating air oven (Fanem 315 SE, Guarulhos, Brazil) at 45 ℃ until constant weight, ground in a knife mill (MA-680, Marconi Equipamentos para Laboratórios Ltda, Piracicaba, SP – Brazil) until pass through a 20-mesh sieve (833 μm). The dried leaves were submitted to dynamic maceration with 70% ethanol in water (v/v) at a plant/solvent ratio of 1:10 (w/v), under constant stirring (Nova Ética, model 119, Vargem Grande Paulista, SP, Brazil) for 60 min, at 50 ℃. The extractive solution was filtered and concentrated in a rotary evaporator (Fisatom 802, São Paulo, Brazil) at 50 ℃ under vacuum, until solid contents reached 12 % (w/w).

The *Psidium. guajava* concentrated extract (PG-CE) was mixed with the following technological adjuvants at a proportion of 8% (wet base): maltodextrin DE10:Aerosil® mixture at 7:1 ratio (MA-80), maltodextrin DE10:Encapsia®:Aerosil® mixtures at a 5:2:1 ratio (MDEA-80), or pure β-cyclodextrin (βCD-80). The compositions were dried in a bench-top Lab-Plant SD-05 spray dryer (Lab-Plant UK Ltd., Huddersfield, UK), equipped with a two-fluid atomizer, with an internal orifice of 0.7 mm. The operating parameters were: inlet air drying temperature, T_{gi} = 150 ℃; drying air flow rate, W_g = 60 m^3/h; extract feed flow rate, W_{susp} = 4 g/min; atomizing air pressure, P_{atm} = 1.5 bar and atomizing air flow rate, W_{atm} = 15 lpm.

2.4 Determination of Antioxidant Activity

2.4.1 DPPH Assay

The DPPH assay was performed as reported previously (Fernandes et al., 2014b). The results were expressed as IC_{50}, which represents the sample concentration in μg/mL required to reduce 50% of the DPPH free radicals added to the reaction medium. All the measurements were performed in triplicate.

2.4.2 ABTS Assay

This study used the ABTS radical cation ($ABTS^{\bullet+}$) decolorization assay protocol reported by Re et al. (1999), with slight modifications. A stock solution of $ABTS^{\bullet+}$ was prepared by mixing ABTS solution with $K_2S_2O_8$ solution (final concentrations of 7 mM and 140 mM, respectively). The mixture was maintained in the dark at room temperature for 16 h before use. To obtain the working $ABTS^{\bullet+}$ solution, the stock solution was diluted in absolute ethanol to achieve an absorbance value of 0.7 (±0.05) at λ = 734 nm, recorded in the UV/vis HP 8453 spectrophotometer (Agilent Technologies, Waldbronn – Germany).

The test-samples (10 μL), ethanol (control), or Trolox® were added to the $ABTS^{\bullet+}$ working solution (3 mL) and, after a 6-min incubation at room temperature, in the dark, the absorbance was recorded at 734 nm. An equivalent volume of ethanol was set as blank. The percentage of absorbance decrease promoted by each sample was interpolated into the standard curve built with Trolox® (0–2000 μM) to calculate their concentration in Trolox® equivalents (μM TE). Each sample was analyzed in triplicate.

2.4.3 FRAP Assay

This study used a modified version of the FRAP assay reported by Benzie and Strain (1996). The working FRAP reagent was prepared daily by mixing 0.3 M acetate buffer pH 3.6, 10 mM 2,4,6-tri(2-pyridyl)-*s*-triazine (prepared in 40 mM HCl), and 20 mM $FeCl_3$ at a proportion of 10:1:1. The test-samples (90 μL) and deionized water (270 μL) were added to freshly prepared FRAP reagent (2.7 mL). After a 30-min incubation at 37 ℃ in a water bath (Fisaton 550, São Paulo, Brazil) the absorbance of the reaction mixture was recorded at 595 nm in the UV/vis HP 8453 spectrophotometer (Agilent Technologies, Waldbronn – Germany), using the FRAP reagent as blank. The ferric reducing capacity of the test-samples was calculated by interpolating the absorbance values in the standard curve built with $FeSO_4$ (250-1500 μM), and expressed as μM Fe^{2+}/g of sample. The assay was performed in triplicate.

2.4.4 ORAC Assay

The ORAC assay was carried out according to the procedure described by Melo et al. (2015), with some modifications. Briefly, the test-samples (30 μL) were mixed with fluorescein (60 μL of a 508 nM solution prepared in 75 mM phosphate buffer pH 7.4) in 96-well dark plates. The reaction was started by adding AAPH (110 μL; 76 mM) and the fluorescence was recorded immediately and every 1 min thereafter for 120 or 160 min, at 37 ℃, at excitation and emission wavelengths of 485 and 528 nm, respectively, using an automated microplate reader (Molecular Devices SpectraMax M3, San Jose, California, EUA). Trolox® (12.5-400 μM) was used to prepare the standard curve. All the samples were analyzed in triplicate.

The area under the curve (AUC) was calculated for each sample by integrating the relative fluorescence curve. The net AUC of the sample was calculated by subtracting the AUC of the blank. The regression equation between net AUC and concentration of Trolox® was used to express the antioxidant activity of the test-samples as μM TE/g dry weight.

2.5 Statistical Analysis

Statistical analysis was performed using the SAS/STAT® software version 9.0 (SAS Institute, Cary, NC, USA, 2002). Statistical differences between groups were analyzed using the Kruskall-Wallis test followed by the Dunn's test. IC_{50} values were calculated by non-linear regression analysis from the concentration-response inhibition curve. The Pearson's correlation coefficient (*r*) was calculated to analyze the correlation between antioxidant capacity assessed using different methods and the content of flavonoids and phenolic compounds.

3. Results and Discussion

The concentrated and spray-dried extracts from *P. guajava* leaves are rich in phenolic compounds (Fernandes et al., 2014; Fernandes et al., 2014b). The total flavonoid and polyphenol content of the concentrated and spray-dried extracts MA-80, MDEA-80 and βCD-80 are respectively (Fernandes et al., 2014b): 22.58 ± 0.08 (mg/g, db) and 23.21 ± 0.72 (% m/m, db), 13.35 ± 0.32 (mg/g, db) and 10.52 ± 0.35 (% m/m, db), 15.48 ± 0.87 (mg/g, db) and 9.67 ± 0.10 (% m/m, db), 12.58 ± 0.17 (mg/g, db) and 12.47 ± 0.66 (% m/m, db). Polyphenol content was expressed in terms of gallic acid equivalents, while total flavonoid content was expressed in

milligrams of quercetin per gram of extract (dry base, db).

Several studies have associated the content of polyphenols with the antioxidant activity of herbal extracts. The redox properties of these compounds enable them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators, and reductants of ferryl hemoglobin (Cáz et al., 2010; Gebicka & Banasiak, 2009). There is not an elementary comprehensive method to accurately estimate and quantify the antioxidant activity, because oxidative processes involve multiple active species, reaction characteristics, and mechanisms (Niki, 2010). Hence, the literature recommends the use of methods with different mechanisms of oxidation inhibition to examine the antioxidant capacity of a given sample (Alañón, Castro-Vázquez, Díaz-Maroto, Gordon, & Pérez-Coello, 2011). The present study used the $ABTS^{\bullet+}$ radical scavenging, FRAP, and ORAC assays to analyze the antioxidant activity of concentrated and spray-dried extracts of *P. guajava* leaves (Table 1).

The ABTS assay principle is based on the ability of a given compound to quench $ABTS^{\bullet+}$ relatively to Trolox®, which is a hydrophilic analogue of vitamin E used as reference antioxidant. The ability of a given compound to reduce a ferric complex (Fe^{3+}-tripyridyltriazine) to a ferrous complex (Fe^{2+}-tripyridyltriazine), at low pH, is the basic principle of the FRAP assay. The ORAC method is based on the thermal decomposition of AAPH to generate free radicals, mainly peroxyl radicals, which react with fluorescein and change its fluorescence emission profile (Floegel, Kim, Chung, Koo, & Chun, 2011). It must be emphasized that the ORAC assay combines both the inhibition time and the degree of inhibition by the antioxidant into a single quantity. It ensures that, at the end of the process, all the antioxidants present in the sample have reacted with the free radicals generated. Compared with the ABTS and FRAP assays, the ORAC method requires a more expensive equipment and longer assay time (Zulueta, Esteve, & Frígola, 2009; Dudonné, Vitrac, Coutière, Woillez, & Mérillon, 2009).

Table 1. Antioxidant activity of the concentrated and spray-dried extracts from *P. guajava* leaves.

Sample	ABTS (μM TE/g)	FRAP (μM FeSO4 E/g)	ORAC (μM TE/g)
PG-SD/MA-80	3,125.13 (173.44)	4,310 (330)	1,820 (20)
PG-SD/MDEA-80	3,406.00 (113.68)	4,210 (190)	1,900 (40)
PG-SD/βCD-80	3,357.99 (301.50)	4,540 (190)	2,020 (120)
PG-CE	3,392.69 (84.61)	8,947 (180)	1,330 (90)

Data are expressed as the mean (standard deviation) of triplicate measurements. PG-CE: concentrated extract. PG-SD: spray-dried extract with the technological adjuvants MA-80 (maltodextrin DE10:Aerosil®), MDEA-80 (maltodextrin DE10:Encapsia®:Aerosil®), or βCD-80 (β-cyclodextrin). TE: Trolox® equivalents.

The antioxidant activity of *P. guajava* concentrated extract (PG-CE) and of the spray-dried products (PG-SD/MA-80, PG-SD/MDEA-80, and PG-SD/βCD-80) were similar to each other, and ranged from 3,125.1 to 3,406.0 μM TE/g dry weight, when assessed by the ABTS assay (Table 1). These results corroborate literature reports on the powerful antioxidant capacity of different *P. guajava* leaf extracts determined by this assay (Nantitanon, Yotsawimonwat, & Okonogi, 2010). The antioxidant activity of the *P. guajava* samples tested herein were (i) sixfold stronger than that exhibited by *Bidens pilosa* extracts spray-dried with Aerosil® as adjuvant, which ranged from 421 to 527 μM TE/g dry weight (Cortés-Rojas & Oliveira, 2012); and (ii) more effective than that displayed by 27 plants used in Peruvian folk medicine, which ranged from 3.7 to 1,045.3 μM TE/g dry weight (Chirinos, Pedreschi, Rogez, Larondelle, & Campos, 2013).

Considering that the antioxidant activity of phenolic compounds usually correlates with their reducing capacity, the FRAP assay represents a reliable and reproducible method to analyze the antioxidant activity of various compounds (Benzie & Strain, 1996; Pérez-Jiménez et al., 2008). The three *Psidium. guajava* spray-dried extracts (PG-SD: MA-80, MDEA-80, and βCD-80) exhibited similar reducing effects, which were twofold weaker than that exerted by PG-CE (8,947 μM $FeSO_4$ E/g), as evaluated by the FRAP assay (Table 1). These findings are in line with a previous report on the twofold stronger antioxidant activity of PG-CE (mean IC_{50} value = 3.34 μg/mL), as compared with the antioxidant activity of PG-SD (mean IC_{50} value = 7.96–9.76 μg/mL) assessed by the DPPH method, which measures the hydrogen donating capacity of a given compound (Fernandes et al., 2014b). It was hypothesized that the drying adjuvants diluted the antioxidant compounds of PG-CE, because the concentration of total phenolic compounds and total flavonoids in the three PG-SD were nearly twofold lower than the ones detected in PG-CE (Fernandes et al., 2014b).

The standard antioxidants ascorbic acid, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) yielded IC_{50} values of 2.96±0.15, 2.97±0.32, and 12.52±0.25 μg/mL, respectively, when assayed using the DPPH method. Therefore, PG-CE was as effective as ascorbic acid and BHA, while PG-SD was more effective than

BHT in donating hydrogen to the DPPH radical. It indicates that the antioxidant efficiency of the concentrated and spray-dried extracts of *P. guajava* was similar to that exhibited by commercial synthetic antioxidant compounds. Compared with synthetic single dietary antioxidants, natural antioxidants from herbal products may be more effective due to the synergistic action among their components (Pérez-Jiménez et al., 2008).

The absolute values of μM TE/g obtained using the ORAC method were lower than those obtained using the ABTS assay (Table 1), for the concentrated and spray-dried *P. guajava* extracts. The antioxidant activity of the PG-SD samples, as determined by the ORAC assay (1,820-2,020 μM TE/g), were comparable to that of açaí berry juice (*Euterpe oleraceae* Mart.) spray-dried with maltodextrin 10 DE (2,376.29 μM TE/g) and Arabic gum (2,408.87 μM TE/g) (Silva et al., 2014). Açaí berries are commonly consumed in human diet due to their high concentration of antioxidant compounds. Compared with PG-SD, *Myrciaria cauliflora* extracts spray-dried with arabic gum and maltodextrin exhibited weaker antioxidant effect (737-900 μM TE/g), as assessed by the ORAC assay (Tonon, Brabet, Pallet, Brat, & Hubinger, 2009). Using this method, the antioxidant activity of *P. guajava* fruit methanol extract was 21.3 μM TE/g fresh mass (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006), which was nearly sixtyfold weaker than that exerted by the PG-CE leaf extract (1,330 μM TE/g) (Table 1).

The PG-SD prepared with different technological adjuvants (MA-80, MDEA-80, and βCD-80) exhibited similar antioxidant activity, regardless the antioxidant assay type. This finding indicates that the type of adjuvant used herein did not significantly interfere with the antioxidant capacity of the dried product. Maltodextrin and cyclodextrins lack functional groups able to donate electrons or hydrogen to free radicals (Phillips, Carlsen, & Blomhoff, 2009; Jullian, Moyano, Yañez, & Olea-Azar, 2007), but Arabic gum has a little protein content, which could exert a weak antioxidant effect mediated by the amino acids tyrosine, histidine, and methionine (Fazaeli, Emam-Djomeh, Ashtari, & Omid, 2012). However, in this study the PG-SD samples with and without Arabic gum (MDEA-80 and MA-80, respectively) exhibited similar antioxidant activity.

It has been previously reported that the total amount of phenolic compounds and flavonoids does not significantly vary across the *P. guajava* spray-dried extracts due to addition of technological adjuvants (Fernandes et al., 2014b). The Pearson's correlation coefficient between the antioxidant capacity of PG-SD assessed by different methods and their content of total phenols and flavonoids was determined, and the results are presented in Table 2. According to the absolute values of *r*, the correlation can be classified as: Negligible (0.00 to 0.30), Low (0.30–0.50), Moderate (0.50-0.70), High (0.70-0.90), Very high (0.90-1.00), being 1.00 a perfect correlation. Values lower or higher than 0.0 imply negative or positive correlations, respectively (Mukaka, 2012). Experimental data of the percentage of maximum DPPH free radical scavenging (DPPH_%), concentration of the sample that scavenges 50% of the DPPH free radical added to the reaction medium ($DPPH\_IC_{50}$), total flavonoids (TF), and total polyphenols (TP) have been reported so far (Fernandes et al., 2014b).

Table 2. Pearson's correlation coefficient between antioxidant activity of *P. guajava* spray-dried extracts assessed by different methods and their total phenol and flavonoid content.

	ABTS	**DPPH_%**	**DPPH_IC50**	**FRAP**	**TF**	**ORAC**	**TP**
ABTS	-	-0.149	-0.019	-0.181	0.065	-0.210	0.062
DPPH_%		-	-0.682	0.364	0.667	0.192	0.580
DPPH_IC50			-	-0.244	-0.976	-0.071	-0.980
FRAP				-	0.290	0.965	0.286
TF					-	0.128	0.947
ORAC						-	0.124

The percentage of DPPH radical scavenging positively correlated with the concentration of total flavonoids (r = 0.667) and phenolic compounds (r = 0.580). As expected, the IC_{50} values for the DPPH radical scavenging ($DPPH\_IC_{50}$) negatively correlated with the content of flavonoids (r = -0.976) and phenolic compounds (r = -0.980), and with the percentage of DPPH radical scavenging (DPPH_%) (r = -0.682). These results corroborate literature reports on the strong positive correlation between the concentration of these compounds and the DPPH radical scavenging capacity, as determined using the Spearman's-Rho coefficient and other correlation parameters; the r values reported ranged from 0.708 to 0.939 (Floegel et al., 2011; Dudonné et al., 2009).

Concerning the methods used to examine the antioxidant capacity of PG-SD, data from the DPPH radical scavenging assay only weakly correlated with data from the FRAP and ORAC methods (r = 0.364 and 0.192,

respectively). In contrast, there was a strong positive correlation between the antioxidant activity of PG-SD assessed by the FRAP and ORAC methods (r = 0.965). Data from these assays correlate moderately in studies of the antioxidant activity of serum (Cao & Prior, 1998), aqueous extracts of 30 plants (r = 0.618) (Dudonné et al., 2009), and oak wood used in wine ageing (r = 0.730) (Alañón et al., 2011). In a study comprising 927 freeze-dried vegetable samples, the results from FRAP and ORAC assays did not correlate well, probably due to the huge variability in chemical composition and reactivity rate among the samples (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). ORAC is considered as the most sensitive method to measure chain-breaking antioxidant function, which involves the hydrogen atom transfer pathway (Cäz et al., 2010).

The total amount of phenolic compounds strongly and positively correlated with the total flavonoid content (r = 0.947), which indicates that flavonoids are the major phenolic compounds that account for the overall antioxidant effect of *P. guajava* extracts. These results agree with literature reports on the marked contribution of phenolic compounds to the antioxidant activity of *P. guajava* leaf extracts (Venkatachalam et al., 2012; Nantitanon et al., 2010). The expressive contribution of phenolic compounds to the antioxidant activity assessed by different methods underscores their multiple mechanisms of action, such as quenching of reactive oxygen species, free radical scavenging, reducing power, and reduction of peroxyl radicals (Cäz et al., 2010).

Considering the high complexity of composition of herbal extracts, the isolation and study of individual antioxidant compounds would be ineffective without understanding the synergistic and/or antagonistic interactions among them within a given matrix (Müller, Fröhlich, & Böhm, 2011). The experimental results reported herein strongly evidences that the *P. guajava* leaf extract is a good source of phenolic compounds, whose total amount and antioxidant activity are preserved after spray-drying with different technological adjuvants (Fernandes et al., 2014b). These features, associated with the fact that the three PG-SD fit the United States Pharmacopoeia recommendations with respect to the product moisture content, water activity, water solubility, and particle size (Fernandes et al., 2014a) make PG-SD promising raw materials to be used as natural antioxidants in food, cosmetic, and pharmaceutical products. Further studies are undertaken to develop practical applications for the products.

4. Conclusions

This study demonstrated the feasibility of spray drying for the production of standardized *P. guajava* leaf extracts that are rich in phenolic compounds and exhibit marked antioxidant capacity. The antioxidant capacity of the products has been confirmed through four *in vitro* assays based on different chemical principles, namely 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity, 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging, ferric reducing antioxidant power (FRAP), and oxygen radical absorption capacity (ORAC). The strong antioxidant capacity and the physicochemical properties of the spray-dried *P. guajava* extracts demonstrate their potential to be used as natural antioxidants in food, pharmaceutical, and cosmeceutical products.

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