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Evaluation of antioxidant potential and total phenolic content of exotic fruits grown in Colombia

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ABSTRACT

Disruption of the balance between reactive oxygen species (ROS) production and endogenous antioxidant defenses leads to oxidative stress, which is related to the appearance of chronic noncommunicable diseases (NCDs). The antioxidant compounds present in fruits, including phenolic compounds, suggest that high fruit consumption may contribute to counteracting oxidative stress. The antioxidant potential of ten fruits grown in Colombia was evaluated by quantifying the content of total phenolic compounds, as well as their free radical scavenging capacity [2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), peroxyl radicals (ROO), and nitric oxide (NO)], the reduction capacity of ferric reducing antioxidant power (FRAP), and the inhibition of the generation of ROS using the 2′,7′-dichlorofluorescein diacetate (DCFH-DA) method in RAW 264.7 mouse macrophages. Regarding phenolic compounds, the extracts of banana passion and sour guava presented the highest content. These extracts also presented the most potent ferric-reducing ability and the best scavenging activity of DPPH, ABTS, NO, and ROO. Moreover, banana passion, yellow pitaya, and golden berry extracts significantly inhibited the production of ROS on LPS-stimulated macrophages. Our results show that banana passion, yellow pitaya, and golden berry are essential sources of molecules with antioxidant activity and their intake could modulate oxidative stress and consequently could help prevent or reduce the incidence of NCDs.

INTRODUCTION

The generation of reactive oxygen species (ROS) by the body's metabolism has a beneficial role in killing infectious agents and regulating cell signaling pathways, as well as genes related to the inflammatory and immune response (Khurana *et al*., 2013). Nevertheless, the imbalance produced by this intracellular accumulation of ROS induces oxidative stress, which is a cellular state in which DNA, carbohydrates, proteins, and lipids are oxidized, resulting in impaired cellular function, mutations, and cell death. These cellular alterations have been related to the initiation and progression of diseases such as diabetes, cancer, cardiovascular disease, and Alzheimer's disease, among others that are classified as noncommunicable diseases (NCDs) (Lee *et al*., 2015; Liguori *et al*., 2018). In this sense, substances capable of inhibiting the excessive production of ROS could become an alternative to reduce the incidence of NCDs.

Results obtained in epidemiological studies showed a correlation between the high consumption of fruits and the prevention of NCDs, especially cardiovascular diseases and cancer (Aune *et al*., 2017; Boeing *et al*., 2012; Wang *et al*., 2014). Based on these studies, the World Health Organization has recommended the consumption of five servings of fruits and vegetables daily as per the Strategy for the Prevention and Control of NCDs (Aune *et al*., 2017). Consistent with this recommendation, the relationship between fruit consumption and health promotion has been evidenced by the increase in the number of bibliographic references. These studies concluded that the positive effect of fruits on health is associated with their content of phytochemicals, mainly phenolic compounds (Côté *et al*., 2010; Hidalgo and Almajano, 2017; Kalaycıoğlu and Erim, 2017; Skrovankova *et al*., 2015). These compounds have a recognized antioxidant effect

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and, therefore, have been associated with the prevention of NCDs (Y Aboul-Enein *et al*., 2013; Zhang *et al*., 2015).

The demand for fruits in national and international markets has continuously grown, perhaps by the publications that show that low consumption of fruits is among the main factors associated with worldwide mortality. On the contrary, fruit intake is related to a decreased risk of NCDs (Khoo *et al*., 2011; Slavin and Lloyd, 2012). Colombia is one of the countries that is considered as megadiverse, since it has a great variety of exotic fruits (Contreras-Calderón *et al*., 2011). Fruits such as yellow pitaya (*Hylocereus megalanthus*), banana passion (*Passiflora cumbalensis*), purple passion fruit (*Passiflora edulis*), golden berry (*Physalis peruviana*), tamarillo (*Solanum betaceum*), lulo (*Solanum quitoense*), soursop (*Annona muricata*), kalipatti sapota (*Manilkara zapota*), sapote (*Pouteria sapota*), and sour guava (*Psidium friedrichsthalianum*) are commonly consumed in Colombia and are used extensively to treat various diseases in different regions of the world. For example, soursop and purple passion fruit are used to treat cancer, hypertension, inflammation, among other diseases (Daddiouaissa and Amid, 2018; Nanda *et al*., 2013; Taïwe and Kuete, 2017). Sapote and tamarillo are useful remedies for patients' gastrointestinal problems (Angulo *et al*., 2012; Ngomle *et al*., 2020; Stanley *et al*., 2009). Golden berry is used to treat asthma, rheumatism, cancer, and leukemia (Hassanien, 2011; Wu *et al*., 2004a). Lulo is used to treat nerve problems and skin diseases (Bussmann *et al*., 2018). Kalipatti sapota is used in the treatment of pulmonary diseases and also inhibits the growth of breast and colon cancers (Bano and Ahmed, 2017). Yellow pitaya has a mild laxative effect (Fratoni *et al*., 2019; Sudha *et al*., 2017). Leaves and flowers of *P. cumbalensis* are widely employed in folk medicine as a poultice for gastrointestinal problems (Jerves-Andrade *et al*., 2014). However, no reports of the species of *P. friedrichsthalianum* were found in the literature.

Regarding studies of antioxidant activity, in the literature, there are reports of the antioxidant potential of some fruits included in this study that are evaluated individually. For example, the content of total phenolic compounds and the uptake of radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) have already been reported for all fruits included in this work (Bravo *et al*., 2016; dos Reis *et al*., 2018; Flores *et al*., 2013; Gordillo *et al*., 2012; Prabhu *et al*., 2018; Torres Grisales *et al*., 2017; Vasco *et al*., 2008; Woo *et al*., 2013), while for yellow pitaya and sour guava, there are no activity reports for the ferric-reducing antioxidant power (FRAP) or the oxygen radical absorbance capacity-fluorescein (ORAC-FL) (Bravo *et al*., 2015, 2016; Espin *et al*., 2016; González *et al*., 2012; Gordillo *et al*., 2012; Penaloza *et al*., 2017; Shafii *et al*., 2017; Vasco *et al*., 2008; Woo *et al*., 2013; Yahia and Barrera, 2009; Yahia *et al*., 2011; Zapata *et al*., 2014). Moreover, the scavenging of nitric oxide radical and the inhibition of the generation of intracellular ROS have been reported only for golden berry, kalipatti sapota, soursop, tamarillo, and purple passion fruit (Gomathy *et al*., 2013; González *et al*., 2016; Kou *et al*., 2009; Maruki-Uchida *et al*., 2013; Orqueda *et al*., 2020; Rop *et al*., 2012; Tan *et al*., 2018; Wu *et al*., 2004b; Zamudio-Cuevas *et al*., 2014). Despite this background, it is complicated to determine which would be the most promising fruits as antioxidants, since the fundamental differences are observed in the extraction methods regarding the used methodology and in the expression of the results. Few reports have studied the antioxidant properties of these fruits under the same experimental conditions (Bravo *et al*., 2016; Contreras-Calderón *et al*., 2011). Taking into account the high demand for these fruits, their ethnopharmacological use, and antioxidant history, in this work, we evaluated the antioxidant potential of ten exotic fruits grown in Colombia to identify the promising fruits for the treatment or prevention of NCDs by quantifying the content of total phenolic compounds, as well as their free radical scavenging capacity (DPPH, ABTS, ROO, NO). Additionally, the reduction capacity of the extracts was measured with the FRAP assay and the inhibition of the generation of ROS in lipopolysaccharide (LPS)-stimulated RAW 264.7 mouse macrophages was measured using the DCFH-DA method.

MATERIALS AND METHODS

Reagents

Gallic acid, Folin–Ciocalteu, sodium nitroprusside dihydrate, sulfanilamide, horseradish peroxidase, guaiacol, catechin, fluorescein, quercetin, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), ABTS, DPPH, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 5,5-dimethyl-1-pyrroline N-oxide, N-(1-naphthyl)ethylenediamine dihydrochloride, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), pyrogallol red, ferric chloride, ferrous sulfate heptahydrate, magnesium sulfate heptahydrate, potassium persulfate, and lipopolysaccharide (LPS) were obtained from Sigma-Aldrich (St. Louis, MO). Phosphoric acid, potassium chloride, and sodium chloride were obtained from JT Baker (Phillipsburg, NJ). Methanol, ethanol, dimethyl sulfoxide (DMSO), Dulbecco's modified eagle medium (DMEM), and fetal bovine serum (FBS) were obtained from Thermo Fisher Scientific (Pittsburgh, PA). Caffeic acid, aluminum chloride, ascorbic acid, sodium hydroxide, sodium nitrite, D-glucose, calcium chloride, potassium phosphate monobasic, potassium phosphate dibasic, sodium phosphate dibasic, sodium phosphate monobasic, hydrochloric acid, acetic acid, hydrogen peroxide, and 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were obtained from Merck Millipore (Burlington, MA).

Fruit samples and determination of physicochemical parameters

Fruits included in this study were obtained from Fusagasugá (Cundinamarca) and Turbana (Bolívar), which involved yellow pitaya (*H. megalanthus*), banana passion (*P. cumbalensis*), purple passion fruit (*P. edulis*), golden berry (*P. peruviana*), tamarillo (*S. betaceum*), lulo (*S. quitoense*), soursop (*A. muricata*), kalipatti sapota (*M. zapota*), sapote (*P. sapota*), and sour guava (*P. friedrichsthalianum*). Representative samples of each fruit were used to determine the soluble solids (ºBrix), titratable acidity, and pH values, which were conducted following the guidelines established by the Association of Official Analytical Chemists (Helrich, 1990). The concentration of the solid soluble content (ºBrix) was measured using a digital refractometer (Sper Scientific 300034) at 25°C and the pH values were determined by a pH meter (Starter 3100, OHAUS) at 25°C.

Preparation of extracts and determination of total phenolics content

Fruits were washed under tap water, weighed, homogenized, frozen at −80°C, and lyophilized. The dried material was extracted with ethanol (96%) at room temperature in stoppered containers for a defined period of time with frequent agitation until exhaustion and the obtained extract was concentrated in a rotary evaporator at controlled temperatures (38°C – 40°C) and reduced pressure. The Folin–Ciocalteu method was used to quantify the total phenolic compounds (Rivera *et al*., 2018). Phenolic compounds were calculated as mg gallic acid equivalents (GAE)/100 g of fresh weight.

Antioxidant activity assays

DPPH and ABTS free radical assays

Free radical DPPH and ABTS scavenging was determined using the standard methods with some modifications (Castro *et al*., 2018). For the uptake of the DPPH radical, 75 **µl** of different concentrations of the fruit extracts were mixed with 150 **µl** of a methanolic solution of DPPH (70 µg/ml). The mixture was allowed to incubate for 30 minutes and the absorbance at 517 nm was measured. While for the ABTS radical, 20 **µl** of the different concentrations of fruit extracts were mixed with 180 **µl** of the ABTS solution. After 30 minutes of incubation, the absorbance was measured at 734 nm in a Multiskan Go microplate reader (Thermo Scientific, Waltham, MA). A Trolox standard curve was used to determine the equivalent antioxidant capacity and the results were expressed as µmol Trolox equivalents/g of fresh weight.

Ferric-reducing antioxidant power assay

In this work, we used the standard method with some modifications (Castro *et al*., 2014). The FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer (pH 3.6) with 1 volume of 10 mM TPTZ in HCl (40 mM) and with 1 volume of 20 mM $FeCl_3 \cdot 6H_2O$. Different concentrations of fruit extracts (10 µl) were mixed with the FRAP reagent (290 µl), incubated independently at 37°C, and the absorbance was determined at 593 nm after 60 minutes of the reaction using an EnSpire Multimode microplate reader (PerkinElmer, Waltham, MA). The FRAP value was determined by plotting a standard curve made by the addition of ferrous sulfate to the FRAP reagent. Results were expressed as µmol of Fe^{+2}/100 g of fresh weight.

Oxygen radical absorbance capacity-fluorescein

The ability of fruit extracts to trap the peroxyl radical was determined according to the method proposed by Ou *et al.* (2001) and validate by Bisby *et al.* (2008). The peroxyl radicals generated by the thermal decomposition of AAPH reacted with fluorescein to produce a nonfluorescent product over time. Briefly, 20 **µl** of different concentrations of fruit extracts were preincubated for 5 minutes with 120 **µl** of fluorescein (100 nM) in a 96-well flat-bottom black plate. After which, 60 **µl** of AAPH (600 mM) was added to each well and the fluorescence was recorded using a Fluoroskan Ascent equipment (Thermo Scientific, Waltham, MA) every 56 seconds for 150 minutes at excitation and emission wavelengths of 485 and 538 nm, respectively. The results were expressed as µmol Trolox equivalents/g of fresh weight.

Hydrogen peroxide scavenging activity

The method described by Doerge *et al.* (1997), with some modifications, was used to determine the hydrogen peroxide scavenging activity of fruit extracts. Briefly, 900 **µl** of HBSS buffer, 100 **µl** of H_2O_2 (10 mM), and 10 **µl** of fruit extracts at different concentrations were incubated for 30 minutes. After incubation, 50 **µl** of guaiacol and 10 **µl** of horseradish peroxidase (0.6 µg/ml) were added and incubated again for 20 minutes. The scavenging activity was determined in a Multiskan EX microplate reader (Thermo Scientific, Waltham, MA) at 450 nm. Results were expressed as µmol Trolox equivalents/g of fresh weight.

Scavenging of the nitric oxide radical

Nitric oxide is generated by solubilizing sodium nitroprusside in an aqueous solution, where it is transformed into nitrite by the oxygen present in the medium. The produced nitrites are easily quantified by the spectrophotometric assay based on the Griess reaction (Rao, 1997). We incubated 990 **µl** of sodium nitroprusside solution (10 mM) in PBS with 10 **µl** of different concentrations of fruit extracts for 4 hours at room temperature. After incubation, equal volumes of Griess reagent and the samples were mixed. The nitric oxide concentration was determined by spectrophotometry at 550 nm in a Multiskan Go microplate reader (Thermo Scientific, Waltham, MA) by comparing to a standard curve of $NaNO_2$ (1–200 µM). Results were expressed as µmol of results were expressed as µmol of caffeic acid equivalents/g of fresh weight.

Cellular ROS determination by DCFH-DA

Cell culture

Murine macrophage RAW cell lines (ATCC® TIB-71™, Rockville, MD) were maintained in the DMEM supplemented with 10% heat-inactivated FBS in a humidified 5% CO_2 atmosphere at 37**°C.**

Assessment of cell viability

The toxicity of fruit extracts on RAW 264.7 macrophages was evaluated using the 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric method (Scudiero *et al*., 1988). RAW 264.7 macrophages were seeded in sterile 96-well plates (2×10^4 cells/well) and incubated at 37°C for 48 hours, then the medium was removed, and cells were washed with phosphate buffer saline (PBS) and treated for 30 minutes with different concentrations of fruit extracts, after which, they were activated with LPS (1 µg/ml) and incubated again for 24 hours at 37°C. Following exposure, the cell culture medium was discarded and 100 µl of MTT solution (0.25 mg/ml) was added to each well of the plate. The plates were incubated at 37ºC for 4 hours in the CO_2 incubator. Finally, 100 µl of DMSO was added to dissolve the formazan crystals, and the absorbance was measured at 550 nm by a Multiskan EX microplate reader (Thermo Scientific, Waltham, MA). In each trial, a group of cells not exposed to the extracts was included as the negative control and a group exposed to Triton X-100 (20%) as control of maximum toxicity. The viability percentages were calculated, considering the negative control group as 100% viability.

DCFH assay

The formation of intracellular ROS was determined using the oxidation-sensitive dye DCFH-DA according to the protocol

described by Park *et al*. (2017), with some modifications. For that, macrophages RAW 264.7 (10×10^4 cells/well) were incubated in 96-well sterile black plates. After 24 hours of incubation, the cells were washed twice with sterile PBS to remove nonadherent cells. The remaining cells were incubated for 30 minutes with different concentrations of fruit extracts and activated with LPS (1 µg/ml). After 24 hours of incubation, the cells were washed three times with sterile PBS, stained with DCFH-DA (20 µM) in PBS, and incubated for 30 minutes at 37°C in the dark. Fluorescence intensity was measured in a 96-well Fluoroskan Ascent plate reader (Thermo Scientific, Waltham, MA) at an excitation wavelength of 485 nm and an emission wavelength of 538 nm. Quercetin was used as the positive control.

Statistical analysis

Antioxidant activity results of three independent assays were reported as mean ± SEM. A one-way analysis of variance was used to analyze the data, followed by Tukey's and Dunnett's comparison tests. *P*-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Fruits, like vegetables, provide nutrients, such as vitamins and minerals, as well as nonnutritive secondary metabolites with biological activity that can contribute to improving people's health and reducing the risk of chronic NCD. Among these metabolites, phenolic compounds stand out, which have several pharmacological properties and are recognized for their potent antioxidant activity (Liu, 2013). In this work, we evaluated the antioxidant potential as well as the content of total phenolic compounds of ten extracts of fruits grown in Colombia using seven different methods. The information of fruits selected for this study and their state of maturation is presented in Tables 1 and 2.

Quantification of phenolic compounds

Results of the quantification of phenolic compounds of fruits under study are presented in Table 3. Considering the broad range of variations in the total phenolic compounds, the fruits were classified into three groups: with a high content (>80 mg GAE/100 g FW), a moderate content (80> mg GAE/100 g FW >40), and a low content (<40 mg GAE/100 g FW). The results show that only banana passion and sour guava presented high contents of phenolic compounds. On the contrary, yellow pitaya, golden berry, purple passion fruit, sapote, kalipatti sapota, tamarillo, and soursop showed moderate content. Lulo had the lowest content of these compounds.

DPPH, ABTS, and FRAP assay

Table 3 presents the results of the scavenger capacity of DPPH and ABTS free radicals as well as the FRAP of the ten fruit extracts under study. It can be observed that the extracts of banana passion and sour guava presented the best scavenging activity of radicals DPPH and ABTS and the best activity of ferric ion reduction. On the contrary, the extracts of yellow pitaya, golden berry, purple passion fruit, sapote, kalipatti Sapota, tamarillo, soursop, and lulo had a moderate activity. These results are related to the content of phenolic compounds, showing high correlation coefficients in all cases (DPPH: $r = 0.978$; ABTS: $r = 0.979$; and FRAP: $r = 0.981$). These results are similar to those reported by other studies, where

Table 1. Information on fruits included in this study.

Fruits	Part used	Extract yields (%)	Voucher number	Collection site
Yellow pitaya *Hylocereus megalanthus*	Pulp and seed	24.54	199083	Fusagasugá (Cundinamarca) (4°20′14″ N, 74°21′52″ O) Altitude: 1,800 masl Precipitation: 950 mm of rain per year Climate: 8°C–28°C
Banana passion *P. cumbalensis*	Pulp	9.78	199085	
Purple passion fruit *P. edulis*	Pulp	13.04	199081	
Golden berry *P. peruviana*	Whole fruit	18.08	199086	
Tamarillo *S. betaceum*	Pulp	9.10	199080	
Lulo *S. quitoense*	Pulp and seed	5.46	199084	
Soursop *A. muricata*	Pulp	13.12	201951	Turbana (Bolívar) (10°16′35″ N, 75°26′19″ O) Altitude: 138 masl Precipitation: 778 mm of rain per year Climate: 23°C–32°C
Kalipatti sapota *M. zapota*	Pulp	32.27	201955	
Sapote *P. sapota*	Pulp	26.26	201953	
Sour guava *P. friedrichsthalianum*	Pulp	12.01	201952	

The part of the fruit used to carry out the study and prepare the extracts was selected based on the way people eat the fruit. Yields were calculated as: [(grams of lyophilized pulp/grams of fresh weight) × 100].

Table 2. Physicochemical parameters of the fruits.

Fruits	Brix	pH	Total acidity (g/100 ml)
Yellow pitaya	17.68 ± 0.27	4.58 ± 0.01	0.11 ± 0.01
Banana passion	10.62 ± 0.11	3.41 ± 0.03	1.23 ± 0.04
Purple passion	15.04 ± 0.46	3.13 ± 0.04	2.97 ± 0.25
Golden berry	14.20 ± 0.06	3.52 ± 0.006	2.15 ± 0.01
Tamarillo	9.61 ± 0.14	3.61 ± 0.004	2.18 ± 0.03
Lulo	9.08 ± 0.16	3.13 ± 0.007	3.33 ± 0.09
Soursop	12.57 ± 0.38	4.06 ± 0.04	0.62 ± 0.04
Kalipatti sapota	25.37 ± 0.30	5.25 ± 0.08	1.80 ± 0.18
Sapote	22.33 ± 0.37	6.75 ± 0.05	1.07 ± 0.02
Sour Guava	8.43 ± 0.20	2.86 ± 0.02	3.80 ± 0.09

The results represent mean ± SEM (n = 9) from three independent experiments.

Table 3. Quantification of phenolic compounds and antioxidant potential of the extracts by the DPPH, ABTS, and FRAP methods.

Fruits	Phenolic compounds	DPPH	ABTS	FRAP
Banana passion	249.5 ± 0.064	1959.7 ± 1.288	4257.3 ± 0.622	5962.6 ± 3.241
Sour guava	80.9 ± 0.010	374.5 ± 0.083	822.3 ± 0.110	1607.4 ± 0.220
Yellow pitaya	59.1 ± 0.013	177.1 ± 0.073	323.8 ± 0.026	811.2 ± 0.201
Golden berry	53.3 ± 0.011	78.9 ± 0.018	228.6 ± 0.034	419.7 ± 0.125
Purple passion fruit	50.3 ± 0.006	93.3 ± 0.030	209.8 ± 0.027	312.4 ± 0.149
Sapote	49.0 ± 0.004	136.5 ± 0.030	310.7 ± 0.015	549.2 ± 0.094
Kalipatti sapota	44.7 ± 0.008	73.2 ± 0.034	193.9 ± 0.044	412.2 ± 0.156
Tamarillo	41.9 ± 0.007	72.0 ± 0.018	197.6 ± 0.023	427.2 ± 0.070
Soursop	40.4 ± 0.008	64.3 ± 0.018	183.8 ± 0.023	204.1 ± 0.091
Lulo	31.8 ± 0.006	84.5 ± 0.036	144.2 ± 0.008	341.2 ± 0.147

Phenolic compounds were calculated as mg gallic acid/100 g of fresh weight. Results of DPPH and ABTS were expressed as micromoles of Trolox equivalents (TEs) per 100 g of fresh weight (µmol of TEs/100 g of FW) and FRAP results as micromoles of Fe^{+2} per 100 g of fresh weight (µmol of Fe^{+2}/100 g of FW). Results represent mean ± SEM (n = 12) from three independent experiments.

the free radical scavenging capacity of phenolic compounds is evident; being able to react with highly ROS, donating a hydrogen atom (HA), or transferring a single electron (SE) is all due to the low redox potential that it possesses (Kurek-Górecka *et al*., 2014). In fact, according to reaction mechanisms, antioxidant activity tests can be classified as a transference assay of an HA or an SE. FRAP and ABTS have been included in the category as the HA assays, while the DPPH assay can be classified in both groups since both reactions can occur, and the antioxidant agent determines the final mechanism (Hidalgo and Almajano, 2017). It should be noted that although the extracts of golden berry, purple passion fruit, and sapote showed a similar content of phenolic compounds, sapote extract presented better free radical scavenging capacity. This may happen because the activity of phenolic compounds also depends on the concentration on the number of hydroxyl groups and the position of the aromatic ring (Kurek-Górecka *et al*., 2014).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) scavenging assays

The excessive production of ROS causes cell toxicity and target organ damage, causing the initiation of NCDs. The results of ROS scavenging activity by those fruit extracts showed that the extracts of yellow pitaya, sour guava, and banana passion exhibited potent peroxyl radical scavenging activity (Table 4). Extracts of sour guava and banana passion significantly decrease the amount of H_2O_2, which is an important precursor of hydroxyl radicals among other free radicals.

The NO radical can act directly as an effector molecule or as a second messenger, intervening in numerous signaling pathways (Zhang *et al*., 2017). When it is produced in excess, it can cause noxious effects, which are related to the capacity of this mediator to react with oxygen, superoxide, and transition metals, generating NOx, peroxynitrite, and heme-complexes, respectively. Peroxynitrite is a powerful, extremely cytotoxic oxidizing agent used by cells of the immune system within its defense mechanisms. However, when produced in excess, it can damage a wide range of molecules in cells, including proteins, DNA, and lipids, which leads to the initiation of a recognized pathophysiological mechanism causing the appearance of chronic NCDs (Pacher *et al*., 2007). Extracts of sour guava and banana passion presented an important nitric oxide radical scavenging effect (Table 4). It is remarkable that, once again, the fruit extracts with the major activity were those with the highest content of phenolic compounds.

Table 4. Scavenging effect on reactive oxygen and nitrogen species.

Fruits	ORAC	H_2O_2	NO
Banana passion	2409.82 ± 120.74	38.04 ± 1.71	70.81 ± 8.58
Sour guava	2602.08 ± 51.94	36.51 ± 1.90	168.81 ± 3.60
Yellow pitaya	2999.77 ±80.56	2.38 ± 0.01	ulq
Golden berry	350.13 ± 5.54	4.78 ± 0.44	ulq
Purple passion fruit	471.69 ± 4.91	13.79 ± 3.80	ulq
Sapote	143.87 ± 2.65	2.63 ± 0.15	31.50 ± 4.22
Kalipatti sapota	697.69 ± 7.82	0.66 ± 0.14	ulq
Tamarillo	169.71 ± 3.15	4.94 ± 0.21	18.41 ± 3.75
Soursop	311.24 ± 3.69	3.84 ± 0.01	ulq
Lulo	133.19 ± 1.84	1.99 ± 0.06	37.81 ± 1.44

ORAC and H_2O_2 scavenging capacities were expressed as micromoles of Trolox equivalents per gram of fresh weight (µmol of TEs/g of FW). NO scavenging capacity was expressed as micromoles of caffeic acid equivalents per gram of fresh weight (µmol of caffeic acid/g of FW). Results represent mean ± SEM (n = 12) from three independent experiments. Ulq = Under limit of quantification.

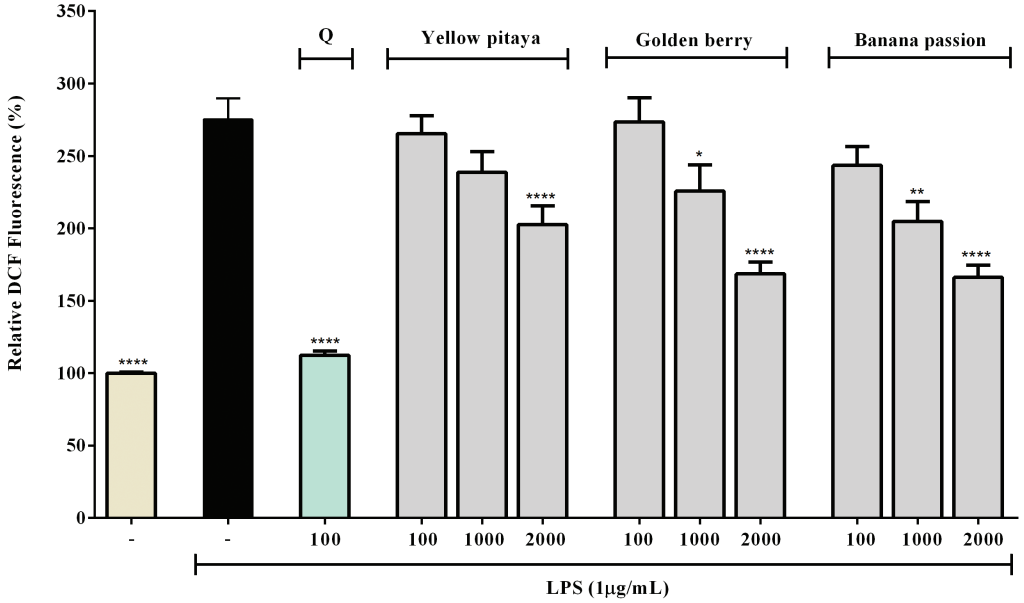

Figure 1. Effect of fruit extracts on the production of intracellular ROS in LPS-stimulated RAW 264.7 macrophages (1 µg/ml) for 24 hours. ROS are expressed as fold increase of 2′,7′-dichlorofluorescein fluorescence over LPS-untreated controls. Fruit extracts were evaluated to 100, 1,000, and 2,000 µg/ml. Quercetin (Q) (100 µM) was employed as the positive control. The results represent mean ± SEM. (*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$ are statistically significant compared to the LPS-treated group).

Production of intracellular ROS

The DPPH, ABTS, FRAP, and ORAC-FL assays are widely used to evaluate the antioxidant capacity of fruits (Hidalgo and Almajano, 2017). However, in recent years, antioxidant activity assays that include cellular models have been widely accepted since it is considered that the results obtained with them could better predict the antioxidant power of fruits.

In order to deepen the understanding of the antioxidant potential of the ten fruit extracts, their capacity to inhibit the generation of intracellular ROS was measured using DCFH-DA. The extracts of golden berry, banana passion, and yellow pitaya were the most active (Fig. 1). The extracts of sour guava, purple passion fruit, sapote, kalipatti sapota, tamarillo, and lulo showed a low inhibition on the formation of intracellular ROS (data not shown). The extract of soursop fruit was not evaluated because it had a toxic effect on the cells. Note that the extract of golden berry was more active than expected, as it was not the extract with the highest content of phenolic and it did not have the best free radical scavenging effect. The opposite happened with the extract of sour guava, which showed the best free radical scavenging activity and the highest content of phenolic compounds, but it did not exhibit a high capacity to inhibit the production of ROS in the cell assay. In such a way that there is no evidence of a correlation between antioxidant activity at the cellular level and the content of phenolic compounds, contrary to what happens with DPPH, ABTS, and FRAP free radical scavenging assays. It could be because the antioxidant properties of polyphenols depend on the physicochemical environment, which is very complex within a biological matrix, while it is more stable in

ABTS, DPPH, and FRAP free radical scavenging assays (Scalbert *et al*., 2005). Furthermore, it is also possible that the antioxidant activity shown by these fruit extracts is due to the presence of other secondary metabolites.

This study provides new information on the antioxidant properties of yellow pitaya, banana passion, lulo, sapote, and sour guava. The results of the scavenging effect on DPPH, ABTS, NO, and peroxyl radicals, as well as the inhibition of the generation of intracellular ROS in macrophages, showed that the total ethanolic extracts obtained from the edible parts of banana passion (*P. cumbalensis*), yellow pitaya (*H. megalanthus*), and golden berry (*P. peruviana*) make them the most promising fruits as antioxidants. These results support the ethnopharmacological use of these three fruits, concerning the treatment of various chronic NCDs, in addition to being an essential foundation to continue promoting their consumption.

CONCLUSION

The fruits like banana passion (*P. cumbalensis*), yellow pitaya (*H. megalanthus*), and golden berry (*P. peruviana*) are a significant source of antioxidant compounds. With the results obtained in this work, we can propose these fruits as functional foods that can be used to enhance health. Nevertheless, these fruit extracts should be evaluated in *in vivo* models to ensure the bioavailability of the active compounds and should be studied more in-depth regarding their phytochemical characterization to identify the compounds responsible for this activity.

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AUTHORS' CONTRIBUTIONS

RS and LF conceived the study; RS, YO, CD, JC, and LF supervised the study and designed the experiments; NM and JC carried out the experiments; JC and LF wrote the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

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