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Antioxidant Profile Characterization of a Commercial *Paullinia cupana* (Guarana) Extracts

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ABSTRACT

The Brazilian guarana has been used since pre-colombian times as a tonic aphrodisiac or stimulating beverage. However, the current literature about the antioxidant properties of guarana is restricted to a few studies and still remains poorly understood. In this study we showed the full guarana commercial extract redox properties against several radicals and reactive species since the overload of these molecules are involved in the pathogenesis of several diseases. All tested concentrations (1, 10, 100 and 1000 μg/mL) guarana extract presented high efficiency in quenching peroxyl radical (~1 μg/mL), malondialdehyde (60 μg/mL), hydroxyl radical (63 μg/mL) and nitric oxide radical (84.1 μg/mL) and chelating iron (46 μg/mL) at low $IC_{50.}$ Since the reactive oxygen/nitrogen species and free radicals have pivotal roles in the initiation and/or progression in several diseases, such as neurodegenerative and cardiovascular diseases it is sovereign importance to know about the chemical profile and redox-active properties regarding medicinal plants candidate to being alternative forms in prevention or treatment of diseases. Thinking about this, our team provided the first milestone regarding the full redox profile of amazon *Paullinia cupana* (guarana) extracts.

1. Introduction

The Plantae Kingdom is considered one of the largest and diverse sources of bioactive molecules. Many plants used in folk medicine were the basis for the discovery and characterization of several drugs clinically used nowadays [1]. In fact, the worldwide use of folk medicinal plants is very significant data from the World Health Organization (WHO) show that about 80% of the world population uses herbal plants to relief diverse painful or unpleasant symptoms. In addition, several plants are usually consumed *in natura* or as a dietary supplement.

Guarana (*Paullinia cupana*), a rain forest bushy plant, from amazon basin cultured by its caffeine-polyphenol rich-seeds [2], which are the most physiologically active ingredient in many energy drinks. The US food and drug administration (FDA) [3] also consider guarana a safe dietary supplement. Despite of being considered a safe supplement there is little information available regarding it bioactive compounds and their biological properties. Although much of the guarana bioactivity is attributed to the caffeine content of the extract a growing scientific literature points out to several other biological active components. On the other hand, it has not been established whether or not these different properties are due to caffeine alone or to the other compounds present in guarana seeds. In a previous characterization study our team identified the catechin, epicatechin, epicatechin gallate as main polyphenol compounds (flavan-3-ols) present in the commercial guarana powder [4]. All of this polyphenols are known as powerful antioxidants. We may also consider the presence of a synergistic effect of all components of the extract it means the synergic effect between polyphenol content and xanthine content.

Previous studies have reported anti-bacterial, antioxidant, chemopreventive and anti-mutagenic activities for guarana extracts [4-10].

It is well known that oxidative or nitrosative stresses probably are pivotal components in the onset and progression of chronic diseases such as atherosclerosis, neurodegenerative disorders (Alzheimer Disease - AD, Parkinson Disease - PD) [11, 12] and diabetes [13]. In this context, supplementation with antioxidants such as vitamin E, ascorbic acid, omegas and glutathione precursors have been extensively studied along the last decades. In the flip side, due to an intrinsic capability of producing a variety of antioxidant compounds mixtures from its secondary metabolism natural functional foods have been considered a promising alternative in the modulation or attenuation of oxidative stress and its associated deleterious effects in chronic disease landscapes [14].

Taking into account that the range of guarana antioxidant properties remains to be investigated the aim of this work was to characterize the redox-active properties of this dietary supplement in an attempt to improve the knowledge about its antioxidant properties. In this intent, we performed some in vitro scavenging activity assays against different oxidants.

2. Experimental Methods

2.1 Chemicals

Guarana (*Paullinia cupana* Mart.) extract powder was obtained from Lifar Ltd. (Porto Alegre, RS, Brazil). Chemicals for oxidation of 2-deoxy-D-ribose assay: The 2-deoxy-D-ribose (31170-5G-F), Phosphoric Acid (W290017), phosphate buffered saline - PBS (P5368), iron II sulfate heptahydrate (215422), hydrogen peroxide (V000194), sodium hydroxide (V000101), 2-thiobarbituric acid (T5500). Chemicals for nitrite assay: Griess reagent (03553), sodium nitroprusside (71778), phosphate buffered saline (P5368). Chemicals for *in vitro* Thiobarbituric reactive substances assay (TBARS): Trichloroacetic acid (T6399), phosphate buffered saline (P5368), 2-thiobarbituric acid (T5500). Chemicals for total reactive antioxidant potential assay/total antioxidant reactivity (TRAP/TAR): Luminol (123072), AAPH (2,2′-Azobis (2-methylproprionamidine) dihydrochloride (440914)), phosphate buffered saline (P5368), chemicals for super-oxide dismutase like activity: (−)-epinephrine (E4250), catalase (C9322), glycine (410225). Chemicals for catalase-like activity: phosphate buffered saline (P5368), hydrogen peroxide (V000194).

Chemicals for ferric reducing antioxidant power assay (FRAP): 2.4.6-tris(2-piridyl)-s-triazine. Chemicals for ferrozine assay: 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4′′-disulfonic acid sodium salt (82959) were obtained from Sigma Chemical Co. (St. Luis, MO, USA). Caffeine were obtained from Sigma Chemical Co. (St. Luis, MO, USA).

Trolox 97% was purchased from ACROS-ORGANICS (New Jersey-USA). The egg yolks used on in vitro TBARS assay were obtained from local commercial establishments.

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2.2 Guarana Aqueous Extract Preparation, Polyphenol Assay and Chemical Characterization

The detailed chemical composition of guarana aqueous extracts and polyphenol identification assay was performed as previously described [4]. The preparation of guarana aqueous extracts was performed as previously described procedures [4, 15].

2.3 Guarana, Caffeine and TROLOX® Concentrations

Briefly, we added 50 mg of guarana powder to 10 mL PBS, vortexed vigorously and incubated to 37 °C for 15 minutes, the solution achieved 5 mg/mL final concentration. From this solution we performed serial dilutions to obtain the concentrations used in this study; 1, 10, 100 and 1000 μg/mL. The caffeine solution was prepared by dissolving 10 mg of caffeine in 10 mL PBS, vortexed and incubated to 37 °C for 15 minutes reaching a 1 mg/mL final concentration. From this, we performed a dilution to achieve 40 μg/mL. This concentration of caffeine was used because it equivalent to those found in the highest concentration of guarana extracts (1000 μg/mL). The TROLOX® final concentrations herein used was 200 nM according reference number [16].

2.4 Total Reactive Antioxidant Potential (TRAP) and Total Antioxidant Reactivity (TAR)

TRAP and TAR were used as an index of non-enzimatic antioxidant capacity and peroxyl scavenging activity of guarana extracts and caffeine. This assay is based on the quenching of luminol chemoluminescence (CL) of AAPH as the peroxyl radical generation source [4, 16-18]. The AAPH solution was prepared by adding 0.0542 g AAPH reagent to 20 mL of PBS pH 8.6 (120 mM AAPH final concentration) followed by 4 μL luminol 50 mM (0.01 mM final concentration) in the dark. The AAPH plus luminol are considered the radical generating system. We left this system to stabilize for additional 2 h before the first reading as previously validated [19]. The tested concentrations of guarana and caffeine were added to a mat 96/well microplate and the luminescence produced by the free radical production was quantified in a liquid scintillator counter (Wallac 1409, Perkin-Elmer, Boston, MA, USA) for 120 minutes.

Total antioxidant reactivity (TAR) were calculated as the ratio of the first reading in absence of samples (I0)/first reading of guarana and caffeine samples. It is important to highlight that TAR and TRAP are different evaluations obtained in the same experiment; TAR indicates the quality of the antioxidants present in the sample based on instant reactivity; TRAP indicates the amount and kinetic behavior of sample antioxidants. TROLOX® 200 nM final concentration was used as standard antioxidant. The results were calculated and expressed as percentage of area under curve (AUC) for TRAP and TAR.

2.5 Hydroxyl Radical-Scavenging Activity

The 2-DR oxidation assay is based on the capacity of a compound or mixture in inhibiting the oxidation of 2-deoxy-D-ribose (2-DR) by hydroxyl radicals. The 2-DR is incubated with a hydroxyl radical generation system, which produces malondialdehyde (MDA) end product. The mixture is then incubated with 2-thiobarbituric acid (TBA), which reacts with MDA and forms a chromophore quantifiable at 532 nm by spectrophotometry [16, 20]. The hydroxyl generating reactions consisted of H_2O_2 (100 μM final concentration), Fe^{2+} ($FeSO_4$ 6 μM final concentration) and 2-DR (5 mM final concentration) solutions in 20 mM PBS (pH 7.4). To measure guarana extracts and caffeine activity against hydroxyl radicals, such extracts were added to the system before H_2O_2 addition. Reactions were carried out for 15 minutes at room temperature and then stopped by the addition of 4% phosphoric acid (v/v). Thereafter, TBA 1% (0.3 g in 30 mL 50 mM NaOH v/v) was added the solutions were incubated for 15 minutes at 95 °C and then cooled at room temperature. The absorbance was measured at 532 nm and the results were expressed as percentage of MDA formed related to the system. Trolox 200 nM were used as standard antioxidant.

2.6 Nitric Oxide ($NO_{\cdot}$) Scavenging Activity

In this assay, Sodium Nitroprusside (SNP) was used as nitric oxide (NO) generating system. Once generated, NO interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction [21]. The reaction mixture (1 mL) containing both 11.11 mM SNP in 20 mM PBS was incubated with guarana and caffeine at 37 °C for 1 h. From this reaction mixture, aliquots of 0.1 mL were taken and mixed with 0.1 mL Griess reagent in a 96/well microplate. The absorbance was measured at 540 nm. Results were expressed as percentage of nitrite formed related to SNP alone. Trolox 200 nM was used as standard antioxidant.

2.7 In Vitro Thiobarbituric Acid Reactive Species (TBARS)

An adapted TBARS method was used to measure the antioxidant capacity guarana and caffeine using egg yolk homogenate as lipid-rich medium [22]. This method is based on measurement of the color produced during the reaction of thiobarbituric acid (TBA) with lipoperoxidation products, such as malondialdehyde and 4-hydroxynonenal [23]. Briefly, egg yolk was homogenized (1% w/v) in 20 mM PBS (pH 7.4) and sonicated at potency 4. 1 mL of homogenate was then homogenized with 0.1 mL of guarana and caffeine to achieve the tested concentrations. Lipid peroxidation was induced by addition of 0.1 mL of AAPH solution (120 mM). Control was just incubation medium without AAPH. Reactions were carried out for 30 minutes at 37 °C. After incubation, samples (0.5 mL) were homogenated with 0.5 mL of trichloroacetic acid (10% final concentration) and after centrifuged at 1200 g for 10 minutes. An aliquot of 0.5 mL from supernatant was mixed with 0.5 mL TBA (0.67%) and heated at 95 °C for 30 minutes. After cooling, 0.2 mL of the misture were added to 96/well microplate and the absorbance was measured at 532 nm. The results were expressed as percentage of MDA formed by AAPH alone (induced control). The TROLOX® 200 nM was used as standard antioxidant.

2.8 Determination of Superoxide Dismutase-Like Activity

The ability of guarana and caffeine to scavenge superoxide anion (“superoxide dismutase-like activity”) was measured as previously described [24]. Guarana and caffeine were mixed with 190 μL glycine buffer (50 mM, pH 10.2) and 5 μL of native catalase 100 U/mL. Superoxide generation was initiated by addition of 5 μL of epinephrine 2 mM, and adrenochrome formation was monitored at 480 nm for 10 minutes (32 °C). Superoxide production was determined by monitoring the reaction curves of samples and measured as percentage of the rate of adrenaline auto-oxidation into adrenochrome [25]. TROLOX® 200 nM was used as standard antioxidant.

2.9 Determinantion of Catalase-Like Activity

The capacity of guarana and caffeine to degrade the hydrogen peroxide (H_2O_2) added in an incubation medium (“catalase-like activity”) was measured as previously described [26]. H_2O_2 were diluted in 50 mM phosphate buffer (pH 7.4), to obtain a 1 mM final concentration was added to microplate 96/well with the guarana and caffeine already placed to achieve the tested concentrations.

The plate was then scanned in a spectrophotometric plate reader (SpectraMax 190. Molecular Devices) at 240 nm every 10 seconds for 5 minutes at 37 °C. Catalase-like activity was monitored based on the rate decomposition of H_2O_2. Data were expressed as percentage of the rate decomposition of hydrogen peroxide.

2.10 Ferric Reducing Antioxidant Power (FRAP)

This assay was used to verify the reductor potential of guarana extracts based on conversion of free ferric iron (Fe^{3+}) to ferrous (Fe^{2+}). Briefly, in dark room, we added 90 μL of diluted guarana extracts to 2.7 mL of FRAP reagent (25 mL 0.3 M acetate buffer pH 3.6, 2.5 mL 10 mM 2.4.6-tris(2-piridyl)-s-triazine and 20 mM ferric chloride). Afterwards, the mixture was homogenized and incubated to 37 °C during 30 minutes. The readings were performed at 595 nm and the FRAP reagent was used as a blank.

2.11 Chelating Activity on Ferrous Ions (Fe^{2+}) – Ferrozine Assay

Metal chelating activity was determined according to the method of Oke et al with some modifications. Briefly, the guarana (400 μL) was mixed with 50 μL $FeCl_2$ (2 mM) and the mixture was incubated at room temperature during 10 minutes. After, 200 μL of 5 mM ferrozine disodium salt (3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4′′-disulfonic acid sodium salt) in each tube. Then the content was shaken vigorously and left standing at room temperature for another 10 min. After the mixture reached equilibrium, the volume was completed to 4 mL with absolute ethanol and the absorbance was then measured at wavelength 560 nm using a microplate spectrophotometer. The chelating activity was calculated as the percentage (%) of inhibition of ferrozine-Fe^{2+} complex formation determined as: 1 – (A sample/A control) × 100, where A control is the absorbance of the only ferrozin-Fe^{2+} complex, and A sample is the absorbance of the guarana extracts and ferrozin-Fe^{2+} mixture. EDTA was used as a standard positive control.

2.12 Statistical Analysis

All Biochemical data were first submitted to distribution analysis test (Kolmogorov-Smirnov) and parametric data were analyzed using the one-way ANOVA followed by Tukey’s post hoc test. All data were analyzed with

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GraphPad Prism Software v.5.0 (GraphPad Software Inc, San Diego, CA, USA). Results were expressed as the mean ± SEM; p values were considered significant when $p < 0.05$.

3. Results and Discussion

3.1 Guarana Extracts Scavenging Activity against Peroxyl Radicals

A peroxyl generation system (AAPH) generates chemoluminiscence (CL) in constant rate and the effect of guarana in free radical CL is expressed as a percentage of area under the curve (AUC) over 120 minutes. The first evaluation of redox properties of guarana was through the TRAP/TAR assays. This in vitro method is suitable to evaluate the peroxyl scavenging activity present in many natural compounds [4, 7, 8, 16, 27, 28]. As presented in Fig. 1A, all tested guarana extract concentrations (1, 10, 100 and 1000 μg/mL) showed significant reduction of the AUC (area under curve) to 22.5, 2.17, 1.7 and 1.49% respectively, suggesting potent non-enzymatic antioxidant activities. Purified caffeine at equivalent concentrations also presented a minor, although significant, peroxyl scavenging activity (36% inhibition of luminescence) (Fig. 1A). Regarding TAR measurements, all the guarana concentrations tested also showed antioxidant capacity higher than purified caffeine (Fig. 2B).

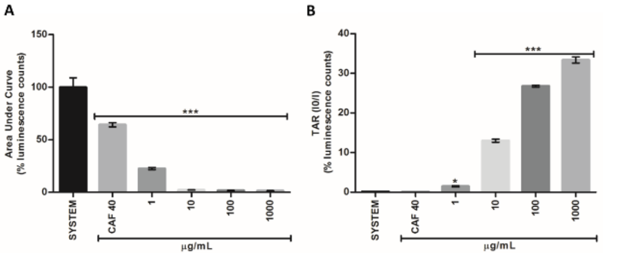

Fig. 1 TRAP and TAR analysis. (A) Total reactive antioxidant potential of different guarana concentrations and caffeine. (B) The TAR measurement was calculated by the ratio of CL intensity in the first reading in absence of extracts (I0)/CL intensity in the first reading in presence guarana and caffeine extracts and expressed as percentage. The vitamin E analogue Trolox® (200 nM) was used as standard antioxidant. The bars represent average ± SEM of three independent experiments. * $p < 0.05$, *** $p < 0.0001$. One-Way ANOVA followed by Tukey's post-hoc test

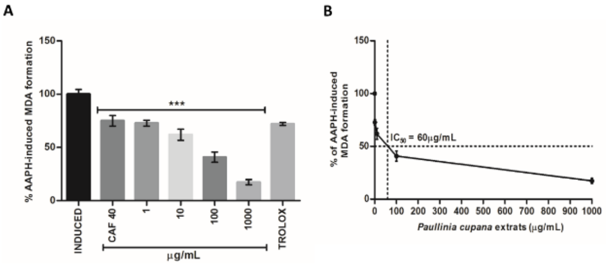

Fig. 2 (A) Percentage of AAPH-induced malondialdehyde (MDA) production. The lipid peroxidation was induced by AAPH free radical source in absence (induced control) and presence of guarana extracts and caffeine. Trolox® 200 nM was used as standard antioxidant. (B) Graphical showing of guarana extracts IC_{50} against peroxyl radicals. The bars represent average ± SEM of three independent experiments. *** $p<0.0001$. One-Way ANOVA followed by Tukey's post-hoc test

3.2 Guarana Prevents AAPH-Induced Lipid Peroxidation

Given the peroxyl scavenging activity of guarana extracts obtained from Fig. 1 experiments, we tested if it could also be able to attenuate the propagation of free radical chain reactions and damage to biomolecules. Then, the protective effects of guarana against AAPH mediated oxidative damage were assessed through measurement of MDA formation in a lipid-rich medium. All tested guarana concentrations (1-1000 μg/mL) were capable to protect lipids against peroxyl-induced damage by decreasing MDA formation in 27.2, 38.1, 59.2 and 82.7% respectively. The guarana extract concentration needed to decrease the MDA formation by 50% (IC_{50}) was 60 μg/mL (Fig. 2B). With a lesser effectivity, purified caffeine was also capable to protect by 25% the lipid environment against oxidative damage (Fig. 2A). Taken together, Figs. 1 and 2 showed that guarana extracts at low concentrations (from 1 μg/mL) are able to scavenge peroxyl radical and protect lipids from peroxidation (Fig. 2). This effect seems to be attributed to other compounds in a higher extent or to the combination of caffeine and the other components than the caffeine itself.

3.3 Guarana Inhibits Hydroxyl Radical Production by Reducing and Chelating Iron

To investigate the ability of guarana to scavenge hydroxyl radicals, we performed the in vitro 2-DR oxidation through Fenton Reaction. Guarana extract concentrations ranging from 1 to 1000 μg/mL were able to inhibit hydroxyl radical production in 41.1, 44, 54.2 and 82.3% of the control, respectively (Fig. 3A). Caffeine was also able to decrease 2-DR oxidation (28.87% of the control), but again, not as efficient as guarana extracts (Fig. 3A). The IC_{50} herein found for guarana extract was 63 μg/mL (Fig. 3B), indicating that guarana extract is able to decrease hydroxyl production at lower concentrations than caffeine (Fig. 3).

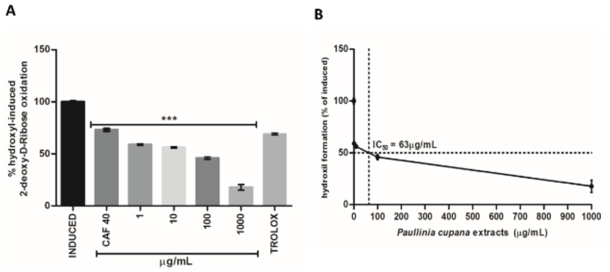

Fig. 3 (A) Hydroxyl-scavenging activity was measured by using hydroxyl-mediated 2-deoxy-D-ribose (2-DR) oxidative degradation. The induced control is MDA production from 2-DR oxidation with $FeSO_4$ and H_2O_2 alone. Other groups represent MDA production by Trolox 200 nM was used as standard antioxidant. (B) Graphic of the guarana IC_{50}. Bars represent average ± SEM of three independent experiments. *** $p < 0.0001$. One-Way ANOVA followed by Tukey's post-hoc test

The redactor potential of guarana extracts was assessed by FRAP assay. Guarana extracts were very efficient in reducing iron (Fe^{3+} to Fe^{2+}) in all tested concentrations. The concentrations of 1 and 10 μg/mL are capable to reduce iron but not as efficient as TROLOX®, while 100 μg/mL showed similar reducing potential to trolox. The higher concentration exhibited iron reducing capacity significantly increased compared to TROLOX®. Caffeine was not able to reduce iron indicating that probably this compound does not possess such property and the phenolic content is the main responsible for such effect (Fig. 4).

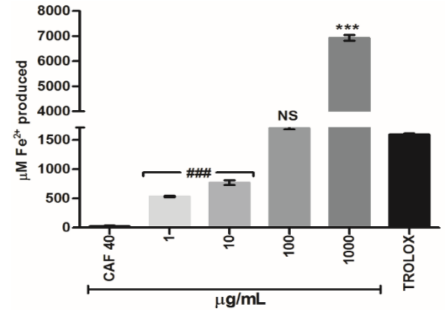

Fig. 4 Iron reducing activity was evaluated through the FRAP assay. The amount of reduced iron was calculated through Fe_2SO_4 standard curve. Bars represent average ± SEM of three independent experiments. ### $p < 0.0001$ indicating less efficiency than Trolox®. NS (Non-Significant related to Trolox®), indicating similar efficiency to Trolox®. *** $p < 0.0001$ indicating higher efficiency than Trolox®. One-Way ANOVA followed by Tukey's post hoc test

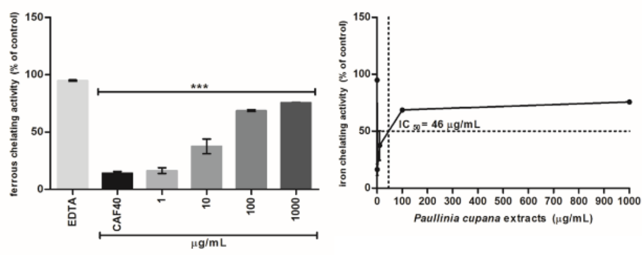

Fig. 5 Chelating activity evaluated by Ferrozine assay. **(A)** The ability of guarana extrats in prevent the Fe^{2+}-ferrozine complex formation, thus decreasing the red color compared to control. **(B)** Graphic of IC_{50}. Bars represent average ± SEM of three independent experiments. *** $p < 0.0001$. One-Way ANOVA followed by Tukey's post-hoc test

It is well known that iron is required to oxygen transport, cellular respiration and enzymatic activity such as catalase. Free Fe^{2+} is also capable to trigger the Fenton reaction thus generating hydroxyl radicals, leading to great oxidative damage. The ferrous-chelating activity is

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considered a suitable indicator in pro-oxidant scenarios where the ferrous specie is an important redox-active catalyst. The Ferrozine assay allows this quantification by the formation of complexes with Fe^{2+}, yielding an intense red chromophore quantifiable by spectrophotometry. However in the presence of chelating agents such as guarana the complex formation is prevented resulting in a decrease in the red color. The measurement of color reduction therefore allows for estimation of the metal chelating activity of the tested extracts. Here we observed for the first time that guarana extract was able to chelate ferrous ions efficiently in all tested concentrations as may be seen in Fig. 5B. The purified caffeine also demonstrated ability as an iron chelator but not as efficient as the guarana extracts showing that this alkaloid alone is not the main responsible for such property.

3.4 Guarana Capacity to Scavenge the Nitric Oxide ($NO_{\cdot}$) Radical

In order to evaluate the effect of guarana extracts against the nitrogen oxidative specie $NO_{\cdot}$, we quantified nitrite accumulation from the spontaneous degradation of Sodium Nitroprusside (SNP) to NO through Griess reaction. As shown in Fig. 6A, all tested guarana extract concentrations were able to significantly inhibit nitrite accumulation in 23.1, 42.92, 51.8 and 58.7% compared to SNP controls, respectively, yielding an 84.1 µg/mL as IC_{50} (Fig. 6B). Purified caffeine also decreased nitrite production, although with a lesser efficacy than guarana (37.3% inhibition).

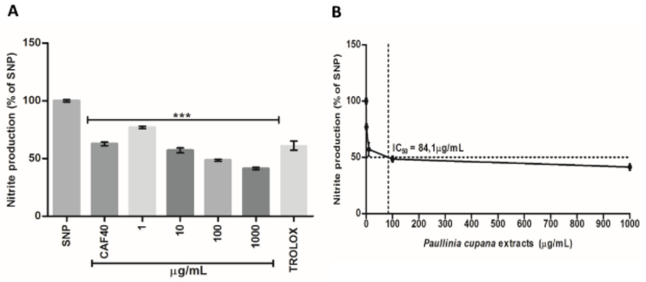

Fig. 6 (A) Nitric oxide was generated from decomposition of sodium nitroprusside (SNP) generating nitrite ions, which were measured by the Griess reaction in 96 well microplate. Nitrite production by SNP alone was compared to nitrite production by SNP in the presence of the tested guarana concentrations and 40 µg/mL caffeine. Trolox was used as standard antioxidant. (B) Graphic of IC 50. Bars represent average ± SEM of three independent experiments. *** $p < 0.0001$. One-Way ANOVA followed by Tukey's post-hoc test

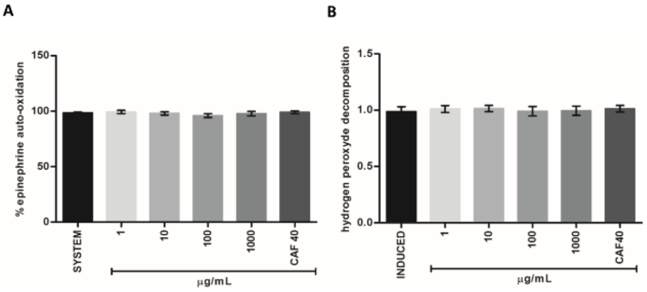

Fig. 7 SOD-like and CAT-like activities. (A) Superoxide dismutase-like (SOD-like) activity was determined by following formation of adrenochrome at 480 nm in absence and presence of guarana and caffeine. (B) CAT-like activity was measured in a phosphate reaction buffer (50 mM) with H_2O_2 with and without guaraná or caffeine. The experiments were performed in triplicate and bars represent average ± SEM of three different experiments. Statistical analysis was performed by One-way ANOVA followed by Tukey's post hoc test

3.5 SOD and CAT Like Activities

To assess the scavenging activity of guarana extracts against superoxide anions (SOD-like activity), we quantified the inhibition of superoxide-dependent adrenaline auto-oxidation to adrenochrome. Moreover, we also tested the ability of guarana extract to decompose hydrogen peroxide in vitro (CAT-like activity). Fig. 7A and B respectively, show that all tested concentrations of guarana extract did not presented any SOD nor CAT like activities.

The growing body of evidence has increasingly placed oxidative stress as pivotal condition for the onset and progression of various pathological conditions such as neurodegenerative and cardiovascular diseases. The plant kingdom is considered a powerful laboratory synthesis of bioactive molecules waiting to being studied from chemical to biological properties. Many plants have increasing attention from the scientific community, which is always in a constant search for properties of main bioactive compounds mainly antioxidant, anti-inflammatory, inhibiting enzymes, etc. This is the case of the Amazon guarana, where studies about it bioactive properties and mechanisms of action were intensified from 2007. But it antioxidant activity remained without the due highlight with few published studies. Regarding this our team provided for the first time the full redox active profile of the Brazilian guarana (*Paullinia cupana*).

It is well known that the peroxyl radical is the responsible for the propagation phase in the lipid peroxidation reaction, contributing to the increased damage to biological membranes and cell injury caused by oxidative species [29]. Taken together, both the Fig. 1A and B suggest that the effect of guarana extracts on non-enzimatic peroxyl scavenging activity is likely attributed to both caffeine and other xanthine and/or polyphenols present and already characterized in guarana extracts [4].

The lipid peroxidation generates as end-products aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), both known as dangerous molecules due to their reactivity [29-31]. These aldehydes can react with proteins modifying their structure and function, with DNA bases generating mutagenic lesions [29] and modifying cellular functions. MDA and 4-HNE are involved in several pathologies such as cancer, neurodegenerative and cardiovascular diseases [31-33]. Lipid peroxides reacts with iron ions generating even more peroxyl and hydroxyl radicals [29]. In this context it is suitable to accept that blocking lipoperoxidation could be an interesting mechanism of guarana antioxidant activity in biological systems.

Hydroxyl is one of the most reactive and damaging radical to the cells. Due to high oxidant power, hydroxyl radicals, attacks DNA molecules, causing strand breakage, contributing to mutagenesis and cytotoxicity [16, 29, 34]. In addition, hydroxyl radicals attack lipids damaging cell membranes increasing lipid peroxidation and cell oxidative damage.

It is well known that iron is required to oxygen transport, cellular respiration and enzymatic activity, such as catalase. Free Fe^{2+} is also capable to trigger the Fenton reaction, thus generating hydroxyl radicals, leading to great oxidative damage. The ferrous-chelating activity is considered a suitable indicator in pro-oxidant scenarios where the ferrous specie is an important redox-active catalyst. The Ferrozine assay allows this quantification by the formation of complexes with Fe^{2+}, yielding an intense red chromophore quantifiable by spectrophotometry. However in the presence of chelating agents, such as guarana the complex formation is prevented, resulting in a decrease in the red color. The measurement of color reduction therefore allows for estimation of the metal chelating activity of the tested extracts. Here we observed for the first time that guarana extract was able to chelate ferrous ions efficiently in all tested concentrations as may be seen in Fig. 5B. The purified caffeine also demonstrated ability as an iron chelator but not as efficient as the guarana extracts showing that this alkaloid alone is not the main responsible for such property.

Several evidences have shown that catechin, epicatechin, epicatechin galate, phenolic compounds present in guarana extracts [35], can reduce or chelate transition metals, as copper and iron, which are known to their harbor redox active properties [36]. In addition, an impairment in brain iron homeostasis is a key factor to early neuropathological events in Alzheimer's disease (AD), including oxidative stress, inflammatory processes, amyloid deposition, tau phosphorylation and neuronal cell cycle regulatory failure, leading to apoptosis [37]. This result suggests that guarana extract may be a useful resource for the prevention of iron metabolism impairment.

NO is a key modulating agent present in acute and chronic inflammation an important component in several pathological conditions, such as neurodegenerative, cardiovascular and pulmonary diseases. It is also an important messenger in Central Nervous System (CNS). In chronic inflammation NO may reach high concentrations thus interacting with superoxide anion, generating a potent oxidizing agent, peroxynitrite ($ONOO^-$). This radical can damage proteins, DNA and lipids, compromising the cellular integrity.

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Several studies have shown the anti-inflammatory benefits of natural compounds present in foods such as black tea, green tea, strawberry and coffee [38-41]. Our group also recently showed evidences about the protective effects of guarana extracts against SNP-mediated cytotoxicity in NIH-3T3 fibroblasts [15]. Another study reported the anti-allergic effects

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of guarana extracts in passive cutaneous anaphylaxis and mast degranulation models [42]. Taking into account the aforementioned results guarana extracts could be a promising alternative in preventing or/and controlling diseases where inflammation is a prevalent feature.

4. Conclusion

In this study, we provided the first milestone about the full redox-active profile of a commercial guarana powder. A growing number of evidences in literature supports that the antioxidant capacity, at low doses, of natural compounds is wide, but the most articles uses isolated methods, such as DPPH, to address this question. It is important to emphasize that oxidative stress is not caused by a unique radical molecule, but a combination of several molecules with differing chemical reactivity, stability and diffusion potentials. Based in this fact, it is more accurate to assess the antioxidant capacity of a natural compound in different contexts, e.g. considering several reactive species and radicals.

Here in we observed that all tested guarana extract concentrations displayed high antioxidant activity against several free radical sources. Such activity may be attributed to it polyphenol and xanthines content, as showed in Figs. 1-6, where caffeine (the main xanthine present in guarana) also presented antioxidant activity. However, our results suggest that caffeine content alone could not fully explain the extension of the observed antioxidant effects of guarana.

Commonly, the polyphenols are the main molecules responsible for the most of antioxidant activity in natural products. The major class of polyphenols found in guarana extracts is the flavan-3-ols (catechins), such as catechin, epicatechin and epicatechin gallate. Such molecules are described as the main responsible for the antioxidant properties found in several natural products. However, the caffeine content also can count to the total antioxidant and we cannot exclude it participation in the overall antioxidant effect of the extract. Our research group has been successful in demonstrating the effectiveness of natural compounds in inhibiting or preventing oxidative stress in diverse pathological scenarios such as cancer, Alzheimer and diabetes models. In addition, our group also has successfully characterized the redox-active properties of other natural compounds such as usnic acid, *Passiflora manicata*, *Hyptis pectinata*, *Lypia sidoides, Remirea maritima* extracts in a scenario where the natural products are gaining even more notoriety and attention of scientific community.

In this study, we observed that guarana extracts exerted antioxidant activity mainly against peroxyl free radicals, the harmful hydroxyl, by scavenging the own radical or reducing and chelating iron and thus preventing the Fenton Chemistry and also decreasing levels of nitric oxide whereas none effect on hydrogen peroxide or superoxide anion was observed. This reveals some important antioxidant mechanism whereby guarana supplements could be beneficial in preventing oxidative stress, a key event in the onset and progression of a variety of diseases.

We are aware that this study is limited to characterization of the redox profile in-vitro, but the first step towards the knowledge of the properties of a particular extract, in this case the guarana extracts is it complete chemical characterization that consist in the identification of main extract bioactive compounds already published in reference number 4, complete redox-active profile based in chemical reactions, enzyme inhibitory assays, etc. Regarding this the mentioned manuscript explored the complete redox-active profile of amazon guarana.

After this step, we set out to study biological properties, which are used experimental models (tissues, cells or animal models) where our group also showed for the first time, some biological properties as, anti-aggregating and protective effects of guarana against amyloid-beta peptide and several toxic aldehydes that occur in brains with Alzheimer's in an in vitro model of neuronal cells.

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