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Thássia Fernandes Santana de Macena [1]
Daiara Rakeli Simão Boyarski [1]
Denise Rocha Ramos Barbosa [1]
Rodolfo Castilho Clemente [1]

[1] Universidade Federal do Tocantins, Curso de Nutrição. Palmas, TO, Brasil.

Article from the dissertation research entitled "*Quantificação de compostos fenólicos, poder antioxidante e teor de açúcares em produtos comerciais a base de Camellia sinensis L.*," (Quantification of phenolic compounds, antioxidant power and sugar content in commercial products based on Camellia sinensis L.), presented in April 2019, at the Universidade Federal do Tocantins, Palmas campus, TO, Brazil..

Correspondence
Thássia Fernandes Santana de Macena
thassiamacena@gmail.com

Quantification of phenolic compounds, antioxidant power and sugar content in commercial products based on Camellia sinensis L.

Quantificação de compostos fenólicos, poder antioxidante e teor de açúcares em produtos comerciais a base de Camellia sinensis L

Abstract

Objective: To analyze and quantitatively compare phenolic compounds, antioxidant capacity and sugars present in infusions and soluble extracts of *Camellia sinensis* L, ***Methods****:* The study presents a completely randomized design, using samples for convenience, Three random samples of each type of tea, The analyzes of total phenolic compounds and flavonoids were determined by the Folin-Ciocalteu colorimetric method and aluminum chloride, respectively, total tannins by complexation with casein and condensates by the butanol-HCl method, The antioxidant capacity, by ferricyanide methodology and free radical scavenging by the radical 2,2-diphenyl-1-picryl-hydrazil, and reducing and non-reducing sugars, through the reagent 3-5 dinitrosalicylic acid. ***Result:****:* The infused extracts showed significantly higher amounts of total phenolic compounds and flavonoids compared to the soluble, This behavior was the same for tannins and antioxidant activity, The infusions obtained greater reducing power and capacity to reduce free radicals, Soluble extracts were highlighted, with a greater presence of sugars, These results were confirmed by the literature and there were no studies carried out with soluble extracts and methodologies similar to that performed here for comparison, ***Conclusion:*** The infusions studied in the present study were richer in bioactive and antioxidant compounds, favoring their benefits for the population, with soluble extracts having a greater presence of additional sugars,

Keywords: *Camellia sinensis*, Antioxidant, Phenolic Compounds, Sugars, Tea.

Resumo

Objetivo: Analisar e comparar quantitativamente compostos fenólicos, capacidade antioxidante e açúcares presentes em infusões e extratos solúveis de Camellia sinensis L. ***Metodologia:*** O estudo apresenta delineamento inteiramente casualizado, utilizando amostras por conveniência. Foram adquiridas três amostras aleatórias de cada tipo de chá. As análises de compostos fenólicos totais e flavonoides foram determinadas pelo método colorimétrico de Folin-Ciocalteu e cloreto de alumínio, respectivamente, taninos totais por complexação com caseína e os condensados pelo método do butanol-HCl. A capacidade antioxidante, pela metodologia do ferricianeto e sequestro de radicais livres pelo radical 2,2-difenil-1-picril-hidrazil, e os açúcares redutores e não redutores, através do reagente ácido 3-5-dinitrossalicílico. ***Resultado***: Os extratos infusos apresentaram quantidades significativamente maiores de compostos fenólicos totais e flavonoides em comparação ao solúvel. Esse

comportamento foi o mesmo para os taninos e atividade antioxidante. As infusões obtiveram maior poder redutor e capacidade de redução do radical livre. Os extratos solúveis foram destaque, com maior presença de açúcares. Esses resultados foram confirmados pela literatura e não houve trabalhos realizados com extratos solúveis e metodologias semelhantes ao realizado aqui para comparação. *Conclusão*: As infusões estudadas no presente trabalho foram mais ricas em compostos bioativos e antioxidantes, favorecendo seus benefícios para a população, tendo os extratos solúveis maior presença de açúcares adicionais.

Palavras-chave: Camellia sinensis. Ação Antioxidante. Compostos Fenólicos. Açúcares. Chá.

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations - FAO, there will be a 7.5% increase in green tea production each year, reaching 3.6 million tons in 2027.[1] The increase in production and consumption is explained by the broad knowledge of the population about the anti-inflammatory and antioxidant benefits of teas.[1] The power known as the natural antioxidant of this drink is due to the presence of active compounds such as polyphenols.[2] Besides that, teas are appreciated for their sensory characteristics of aroma and flavor and the presence of vitamins and minerals.[2]

ANVISA, through RDC nº. 277, of September 22, 2005, conceptualizes tea as the drink originated from vegetable species (s) used in fragments, smaller pieces or whole, having undergone some heat treatment, such as toasted, dried or fermented, and whether or not plus condiments that add flavor or aroma.[3] It also considers soluble products as the result of the dehydration process of an aqueous extract carried out by some physical method, from plant species (s) contained in the specific Technical Regulation for the tea.[3]

The leaves of *Camellia sinensis* L. (*C. sinensis* L.) are among the most consumed.[4,5] Common in the East, this plant belongs to the family of Theaceae and according to the type of processing to which it is submitted, it can give rise to different types of teas, including oolong tea, which undergoes a lighter fermentation process; black tea, which is fully fermented; and green tea, which is notfermented.[4-6]

Green tea undergoes an enzymatic inactivation process shortly after being harvested, making the enzyme polyphenoloxidase inert due to vaporization and drying.[5] This heating method prevents oxidation of phenolic compounds, such as catechins, polyphenols present in greater quantity, with about of 30% of the weight of its dried leaves.[2,5] Proteins, amino acids, lipids, polysaccharides, minerals and caffeine can also be found in the leaves of this tea.[7]

The antioxidant effect of tea from *C. sinensis* L. is the result of the great presence of phenolic compounds, which in turn are capable of sequestering free radicals, preventing oxidative reactions harmful to the functioning of the organism.[8,9] This fact generates health benefits, being associated with chemoprotective actions, thermogenic, anti-inflammatory, anticarcinogenic, antitumor, antiallergic and antiviral.[10,11] The literature also mentions its performance as a preventive for cardiovascular diseases, hypercholesterolemia, antibacterial activity, as well as the presence of minerals and vitamin K.[11]

For consumption, teas are found on the market in the form of fragmented and / or ground leaves or in sachets prepared by infusions.[12,13] Already available form of green tea is its configuration in soluble tea, presented in powder form, obtained through of the lyophilization process, which favors the conservation and longer storage of products.[14]

These forms of consumption of *C. sinensis* L. are carried out by the population with the addition of water for infusions or reconstitution of soluble extracts. Thus, there is a need for research related to soluble and infused green tea common in commerce, regarding its antioxidant capacity and bioactive compounds present, using methodologies that bring it closer to the way teas are consumed by the population.

Thus, this article aims to analyze and quantitatively compare phenolic compounds, antioxidant capacity and sugars present in infusions and soluble extracts of *C. sinensis* L.

METHODS

This study presents a completely randomized design, using samples for convenience. The analyzes took place at the Laboratório de Ciências Básicas e Saúde (Basic Sciences and Health Laboratory) (LACIBS) and

Complexo Laboratorial de Nutrição (Nutrition Laboratory Complex), both located at the Universidade Federal do Tocantins (Federal University of Tocantins), Campus Palmas.

Selection of raw material

Three samples of dry leaves of *C. sinensis* L. were purchased in natural products stores for the preparation of infusions and three samples of soluble powder extracts of *C. sinensis* L. for the preparation of reconstituted soluble extracts. For the choice of teas, the distinction of brands and suppliers was taken into account.

The soluble extracts had in their composition, according to the list of ingredients, in addition to the green tea extract (*C. sinensis* L.): maltodextrin, sucralose sweetener, citric acid acidulant, artificial flavorings and natural green dye.

Sample preparation

For the preparation of the infusions, 5 grams of dry leaves were weighed on a precision scale in a glass beaker, then drinking water was filtered and filtered from the network, heated (~ 95ºC) until completing 1L and left in contact for 5 minutes at room temperature. The content was filtered with commercial filter paper, obtaining a final concentration of 5 g / L of dry matter.

To prepare the reconstituted extracts, 5 grams of the samples of soluble extracts of *C. sinensis* L. were weighed in a glass beaker, diluted in drinking water and filtered from the network at room temperature until completing 1L and stirred manually with aid of the glass rod until complete dissolution. Soon after, they were filtered on commercial filter paper, obtaining a final concentration of 5 g / L of dry matter.

The values of the ratio of tea (g) to water (L) for the preparation of the samples were chosen taking into account their similarity in terms of the preparation and overall consumption of teas by the population. Likewise, drinking and filtered water from the network was used for infusions and reconstitutions of soluble extracts, and as a solvent in the methodologies applied for the experiments.

Experiment

For each analysis, the infused tea and reconstituted extracts were prepared before the beginning of the analysis. Thus, before the experiment was carried out, the teas remained in a glass beaker at room temperature, for immediate use. At the end of the procedure, the teas were discarded. There was no storage of teas already prepared for use the next day.

Quantification of total phenolics: used the methodology described by Bonoli et al.[15] Aliquots of 0.1 mL of sample were diluted in 0.5 mL of the reagent Folin-Ciocalteu and, later, 6 mL of distilled water were added, being vortexed for 1 minute. That done, 2 ml of 15% sodium carbonate solution were added, and again stirred for 30 seconds. After standing in the dark for 2 hours, the reading was performed on a spectrophotometer at 750 nm, using all reagents except the extract as "white". The total phenolic content was determined by interpolating the absorbance of the samples compared to a calibration curve made with gallic acid at concentrations of 10 to 1000 g/mL and expressed as µg equivalent of gallic acid (EAG) per mL of sample.

Quantification of total tannins: adaptation of the methodology of complexing tannins with proteins was used.[16] In tubes containing 1 ml of the samples, 100 mg of casein and 1 ml of distilled water were added, being vortexed for 1 minute and later resting at sheltered from light for 15 minutes for the complexation of tannin-protein. After resting, they were stirred again for 30 seconds, then centrifuged at 5000 rpm for 4 minutes. 0.2

ml of the supernatants were transferred in tubes and the protocol of total phenolics was repeated for the determination of simple phenolics.

The content of total tannins was calculated through the difference between total phenols and simple phenols, since the tannins were complexed and precipitated with casein.

Quantification of condensed tannins: it was performed using the butanol-HCl method.[17] Tubes containing 0.3 mL of the samples were added with 1.8ml of butanol-HCL 5%, vortexed and placed in a water bath at 100º C for 70 minutes. That done, they were cooled in a cold bath for 5 minutes to interrupt the reaction. The samples were read on a spectrophotometer at 550 nm, the blank of each sample being the same components, but without being subjected to a water bath. The content of condensed tannins was determined by interpolating the absorbance of the samples on a calibration curve, made with purified tannin from *Pinus pinaster* in concentrations of 10 to 500□g/mL and expressed as □g of tannin / mL of the samples.

Quantification of flavonoids: the methodology described by Dewanto et al.[18] Tubes containing 0.25 ml of the samples were added with 1.25 ml of distilled water and agitated with 75□l of 5% aqueous sodium nitrite solution and left to rest for 6 minutes. After that, 150□l of 10% aqueous aluminum chloride solution were added, stirred and left to stand for another 5 minutes. Then, 0.5 ml of 1M aqueous sodium hydroxide solution was added and stirred again. Then, the absorbances were read in a spectrophotometer at 510 nm, with the flavonoid content determined by interpolating the absorbance of the samples in a calibration curve made with quercetin in concentrations of 0.1 to 5 mg / mL, expressed as mg of flavonoids per mL of sample.

Ferric ion reducing power test: the ferricyanide methodology was used, optimized by Berker et al.[19] In tubes containing 1 mL of the samples (1 mL of distilled water to the blank), 6.3 mL of water, 0.2 mL were added of 1M hydrochloric acid, 1.5 ml of 1% potassium ferricyanide and 0.5 ml of 1% sodium dodecyl sulfate. After stirring, 0.5 ml of 0.2% ferric chloride was added and stirred again. After resting in the dark for 30 minutes, absorbances at 750 nm were read. The results were expressed as a percentage of iron reduction compared to the quercetin and ascorbic acid standards, both used in the same concentration of the extracts (1 mL).

Free radical scavenging: methodology was used using the radical 2,2-diphenyl-1-picryl-hydrazil (DPPH).[20] 250 ml of DPPH stock solution in absolute ethanol were prepared in a concentration of 40 µg / mL, maintained refrigerated and protected from light. A calibration curve was constructed at concentrations of 40, 35, 30, 25, 20, 15, 10, 5 and 1 µg / mL, from the absorbance values at 515 nm of all solutions, with ethanol as "white".

Dilutions of samples and standards in concentrations of 4, 8, 16, 31, 63, 125, 250, 500 and 1000 µg / mL were obtained and 0.3 mL of each dilution was reacted with 2.7 mL of stock solution DPPH. For “white”, ethanol was used instead of DPPH, and a “white” tube was made for each concentration. The readings of the absorbances of the reaction mixtures were performed at 515 nm after 1 hour. From the equation of the DPPH calibration curve and the absorbance values over 1 hour, for each concentration tested, the values of DPPH60 were determined, which is the remaining concentration of DPPH in the reaction medium after 60 minutes. That done, the percentage of remaining DPPH (% DPPHRem) was calculated, according to the equation:

$$\%DPPHREM = [DPPH]T=t /[DPPH]T=0x\ 100$$

where [DPPH] T = t corresponds to the concentration of DPPH after the reaction with the extract and [DPPH] T = 0 is the initial concentration of DPPH, that is, 40 mg / mL.

The efficient concentration, amount of sample needed to decrease the initial DPPH concentration by 50% (EC_{50}), was determined from an analytical curve, obtained by plotting the sample concentrations (µg / mL) or positive control on the abscissa. and in the ordinate, the percentage of DPPH remaining (% DPPHREM).

Reducing and non-reducing sugars: it was carried out using the reagent 3- 5-dinitrosalicylic acid (DNS) .[21,22] For the quantification of reducing sugars (AR), 0.5 ml aliquots of the extracts (0.5 ml of distilled water as white) and stirred 0.5 mL of the reagent, being placed in a water bath at 100ºC for 15 minutes and, subsequently, submitted to an ice bath for 5 minutes to interrupt the reaction. The absorbances were read at 540 nm and interpolated on a calibration curve made with anhydrous glucose solution at 2 mg / mL, the results being expressed in mg of AR / mL of extract.

For non-reducing sugars (ANR), 2 ml aliquots of the samples were added with 2 ml of 2M HCl and subjected to a water bath at 100ºC for 10 minutes for the hydrolysis of the sugars, being subsequently submitted to an ice bath for 5 minutes and stirred with 2 mL of 2M NaOH to neutralize the acid. Then, 0.5 mL aliquots of these mixtures were subjected to the same AR treatment. The absorbances were plotted on the same calibration curve previously used, and the results were obtained using the equation ANR = A * 3 - AR, with A being the result obtained by interpolating the sample absorbance on the curve, multiplied by 3 due to the dilution process. necessary for the hydrolysis process of the samples, being subsequently subtracted by the content of reducing sugars.

For the discussion, articles in English and Portuguese were selected with methodologies more similar to those used in this work, on the platforms: Google Scholar, PubMed, SciELO and ScienceDirect, in the years 2006 and 2017, since no more current articles were found using the same sample and methodology of this study.

Statistical analysis

All analyzes were performed in triplicates. The data obtained were grouped, calculated and analyzed using descriptive statistics, and for comparison between means, two-way analysis of variance - ANOVA, followed by the Tukey test, with a significance level of 5%. The calculations of the calibration curves and statistical analyzes were performed using Microsoft Office Excel® 2013 software and the Graph Pad Prism® 7.0 statistical program.

RESULTS

Phenolic compounds

After preparing the samples of the infused tea and the soluble extracts, analyzes were performed in triplicates. Table 1 shows the results of total phenols, flavonoids and total tannins for infusions and soluble extracts of *C. sinensis* L.

Table 1. Quantification of total phenolics, flavonoids and total tannins between infusions and soluble extracts of *C. sinensis* L., Palmas-TO, 2019.

	Total Phenolics (µg EAG/mL) Average±SD*	flavonoids (mg/mL) Average±SD*	Total Tannins (µg/mL) Average±SD*
Infusions			
Infusion 1	818.3 ± 18.7^{a}	1.77 ± 0.02^{b}	640.8± 33.7^{a}
Infusion 2	206.2 ± 9.8^{c}	1.54 ± 0.04^{c}	105.4± 9.15^{c}
Infusion 3	250.0 ± 4.0^{b}	2.20 ± 0.09^{a}	131.0± 7.91^{b}

Table 1. Quantification of total phenolics, flavonoids and total tannins between infusions and soluble extracts of *C. sinensis* L., Palmas-TO, 2019.(Continues)

	Total Phenolics (µg EAG/mL) Average±SD*	flavonoids (mg/mL) Average±SD*	Total Tannins (µg/mL) Average±SD*
Soluble Extracts			
Soluble 1	129 ± 4.5^{d}	0.20 ± 0.01^{d}	92.78± 2.32^{d}
Soluble 2	78.4 ± 7.6^{e}	0.09 ± 0.00^{d}	56.62± 9.88^{de}
Soluble 3	14.36 ± 1.2^{f}	0.06 ± 0.09^{d}	7.46± 1.52^{e}

*SD=Standard Deviation. Average columns with similar letters do not differ statistically from each other by the Tukey test (p<0.05).

According to table 1, the infused extracts had a greater relevance in amounts of total phenolics, with emphasis on the infused 1, which also had a higher content of tannins and flavonoids, with the infused 3.

The condensed tannin test was also performed, but only the infusion1 obtained a positive result, with 64.3 ± 2.91 µg of tannins / mL of sample. It must be taken into account that the total tannin methodology is not able to differentiate the type of tannin present in the sample; in this way, the extracts can be rich in hydrolyzable tannins, for example, but poor in condensed tannins.

Ferric ion reducing power test

Table 2 shows the antioxidant activity of infusions and soluble extracts of *C. sinensis* L. by the test of the reducing power of the ferric ion, in comparison to the quercetin and ascorbic acid patterns.

Table 2. Reducing power between infusions and soluble extracts of *C. sinensis* L., compared to quercetin and ascorbic acid standards. Palmas-TO, 2019. Source: Data found by the author.

	Reducing power (Absorbance) Average±SD*	% Reduction (Quercetin standard)	% Reduction (ascorbic acid standard)
Standards			
Ascorbic acid	3.18 ± 0.13^{a}	--	--
Quercetin	1.79 ± 0.0^{b}	--	--
Infusions			
Infusion 1	2.92 ± 0.13^{c}	162	88.4
Infusion 2	2.3± 0.04^{d}	128.3	75.7
Infusion 3	2.05 ± 0.08^{e}	114.6	63.9
Soluble Extracts			
Soluble 1	1.77 ± 0.01^{a}	98.1	53.6
Soluble 2	1.47 ± 0.06^{f}	82.2	48.5
Soluble 3	0.22 ± 0.0^{g}	12.4	6.9

*SD= Standard Deviation.
Average columns with similar letters do not differ statistically from each other using the Tukey test (p <0.05).

According to the table above, all infused extracts showed a greater reduction power than soluble extracts. Infusion 1 stood out for presenting a reduction percentage closer to the ascorbic acid gold standard (88.4%).

Free radical scavenging

Table 3 shows the antioxidant capacity of infusions and soluble extracts of *C. sinensis* L., through the percentage of DPPH remaining compared to the quercetin and BHT standards, by the free radical scavenging experiment.

Table 3. DPPH values remaining from infusions and soluble extracts of *C. sinensis* L. and BHT and quercetin standards. Palmas-TO, 2019. Source: Data collected by the author

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	Samples Concentrations (%)								
	04 µg/mL	08 µg/mL	16 µg/mL	32 µg/mL	63 µg/mL	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
Infusions									
Infusion 1	79.7^{aA}	68^{aA}	57^{aA}	30^{abA}	6.8^{aA}	5.9^{aA}	5.8^{aA}	4.8^{aA}	4.4^{aA}
Infusion 2	89.8^{aB}	89.2^{aB}	88.7^{aB}	84.9^{aB}	73.8^{bB}	61.2^{abB}	32.3^{abB}	8.6^{aB}	7.1^{aB}
Infusion 3	86.4^{aB}	84.9^{aB}	81.0^{aB}	77.6^{aB}	68^{bcB}	49.0^{abB}	15.2^{aB}	6.2^{aB}	5.3^{aB}
Soluble Extracts									
Soluble 1	88.9^{aB}	86.7^{aB}	79^{aB}	75.6^{aB}	69^{bB}	52^{abB}	29.2^{abB}	5^{aB}	4.7^{aB}
Soluble 2	90^{aBC}	90^{aBC}	88^{aBC}	83^{aBC}	76^{bBC}	61.4^{abBC}	41^{abBC}	15.2^{abBC}	5.3^{aBC}
Soluble 3	91.1^{aC}	88^{aF}	86.5^{aC}	86.1^{aC}	85.8^{bC}	85^{bC}	77.5^{bC}	68.2^{bC}	51.3^{aC}
Standards									
BHT	92.7^{aB}	90.6^{aB}	87.9^{aB}	80.9^{aB}	70.2^{bB}	52.3^{abB}	38.5^{abB}	20.3^{abB}	5.6^{aB}
Quercetin	61.2^{aA}	44.7^{aA}	40.8^{aA}	6.9^{bA}	5.4^{adA}	5.4^{acA}	5.4^{aA}	5,4^{aA}	5,4^{aA}

Columns and lines of means with similar letters do not differ statistically from each other by the Tukey ($p < 0.05$).
Lower case letters correspond to statistics between concentrations, according to the columns. Capital letters correspond to statistics between groups, according to the lines.

As for table 3, we noticed that infusion 1 was the one with the best capacity for free radical scavenging (DPPH). The% DPPHR was being reduced proportionally to the increase in the concentration of the infusion, presenting antioxidant activity similar to the quercetin pattern and superior to the standard BHT. Soluble 3 stood out because, even at higher concentrations, its antioxidant activity was lower compared to infusions and other soluble extracts.

The antioxidant activity of infusions and soluble extracts of *C. sinensis* L. was also expressed in EC_{50} values, as shown in Table 4.

Table 4. EC_{50} values of infusions and soluble extracts of *C. sinensis* L. Palmas- TO, 2019. Source: Data found by the author

	CE50(mg/mL) Average±SD*
Infusions	
Infusion 1	17.49 ± 0.09
Infusion 2	154.92 ± 10.35
Infusion 3	109.38 ± 8.32
Soluble Extracts	
Soluble 1	116.47 ± 7.45
Soluble 2	190.11 ± 4.43
Soluble 3	1026.15 ± 94.09
Standards	
Quercetin	7.606 ± 0.83
BHT	168.74 ± 0.07

*SD= Standard Deviation.

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The results of antioxidant capacity expressed in efficient concentration (EC_{50}) (Table 4) or also called the inhibition coefficient (IC_{50}), represents the concentration capable of inhibiting 50% of the initial concentration of the free radical DPPH.[23] Lower values for EC_{50} indicate greater antioxidant activity.[24]

Infusion 1 showed greater antioxidant capacity compared to the other samples, with the lowest value for EC_{50} (Table 4).

Sugars

Table 5 describes the results in averages of the levels of reducing and non-reducing sugars of infusions and soluble extracts of C. sinensis L.

Table 5. Content of reducing and non-reducing sugars in samples of infusions and soluble extracts of *C. sinensis* L. Palmas-TO, 2019

	REDUCING SUGARS (mg/mL)	NON-REDUCING SUGARS (mg/mL)
	AVERAGE ± SD*	
Infusions		
Infusion 1	1±0.01^{d}	0.3±0.06^{c}
Infusion 2	0.3±0.01^{f}	0.2±0.02^{c}
Infusion 3	0.9±0.00^{e}	0.1±0.01^{c}
Soluble Extracts		
Soluble 1	1.9±0.04^{b}	2.0±0.24^{a}
Soluble 2	2.1±0.03^{a}	1.6±0.07^{b}
Soluble 3	1.4±0.03^{c}	2.0±0.22^{a}

*SD= Standard Deviation.
Average columns with equal letters do not differ statistically from each other using the Tukey test ($p < 0.05$).

Unlike the other tests, for the amount of reducing and non-reducing sugars per mL of the sample, soluble extracts stood out with high levels.

DISCUSSION

Phenolic compounds

A high content of total phenolics was also found in the literature in aqueous infusions of C. sinensis L. Moraes-De-Souza et al.[23] used the same methodology, but with a concentration of 1: 100 (w / v). herbs, found higher content of total phenolics in green tea, between 59.18 ± 0.78 to 103.98 ± 0.19mg EAG / g (59.180 ± 0.78 to 103.980 ± 0.19µg EAG / mL) of tea. Sousa[7] analyzed the content of total phenolics at a concentration of 1:10 (w / v), with a result of 6,519.12mg EAG / L (6,519.12µg EAG / mL). Both performed the tests with more concentrated extracts in relation to this study, which is 5: 1000 (w / v), and achieved much higher values of total phenolics.

Camargo et al.[25] also studied *C. sinensis* L. infusions, this time at a concentration of 5:25 (w/v), found total phenolic values of 76.00 ± 0.162µg/mL. And Zielinski et al.,[26] in studies with several teas under water infusion of 2:100 (w/v), obtained values for total phenolics from 100.45 to 1.034.48mgEAG / L (100.45 to 1.034.48µg EAG/mL). Despite the divergences regarding the concentrations used both of infused extracts and of the reagents used in the methodology, these authors achieved results converging to the present study.

Zielinski et al.[26] also quantified the flavonoid content in the same concentrations as the phenol test, finding values between 0.034 to 0.179 mg of catechins/mL, using similar methodology, but with results below those found in this study with infusions, approaching of the values found here in samples of soluble extracts. Pereira et al., 24 in a study with extracts of *C. sinensis* L. prepared under aqueous infusion at a concentration of 1: 100 (w / v), found mean flavonoid values of 8.30 mg/g (0.008 mg/mL), and when compared with black and white tea, green and white tea showed higher levels of flavonoids, these values being lower than those found here. However, studies confirm, as well as the current one, that tea from *C. sinensis* L. has significant amounts of total phenolics and flavonoids.

On average, much higher values were found in the literature compared to the present study. This can be explained by differences in concentrations, variation in the method of preparation as to the temperature and time of infusion, and the different types of brands, method of storage, processing and cultivation of the samples used.[27]

According to Nibir et al.,[28] infusions of different varieties of *C. sinensis* L. at a concentration of 50: 300 (w / v), with subsequent lyophilization, obtained results for total phenolics of 26.33 ± 1.73mg EAG/g extract (26,330 ± 1,73µg EAG/mL) extract. That same study also quantified flavonoids, resulting in 50.12 ± 0.6 mg/g (0.050 ± 0.6 mg/mL) for green tea, which in comparison with other varieties was the one that achieved the greatest amount of phenolic and flavonoid compounds. In comparison to the soluble extract carried out in the present study, the values found for total phenolics were higher, while for flavonoids they were close.

It must be taken into account, however, that the soluble extract acquired here did not undergo the same lyophilization process as the aqueous extract, it has already been acquired in soluble powder, which corroborates for a probable reduced quantification of phenolic compounds. However, studies with soluble extracts that are similar to those carried out in this article were not found in the literature, making comparison between samples difficult.

Regarding total tannins, studies using *C. sinensis* L. in both infusions and soluble extracts for such quantification were not found in the literature. Thus, the results found are of great value for the characterization of this herb.

Ferric ion reducing power test

In the literature, when analyzing the ferric reducing power (FRP) in leaves of *C. sinensis* L., different ways of performing the method and expressing the results are found, which makes it difficult to obtain an accurate comparison.

Chan et al.[29] compared tropical and spiced herbal teas with *C. sinensis* L. tea variations, green, oolong and black in 1:50 (w/v) concentrations, using the same method used here, but with results expressed in mg GAE/g. Despite this, tea from *C. sinensis* L. obtained superior results than the other samples, with the green tea infused extract having a greater reducing activity than oolong and black tea. Chan et al.,[30] on the other hand, used infusions in the same concentrations, but performed aqueous and methanolic extractions of 1:50 (w/v) for both; and a method different from that used in the present study used the iron-reducing antioxidant power (FRAP). The values found were 126 ± 4.5mg GAE/g for extraction with methanol and 123 ± 10.8mg EAG/g for aqueous extraction.

Nibir et al.,[28] who used powdered extract from the freeze-drying of the aqueous extract, already described in the discussion of total phenols and flavonoids, also quantified the antioxidant capacity by the

FRAP method. Green tea exhibited the greatest reducing activity, 102.33 ± 1.02 mg EAG / g, differing from other tea varieties.

When comparing with other literature, it must be taken into account that the reducing power of *C. sinensis* L. or other samples can occur through various compatible methodologies. The literature cited addressed these results in different methodologies, types of extracts and divergent concentrations, in addition to samples from and grown in different places. All of this compromises data comparison.

In this study, aqueous extraction was chosen, as it relates to the way in which *C. sinensis* L. teas are consumed, and thus, to know their properties in this condition. In addition, the use of organic solvents in methodologies can make them more costly, time-consuming, dangerous because they contain toxic substances, in addition to generating polluting residues.[31]

Free radical scavenging

For the percentage of antioxidant activity, higher antioxidant capacity was found in the literature for infusions of *C. sinensis* L., as in this study. Zielinski et al.,[26] with samples in concentration 2: 100 (w / v), found percentages between 12.64 and 68.60% of reduction. Guimarães,[32] using concentrations 1: 100 (w / v) for green tea, reached 89.26%.

In relation to the EC50, values similar to the results highlighted here are described in the literature. Studies of *C. sinensis* L. infusions, compared to other teas, stood out with the best antioxidant capacity. Pereira et al.[24] found EC50 values varying between 13.51 and 35.10µg/mL, in extracts with concentrations of 1: 100 (w/v). At the same concentration, Moraes-De-Souza et al.[23] found values below 150 µg/mL and Camargo et al.,[25] using concentrations of 5:25 (w/v), found 14.45 ± 0.09 µg/mL.

For the DPPH method, no studies were found in the literature that used soluble extracts of *C. sinensis* L. commercially acquired as the one studied here. The importance of this study in terms of its use by the population is highlighted.

Sugars

Higher values of sugars found in samples of soluble extracts may suggest the presence of maltodextrin and sucralose sweetener, added in drinks as a way to sweeten them, as observed in the list of ingredients in the samples in this study, unlike infusions, which use parts of fresh plants. This can serve as an alert for consumers, who using soluble teas as a way to enjoy their antioxidant benefit, may also be consuming carbohydrates and additives added to these products.

It was not possible to compare and discuss the results of reducing and non-reducing sugars with the literature. Due to the absence of studies investigating sugars in samples of *C. sinensis* L. This fact indicates the importance of this study for the scientific field.

CONCLUSION

In view of the results obtained in the present study, the greater antioxidant potential and the presence of phenolics in *C. sinensis* L. infusions are evident when compared to soluble extracts, which in turn had a higher content of reducing and non-reducing sugars.

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Contributors

Boyarski DRS and Barbosa DRR acted in the performance of laboratory tests, analysis and interpretation of results and assisted in the formatting and writing of the article; Clemente RC participated as an advisor, assisting in all stages of the article. He acted directly from the choice of the theme, objectives and methodologies to the review and correction of the writing and formatting of the final version of the article.

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