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Original Article
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Inhibition of Glycation End Products Formation and Antioxidant Activities of *Ilex paraguariensis*: comparative study of fruit and leaves extracts

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Objectives: *Ilex paraguariensis* (Aquifoleaceae) is cultivated to produce "yerba mate". Due to its nutritional, energizing, hypoglycemic and antioxidant effects, it is used in the elaboration of food, pharmaceuticals, and cosmetics. The oxidative stress related to protein glycation and production of advanced glycation end products (AGEs) leads to the development of several diseases. The objective of this work was to compare the antioxidant and anti-AGEs activity of a decoction of fruits (F) and leaves (L).
Methods: The antioxidant activity was assayed by the DPPH assay and the inhibition of egg yolk lipid peroxidation (ILP), and anti-AGEs activity, through the inhibition of the formation of fructosamine (IF), β-amyloid (Iβ), protein carbonylation (IC) and AGEs (IA). Polyphenols were quantified by HPLC.
Results: Maximum response ± SEM: For F 0.01 μg/mL: IF = 42 ± 4%, IC = 17 ± 2% and for 10 μg/mL: IA = 38 ± 4%, Iβ = 67 ± 7%. For L 0.1 μg/mL: IF = 35 ± 2%, IC = 19 ± 2% and for 100 μg/mL: IA = 26 ± 3%, Iβ = 63.04 ± 2%. The DPPH IC_{50} = 134.8 ± 14 μg/mL for F and 34.67 ± 3 μg/mL for L. The ILP IC_{50} = 512.86 ± 50 μg/mL for F and 154.8 ± 15 μg/mL for L. By HPLC L presented the highest amounts of flavonoids and caffeoylquinic acids. F and L showed strong anti-AGEs activity, affecting the early stages of glycation at low concentrations and the late stages of glycation at high concentrations. The highest activity for both F and L was seen in the IF and Iβ. F presented the highest anti-AGEs potency. L presented the highest antioxidant potency, which was related to the highest content of polyphenols.
Conclusion: The fruits of *I. paraguariensis* could be a source of antioxidant and anti-AGEs compounds to be used with medicinal purposes or as functional food.

Keywords: anti-AGEs activity, antioxidant activity, fruits extract, *Ilex paraguariensis*

INTRODUCTION

Ilex paraguariensis (Aquifoleaceae) is a plant cultivated in Misiones and northeast regions of Corrientes provinces in Argentina for its leaves and young branches known as "yerba mate" worldwide [1]. For its various health benefits, this plant is commonly prepared into beverages through infusion and decoction methods [2]. Furthermore, it is often used in food, pharmaceutical, and cosmetic products [3-5]. It has been reported that yerba mate has nutritional, energizing, hypoglycemic, analgesic, diuretic, antiulcer, antirheumatic, digestive, and antioxidative properties. Due to these beneficial properties and applications, the plant is listed in the Argentine Food Code (Código Alimentario Argentino, CAA) [6], the Argentine Pharmacopoeia [7], and the regulatory codes of various other countries.

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The bioactive properties of yerba mate are attributed to the presence of various organic compounds like quercetin, epicatechin, gallocatechin [4], naringenin, caffeoylquinic acids, xanthines, triterpenoids, organic acids, and saponins [8-10].

While the phytochemical and pharmacological properties of the leaves of *I. paraguariensis* have been extensively studied, the bioactive properties of its fruits have not yet garnered comparable attention. In fact, the fruits of *I. paraguariensis* are considered waste products with no commercial value in the yerba mate industry and are separated from the products to meet the specifications of the CAA. It has been reported that approximately 560 tons of *I. paraguariensis* are produced annually, with the fruit portions either being discarded or utilized as fertilizers. Despite this lack of appreciation of the fruit portion of *I. paraguariensis*, we were intrigued by its considerable content of total dietary fiber (42.0 ± 1.6 g/100 g) and nutritionally valuable minerals: potassium (1324 ± 15 mg/100 g), iron (6.4 ± 0.5 mg/100 g), magnesium (168 ± 15 mg/100 g), calcium (150 ± 12 mg/100 g), copper (1.1 ± 0.1 mg/100 g), zinc (2.3 ± 0.3 mg/100 g), and sodium (1.3 ± 0.1 mg/100 g) [11]. Considering such an ample amount of health-beneficial contents is available in the fruit portion of the plant, we hypothesized that it may also have bioactive properties – similar to those of yerba mate – that are yet to be explored by the scientific community.

Oxidative stress is closely related to the glycation of proteins and the production of advanced glycation end products (AGEs), which result in aberrant alterations of proteins, lipids, and nucleic acids. Ultimately, excessive oxidative stress disrupts cell functionalities and leads to the development of various diseases like metabolic syndrome and cancer. The antioxidative and anti-AGEs action of the fruits has not been previously studied. The objective of the present study was to evaluate the antioxidative and anti-AGEs activities of the decoction obtained from *I. paraguariensis* fruits (F) and compare them with those of the decoction obtained from the plant's leaves (L). The antioxidative activity was evaluated by testing the bleaching of the DPPH radical and the degree of inhibition of egg yolk lipoperoxidation (ILP) in the presence of the decoctions. The anti-AGEs activity was evaluated by the degrees of inhibition of fructosamine (IF) and β-amyloid (Iβ) formations, protein carbonylation (IC), and AGEs formation (IA) by UV-Vis and fluorescence spectroscopy in a bovine serum albumin (BSA) glycation model supplemented with 5% glucose. Finally, the polyphenol contents (flavonoids and caffeoylquinic acids) in the plant components were also quantified by high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

1. Plant material and extract preparation

Ripe fruits and leaves of *Ilex paraguariensis* St. Hilaire var. *paraguariensis* were kindly provided by "Las Marías" establishment (Gobernador Virasoro, Corrientes, Argentina). The fruits were collected in April 2010, and the leaves were harvested in March of the same year.

The plant components were identified by morphological, anatomical, and histochemical criteria by Dr. Gustavo Giberti † at the Museum of Pharmacobotany, School of Pharmacy and Biochemistry, University of Buenos Aires. The identification with BACP numbers were as follows: fruits, BAF 2 (series 2010); leaves, BAF 20121. The fruits were dark reddish-brown in color, with an average diameter of around 4 to 6 mm.

For the extract preparations, 25 g of ground fruits or leaves were first dried in a stove by hot air circulation and heating at 40℃ until 2% moisture level was reached. Next, the plant components were boiled with 500 mL of distilled water for 20 minutes, followed by filtration with a filter paper (Whatman No.1) and lyophilization.

2. Phytochemical studies: HPLC analysis

The polyphenol contents in the plant components (caffeoylquinic acids and flavonoids) were analyzed by HPLC [9]. A Varian ProStar HPLC system equipped with a 20 μL Rheodyne injector and photodiode array detector was used. The detection was carried out at 325 nm for caffeoylquinic acids and 255 nm for rutin and quercetin compounds. Varian Star 5.5 software was used for data analysis.

For chromatography, a reversed-phase C18 column (Agilent Zorbax Eclipse XDB-18; 250 mm × 4.6 mm, 5 μm particle size) was used. The mobile phase consisted of (A) water with 2 vol% acetic acid and (B) methanol with 2 vol% acetic acid, which was eluted in a gradient manner from 15% B to 40% B in the first 30 minutes, 40% B to 75% B in the next 10 minutes, 75% B to 85% B in the next 5 minutes, and to 100% B in the final 5 minutes. The flow rate was set at 1.2 mL/minute and the column temperature at 30℃. The standard samples (0.02 mg/mL) or lyophilized extracts (1 mg/mL) were dissolved in a mixture of methanol and water (70:30; *v*/*v*). The analyses of all samples

were carried out in triplicate, in which the variation coefficient of ≤ 2% was considered acceptable.

Solvents: HPLC-grade methanol (J.T. Baker, Mexico) and acetic acid (Merck, Argentina) were used for HPLC. Water was of ultrapure quality obtained from a Milliq water purifier system. The standards were purchased from Sigma-Aldrich (USA).

3. Anti-AGEs activity

1) Albumin glycation

In vitro albumin glycation model was established by adding 1 mL of BSA (5%) in 1 mL of glucose (3%) dissolved in potassium phosphate buffer (200 mM, pH = 7) and incubating the mixture at 37℃ for 5 days in either the presence or absence of the plant extracts [12]. The time of incubation was optimized through a kinetics study (data not shown). Before incubation, all solutions were filtered through a 0.22 μm membrane to prevent microbiological contamination.

2) Estimation of fructosamine levels

The levels of fructosamine were determined by the nitroblue tetrazolium assay [13]. Briefly, 40 μL of a glycated sample or positive control was mixed with 800 μL of a nitroblue tetrazolium solution (0.75 mM in 100 mM sodium carbonate buffer, pH = 10.35). This mixture was incubated at 37℃ for 30 minutes. Next, the absorbance of the mixture at 530 nm was measured (UV-10 Spectrophotometer, Thermo Scientific, USA). To calculate the percentage of inhibition of fructosamine formation, the following equation was used:

$$\text{Inhibitory activity (\%)} = [(A_0 - A_1) / A_0] \times 100,$$

where A_0 is the absorbance value of the positive control (fructosamine) and A_1 is absorbance of a glycated albumin sample incubated with a plant extract.

3) Estimation of carbonyl groups

Protein carbonyl compounds are considered as intermediates of the glycation process and their formations were determined by the DNPH (2,4-dinitrophenylhydrazine) method [14]. In this assay, 0.5 mL of glycated albumin samples were added to 0.5 mL of 2,4-DNPH (10 mM in 2.5 M HCl) and the mixture was incubated for 1 hour at room temperature. Next, 0.5 mL of trichloroacetic acid (20%) was added to the mixture to precipitate the proteins and the resulting precipitates were washed three times with a mixture of ethanol:ethyl acetate (1:1, *v*/*v*). Next, 1 mL of urea (8 M) was added to the precipitates and the absorbance of the mixture at 365 nm was measured. The molar extinction coefficient (ε at 365 nm) of 21 mM/cm was used to calculate the concentration of protein carbonyl compounds. The following equation was used to calculate the percentage of inhibition of protein carbonyl formation:

$$\text{Inhibition \%} = [(A_0 - A_1) / A_0] \times 100,$$

where A_0 is the absorbance of the solution incubated without an extract and A_1 is the absorbance of the solution with an extract.

4) Estimation of amyloid β-structures

Congo Red was used to measure the aggregation levels in the glycated samples [14]. In brief, 100 μL of Congo Red (100 mM in 10 vol% ethanol in phosphate-buffered saline) was mixed with 0.5 mL of each glycated sample and the mixture was incubated at room temperature for 20 minutes. The absorbance of the mixture at 530 nm was measured. The following formula was used to determine the percentage of inhibition of amyloid formation:

$$\text{Inhibition \%} = [(A_0 - A_1) / A_0] \times 100,$$

where A_0 is the absorbance of the solution incubated without an and A_1 is the absorbance of the solution with an extract.

5) Determination of AGEs levels

The production of fluorescent products of glycated albumin was monitored to assess the formation of total AGEs. A LS50B Perkin Elmer luminescence spectrometer (USA) was used for the analysis, in which the excitation and emission wavelengths were set at 370 and 440 nm (slit width = 0 nm), respectively [15]. The following formula was used to determine the percentage of inhibition of total AGEs formation.

$$\text{Inhibition \%} = [(F_0 - F_1) / F_0] \times 100,$$

where F_0 is the fluorescence emission of the positive control (a glycated albumin sample without an extract) and F_1 is the fluorescence emission of a glycated albumin samples with a plant extract.

4. Antioxidative activity

1) DPPH radical scavenging capacity

The decrease in the absorbance of a methanolic solution of DPPH was measured to determine the radical scavenging activity of the samples [16]. Briefly, 5 mL of a DPPH solution (3.3 mg of DPPH in 100 mL methanol) was added to 1 mL of an extract and incubated for 30 minutes in the dark. The absorbance (A_1) of the mixture was then measured at 517 nm. The absor-

bance of the control containing blank methanol was measured at the same wavelength. The percentage of radical scavenging capacity was calculated using the following equation:

$$\text{DPPH radical scavenging capacity (\%)} = [(A_0 - A_1) / A_0] \times 100.$$

2) Egg yolk lipid peroxidation

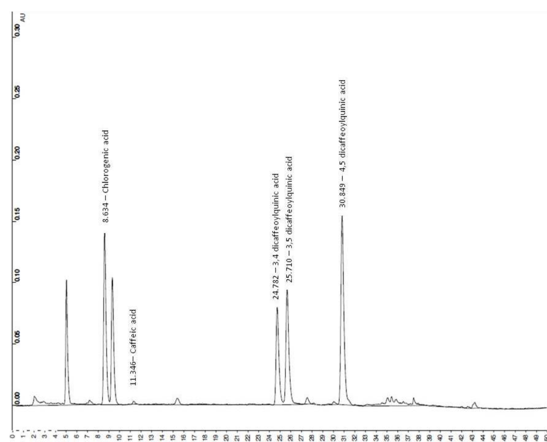
The egg yolk lipid peroxidation level was assessed by the method described by Placer et al. (1966) [17], which measures the level of malondialdehyde (MDA) – an end product of fatty acid peroxidation. Briefly, 500 μL of an egg yolk emulsion in phosphate buffer pH = 7.4 (final concentration of 25 g/L) was added to 500 μL of an unknown sample dissolved in the same buffer. The mixture incubated for 1 hour at 37℃. Next, 250 μL of 15% trichloroacetic acid and 500 μL of 1% thiobarbituric acid were added to the mixture, which was further incubated at 100℃ for 20 minutes. The mixtures were then centrifugated for 10 minutes at 3,500 × *g*, and the supernatants were collected for absorbance measurements at 532 nm. The percentage of inhibition of lipoperoxidation was calculated using the following equation:

$$\text{lipoperoxidation (\%)} = (A_0 - As / A_0) \times 100,$$

where A_0 is the absorbance of an egg yolk emulsion in a blank buffer without an unknown sample and As is the absorbance of an egg yolk emulsion with an unknown sample.

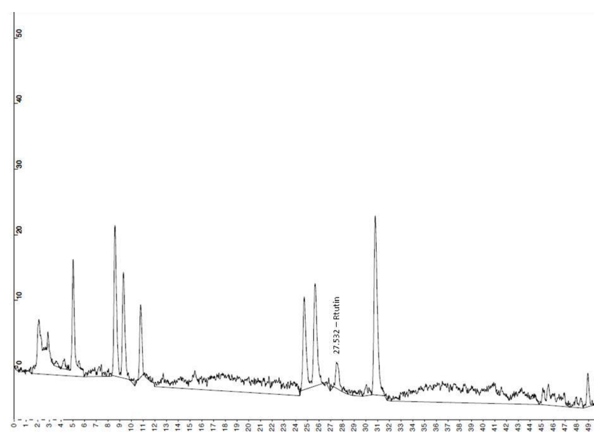
Fruits identified caffeoylquinic acids

A



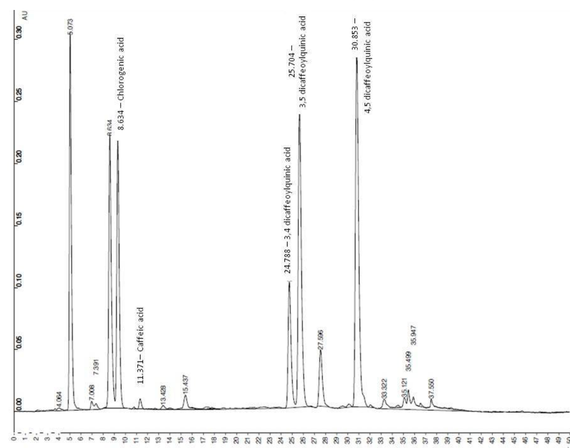
Fruits identified flavonoids

B



Leaves identified caffeoylquinic acids

C



Leaves identified flavonoids

D

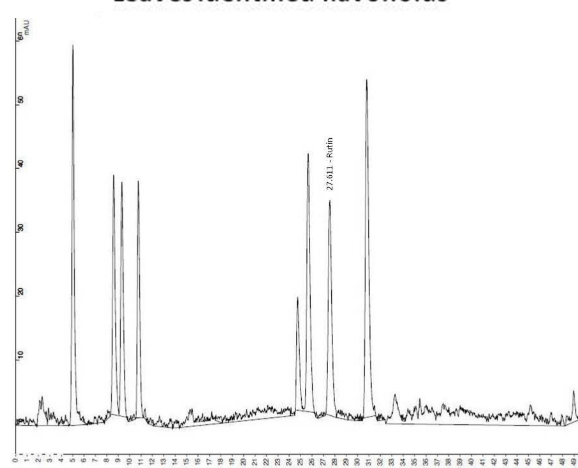


Figure 1. HPLC analysis of fruits and leaves extracts to determine the content of caffeoylquinic acid and flavonoids. (A, C) Caffeoylquinic acids content in fruits and leaves, respectively. (B, D) Flavonoids content in fruits and leaves, respectively. Figures are representative of three experiments.

5. Statistical analysis

All experimental data are expressed as mean ± standard error of the mean (SEM) of at least 3 independent experiments performed in duplicate. The significance level was set at $p < 0.05$.

RESULTS

Polyphenol contents (caffeoylquinic acids and flavonoids) in the extracts of fruits (Fig. 1A, B and Table 1) and leaves (Fig. 1C, D and Table 1) of *I. paraguariensis* were detected and quantified by HPLC. The identified caffeoyl derivatives were as follows: chlorogenic acid at retention time (RT) of 8.6 minutes, caffeic acid at RT of 11.3 minutes, 3,4 dicaffeoylquinic acid at RT of 24.8 minutes, 3,5 dicaffeoylquinic acid at RT of 25.7 minutes, and 4,5 dicaffeoylquinic acid at RT of 30.8 minutes. The flavonoid rutin at RT of 27.6 minutes was also identified.

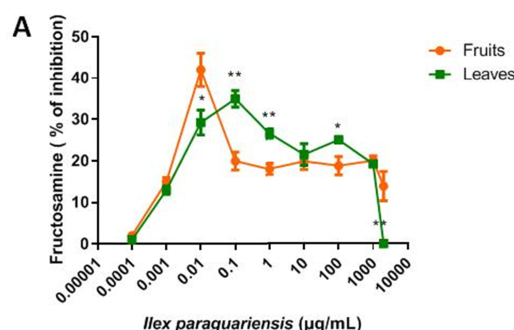
The formation of fructosamine, which occurs at an early stage of the glycation process, was evaluated. Both F and L extracts effectively inhibited the formation of fructosamine, showing a maximum effect. The F extract displayed up to 42% inhibition at 0.01 μg/mL, while the L extract displayed up to

Table 1. Caffeoyl derivatives and flavonoids in *Ilex paraguariensis* fruits and leaves extracts

Compounds	% (mean ± SEM) Fruits	% (mean ± SEM) Leaves
Chlorogenic acid	1.31 ± 0.01	4.13 ± 0.04****
Caffeic acid	0.012 ± 0.001	0.076 ± 0.004****
3,4-Dicaffeoylquinic acid	0.71 ± 0.04	1.7 ± 0.1***
3,5-Dicaffeoylquinic acid	0.86 ± 0.07	4.1 ± 0.3***
4,5-Dicaffeoylquinic acid	1.43 ± 0.09	5.2 ± 0.3***
Rutin	0.191 ± 0.003	3.10 ± 0.03****

Results were expressed as mean ± SEM of three experiments made in duplicate. ***$p < 0.001$, ****$p < 0.0001$ significant differences between fruits and leaves, in accordance to Student's *t* test.

Early glycation stage



Intermediate glycation stage

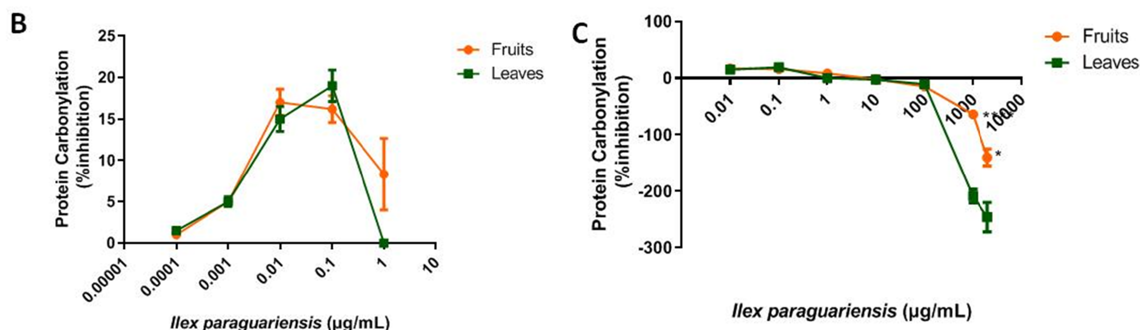


Figure 2. Early and intermediate glycation stage. (A) Effect of *Ilex paraguariensis* fruits and leaves on fructosamine formation. (B) Effect of *Ilex paraguariensis* fruits and leaves on protein carbonylation low concentrations. (C) Effect of *Ilex paraguariensis* fruits and leaves on protein carbonylation high concentrations. Results were expressed as mean ± SEM (%) three experiments made in duplicate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ significant differences between each concentration of fruits and leaves, in accordance to Student's *t* test.

35% inhibition at 0.1 μg/mL (Fig. 2A). There was no significant difference in the IC_{50} values of the F and L extracts indicating both plant components displayed comparable efficacies in the inhibition of fructosamine formation.

In terms of protein carbonylation inhibition, the F extract showed up to 17% inhibition at 0.01 μg/mL, while the L extract showed up to 19% inhibition at 0.1 μg/mL (Fig. 2B, C and Table 2). This result indicated that there was a difference in inhibitory potencies between the F and L extracts. Moreover, it was demonstrated that higher concentrations of the F and L extracts did not lead to inhibition of protein carbonylation (Fig. 2C and Table 2). There was no significant difference in the protein carbonylation IC_{50} values of the F and L extracts, but the fructosamine IC_{50} value of the L extract was significantly lower than its

Table 2. Maximum response and IC_{50} of anti-AGEs and antioxidant activity of fruits and leaves from *Ilex paraguariensis*

Activities	Maximum response (% inhibition) and concentrations (μg/mL)		IC_{50} (μg/mL)	
	Fruits	Leaves	Fruits	Leaves
Fructosamine inhibition	42 ± 4 (0.01)	35 ± 2^{NS} (0.1)	0.0017 ± 0.0002	0.0016 ± 0.0001$^{\#}$
Protein carbonylation inhibition	17 ± 2 (0.01)	19 ± 2^{NS} (0.1)	0.0020 ± 0.00015	0.0028 ± 0.0004^{NS}
AGEs inhibition	38 ± 4 (10)	26 ± 3* (100)	0.80 ± 0.07	0.0034 ± 0.0002****
β-amyloid inhibition	67 ± 7 (10)	63.04 ± 2^{NS} (100)	0.0022 ± 0.00015	2.5 ± 0.19
DPPH scavenger activity	80 ± 7	80 ± 8^{NS}	134.8 ± 14	34.67 ± 3***
Lipid peroxidation	100	100	512.86 ± 50	154.88 ± 15***

Results were expressed as mean ± SEM of inhibition (%) or IC_{50} (μg/mL) of three experiments made in duplicate. *p < 0.05, ***p < 0.001, ****p < 0.0001 significant differences between Fruits and Leaves; $^{\#}$p < 0.05 significant differences between IC50 for frusctosamine inhibition and protein carbonylation inhibition in leaves. NS: no significant differences in accordance to Student's *t* test.

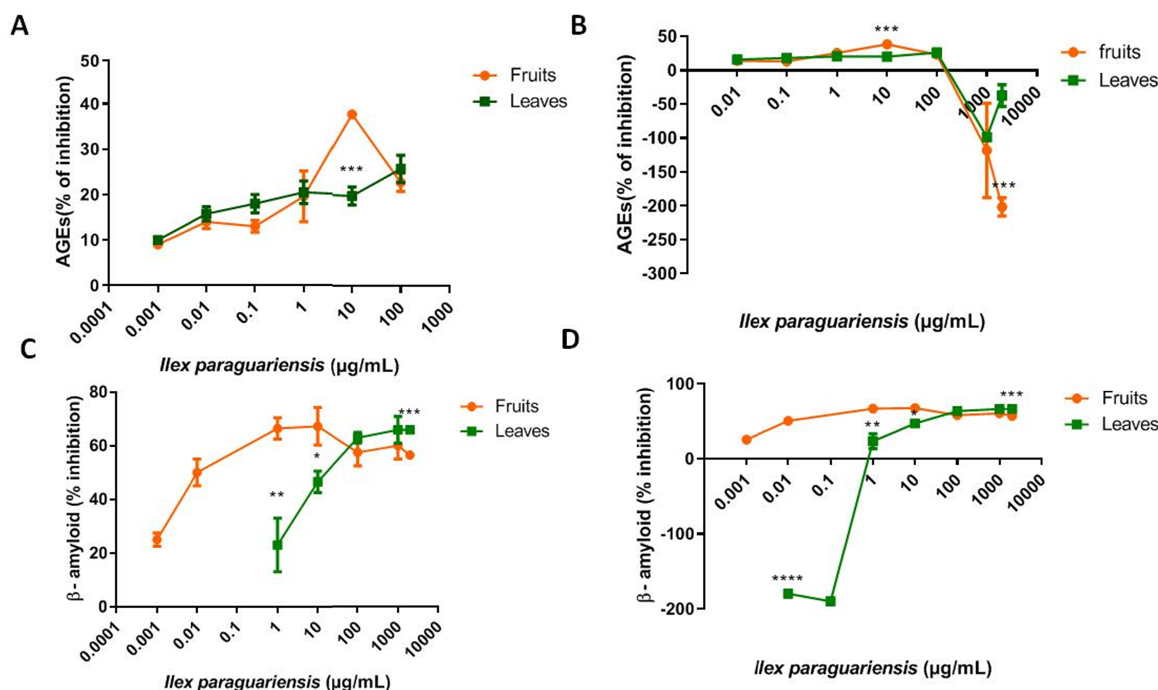


Figure 3. Late glycation stage. (A, B) Low and high concentrations, respectively, AGEs formation. (C, D) Low and high concentrations, respectively, β-amyloid formation. Results were expressed as mean ± SEM of inhibition (%) of three experiments made in duplicate. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 significant differences between each concentration of fruits and leaves, in accordance to Student's *t* test.

protein carbonylation IC_{50} value (Table 2).

In terms of total AGEs and β-amyloid formation inhibition, the F extract displayed up to 38% AGEs inhibition (Fig. 3A, B and Table 2) and 67% β-amyloid inhibition (Fig. 3C, D and Table 2). On the other hand, the L extract displayed up to 26% AGEs inhibition (Fig. 3A, B and Table 2) and 63% β-amyloid inhibition (Fig. 3C, D and Table 2). The F extract displayed maximum inhibition efficacies at 10 μg/mL, while the L extract exerted maximum efficacies at 100 μg/mL for the both total AGEs and β-amyloid formations, with these two concentrations being significantly different ($p < 0.05$). In terms of total AGEs IC_{50} values, that of the L extract was lower than that of the F extract. Conversely, for β amyloid IC_{50} values, that of the F extract was lower than that of the L extract (Table 2).

At concentrations above 100 μg/mL, both F and L extracts failed to inhibit the formation of total AGEs (Fig. 3B and Table 2). In addition, lower concentrations of the L extract failed to inhibit to formation of β-amyloid (Fig. 3D and Table 2).

Both F and L extracts exerted notable radical (DPPH) scavenging and lipid peroxidation inhibitory activities in concentration-dependent manners (Fig. 4A, B). Importantly, the L extract displayed lower IC_{50} values than the F extract for both radical scavenging and lipid peroxidation inhibition ($p < 0.0001$, Table 2).

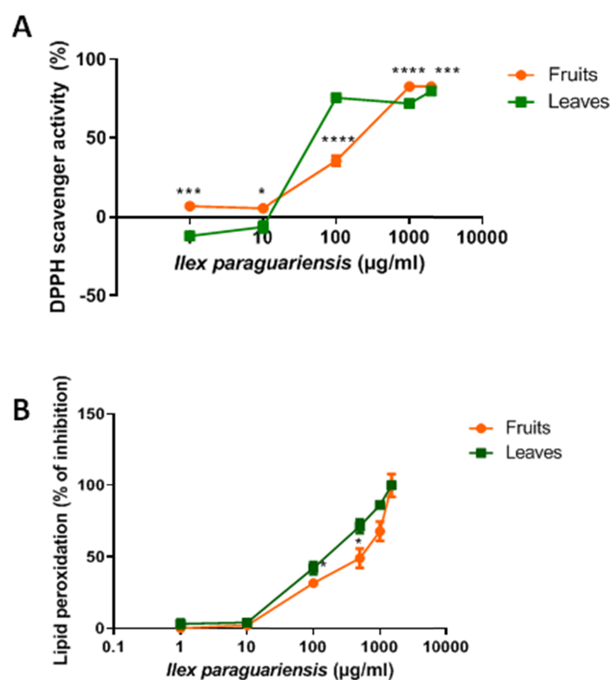


Figure 4. Effect of *Ilex paraguariensis* fruits and leaves. (A) DPPH scavenger activity. (B) Lipid peroxidation. Results were expressed as mean ± SEM of inhibition (%) of three experiments made in duplicate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ significant differences between each concentration of fruits and leaves, in accordance to Student's *t* test.

DISCUSSION

To the best of our knowledge, this is the first study that reports the anti-AGEs and antioxidative properties of the F extract of *I. paraguariensis*. Here, we systematically analyzed the bioactive properties of the plant's fruit portion and compared its efficacies with those of the plant's leaf portion to demonstrate the therapeutic potential of the F extract. Specifically, the F extract exerted enhanced anti-AGEs activity compared with that of the L extract, while the latter exerted a more powerful antioxidative effect.

The anti-AGEs activities were evaluated using an *in vitro* model of albumin glycation. It is known that the glycation of proteins leads to a partial loss of protein activity, with circulating glycated proteins like albumin being involved in the development and exacerbation of diabetes [18]. Due to its concentration and half-life, albumin is one of the proteins that is most affected by glycation [19]. Glycated albumin undergoes conformational changes, oxidation of free thiol groups forming disulfide bonds or thiol radicals, and transformation from its globular structure into amyloid fibrils characterized by a cross β-structure. These aberrant alterations adversely affect the protein's ability to transport drugs and small molecules in circulation, contributing to the onset of heart, kidney, and ocular diseases [20]. Moreover, the interaction between glycated albumin and erythrocytes is known to cause oxidative stress in the vessel wall, subsequently leading to diabetic complications.

The glycation process is divided into three main stages: initiation, propagation, and advanced [13]. During the initiation stage, reducing sugars like fructose engage with free amino groups of proteins to form Schiff base and Amadori product. During the propagation stage, Amadori product undergoes transformation into carbonyl compounds, such as glyoxal, methylglyoxal, and deoxyglucosones, facilitated by catalytic metal ions or oxygen. In the advanced stage, irreversible fluorescent AGEs are formed, including N-ε-carboxyethyllysine, N-ε-carboxymethyllysine, 3-deoxyglucosone, 3-deoxyglucosone lysine dimer, glyoxal, glyoxal-lysine dimer, methylglyoxal, MG-derived-hydroimidazalone, and methylglyoxal-lysine dimmer. These compounds have the potential to form crosslinks with other proteins to yield β-amyloid compounds. Here, oxi-

dative stress is not only involved in the development of protein glycation but can also be a result of the respective process.

Both F and L extracts demonstrated anti-AGEs activity at different stages of glycation and such inhibitory activities were shown to be concentration-dependent. The F extract was more effective in inhibiting the formation of fructosamine, protein carbonylation, and β-amyloid relative to the extract's anti-AGEs activity. The IC_{50} values for the F extract's inhibition of fructosamine, protein carbonylation, and β-amyloid were around 500 times lower than the extract's IC_{50} for AGEs inhibition. The L extract was found to be significantly more potent for the inhibition of fructosamine formation than for protein carbonylation, AGEs, or β-amyloid formation, showing a significantly lower IC_{50} value than those of the latter three compounds.

Although the maximum inhibitory concentration of the L extract was ten times higher than that of the F extract, no significant difference was observed between the F and L extracts' fructosamine and protein carbonylation inhibition efficacies. The F extract was found to be more effective in the inhibition of β-amyloid formation than the L extract in terms of both inhibition percentage and IC_{50} values. On the other hand, the L extract was more effective in the inhibition of AGEs formation than the F extract, with its IC_{50} value being around 230 times lower than that of the F extract. Considering the maximum inhibitory concentrations, it was evident that lower concentrations of both F and L extracts exerted a more pronounced inhibition of the early intermediates of glycation, whereas their higher concentrations affected later stages of the process.

Various targets can be inhibited to block the production of AGEs. Reactive oxygen species (ROS) can be scavenged to prevent carbonyl production, while carbonyl groups can be blocked or detoxified. In addition, inhibition of protein cross-linking is another target, as well as blocking the receptors of AGEs. Among these approaches, the reduction of ROS and carbonyl groups are considered crucial in the inhibition of AGEs formation. To that respect, both F and L extracts effectively scavenged ROS and inhibited carbonyl production. Moreover, both extracts simultaneously inhibited other critical intermediates of AGEs. This is the first time that the anti-AGEs activity of *I. paraguariensis* fruits is demonstrated.

The antiglycant activity of the L extract of *I. paraguariensis* has previously been demonstrated in a model of BSA glycation with methylglyoxal. In this experimental model, the extract inhibited the formation of AGEs adducts in a dose dependent-manner, and the effect was comparable to that of aminoguanidine [21, 22]. In addition, the L extract of *I. paraguariensis* was found to inhibit AGEs formation by 53% and 78% in oil cooked eggs, depending on the employed concentration. Moreover, we observed that the L extract could also inhibit eggs yolk lipoperoxidation (data not shown).

While the antioxidative activity of the L extract of *I. paraguariensis* is well-known, that of the F extract has not been reported to date. In this investigation, the L extract displayed around 3 to 4 times more effective antioxidative activity than the F extract, as demonstrated by their IC_{50} values. It has been demonstrated that the L extract is more potent than ascorbic acid in preventing copper- or hydrogen peroxide (H_2O_2)-induced LDL autoxidation [23]. In addition, *I. paraguariensis* extract can significantly inhibit lipid oxidation, as previously evaluated by the oxidation of liposomes using 2,2'-azobis-2-amidinopropane dihydrochloride (AAPH) [24]. Furthermore, the extract can protect the activity of paraoxonase-1 (PON-1), an antioxidative enzyme found in HDL particles with cardio-protective effects, from oxidative stress induced by AAPH [25]. Moreover, the L extract has been shown to display peroxidase-like activity and stimulate peroxidase secretion in rat submandibular glands, thereby exerting a protective antioxidative activity [26]. This antioxidative activity has been attributed to the presence of polyphenols [27] and has been demonstrated to prevent the H2O2-induced lysis of red blood cells [28].

Importantly, the bioactive properties of the plant extracts discussed in this work could also be attributed to the presence of polyphenols. The respective compounds, along with other compounds with nutritional properties like oleic acid, have previously been found in ripe fruits [11]. Previously, polyphenols have been found in the L extract [8-10]. A comparative study was conducted on the antioxidative activity and phenolic compounds in both natural and processed yerba mate leaves. Additionally, polyphenols and various other compounds have been found in the fruit portion of *I. paraguariensis* in a stage of physiological immaturity. The presence of free triterpenes, including ursolic acid, oleanolic acid, rotundic acid, and derived saponins [29], along with chlorogenic acid, caffeine, and theobromine [30] have also been found in the fruit portion of the plant. In this work, the elevated antioxidative activity and anti-AGEs effect exerted by the L extract could be attributed to its abundant polyphenol content. The antioxidative, anti-inflammatory activity, and anti-glycation activities of polyphenols have been extensively investigated in previous reports.

In summary, our results demonstrated that polyphenols can

inhibit the biosynthesis of AGEs through their antioxidative and metal-chelating capabilities, interaction with proteins, methylglyoxal trapping, and/or the receptor blockade for advanced glycation end products [31]. The potent inhibition of β-amyloid formation exerted by the F extract could be also be attributed to the presence of polyphenols and various other compounds like unsaturated acids like oleic acid [11, 32], which can solubilize bovine α-lactalbumin that facilitates fibrillation and β-amyloid formation.

CONCLUSION

In conclusion, we demonstrated that both F and L extracts of *I. paraguariensis* show strong anti-AGEs activity at low concentrations, affecting the early stages of glycation process. For the later stages of glycation, higher concentrations of the extracts were required for effective inhibition. The maximum inhibitory activities of both F and L extracts were associated with the inhibition of fructosamine and β-amyloid aggregation. The L extract displayed a potent antioxidative activity due to its high polyphenol content. The F extract's potent anti-AGEs activity was predicted to be due to the presence of other compounds like unsaturated fatty acids, which are not found in the L extract. Collectively, our findings suggest that the fruit portion of *I. paraguariensis* posseses great therapeutic potentials, warranting its suitability for applications in medicine or functional food.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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