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(RESEARCH ARTICLE)

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Analysis of the inhibition of lipoperoxidation and carbonylation in human plasma by natural infusions used in Mexico

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Abstract

Chronic diseases, including cancer, cardiovascular diseases and aging. These processes are related to the accumulation of reactive oxygen species (ROS) and lipid and protein oxidation products, which can cause cellular and tissue damage. Therefore, the search for natural compounds with antioxidant properties that can mitigate these oxidative processes is of great interest in the field of health and nutrition. Mexico is a country known for its rich biodiversity and its tradition in the use of medicinal plants and natural infusions with therapeutic properties. In this context, this article focuses on the analysis of the capacity of natural infusions used in traditional Mexican medicine to inhibit lipoperoxidation and carbonylation in human plasma. These infusions, derived from native plants and aromatic herbs, have been passed down from generation to generation as natural remedies and have been used to treat a wide range of health conditions.

Keywords: Natural Infusions; Lipoperoxidation; Carbonylation; Antioxidants; Oxidative Stress

1. Introduction

Oxidative stress has been linked to a variety of metabolic diseases, including diabetes, atherosclerosis, and metabolic syndrome. This phenomenon occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the body. ROS, which include free radicals and peroxides, can damage biomolecules such as lipids, proteins, and DNA. One of the most detrimental outcomes of oxidative stress is lipoperoxidation, which leads to lipoperoxidation end products (PFAL) and protein carbonylation, thus contributing to the development of metabolic diseases.

1.1. Oxidative Stress and Metabolic Diseases

Oxidative stress has become a determining factor in the development of various metabolic diseases, such as type 2 diabetes mellitus, obesity and cardiovascular disease [1,2]. The imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the organism can cause cellular and tissue damage, contributing to the pathogenesis of these diseases [3,4,5].

1.2. Free radicals

Free radicals are highly reactive molecules with unpaired electrons in their external orbit. The overproduction of free radicals can trigger harmful oxidation reactions in lipids, proteins and nucleic acids, contributing to oxidative stress [6,7]

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1.3. Reactive Oxygen Species

Reactive oxygen species, including hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH), are products of redox reactions that can damage biomolecules [8, 9]. Understanding its production and regulation is crucial to understanding oxidative stress [10,11, 12].

1.4. Lipoperoxidation (final products derived from lipoperoxidation)

Lipoperoxidation is a lipid oxidation process that produces end products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) [13,14]. These reactive compounds can damage cell membranes and proteins, contributing to oxidative stress. [15]. The accumulation of these lipoperoxidation end products has been associated with a number of negative effects on human health. [16,17]. Recent studies have shown that the elevated presence of MDA and other derivatives of lipoperoxidation correlates with chronic diseases, including cardiovascular, neurodegenerative diseases and various forms of cancer [18]. Furthermore, lipoperoxidation plays a significant role in cellular aging and has been linked to inflammatory processes that contribute to the development of pathologies such as rheumatoid arthritis and inflammatory bowel disease [19].

1.5. Blood

Blood plasma is a critical component for the distribution of nutrients and the elimination of waste products in the body [20]. This biological fluid comprises blood cells, such as erythrocytes, leukocytes and platelets, suspended in a plasma containing water, proteins and electrolytes [21, 22]. Blood homeostasis is essential for the proper functioning of the body and its ability to respond to internal and external challenges [23,24]. Oxidative stress in plasma can have significant systemic effects [25]

1.6. Carbonylation

Carbonylation is a post-translational process that involves the attachment of carbonyl groups (-C=O) to proteins and other biomolecules [26,27]. It is associated with cellular dysfunction and has been observed in various diseases, including Alzheimer's and diabetes [28]. Recent research has identified carbonylation as a marker of oxidative damage in various pathological conditions. In neurodegenerative diseases, such as Alzheimer's and Parkinson's, carbonylation of specific proteins has been linked to the formation of toxic aggregates and neuronal dysfunction. Furthermore, in cardiovascular diseases, lipoprotein carbonylation and lipid oxidation play a role in atherosclerosis and endothelial dysfunction [29,30].

1.7. Antioxidants

Antioxidants, compounds that neutralize free radicals and reduce oxidative stress in the body, play a crucial role in promoting health. In particular, antioxidants derived from plants, present in various natural infusions consumed in Mexico, have been the subject of attention in scientific research due to their potential health benefits [31].

Recent studies have highlighted the richness of antioxidants in herbs and plants commonly used in Mexican infusions. For example, chamomile tea (*Matricaria chamomilla*) has demonstrated antioxidant properties, attributed to its flavonoids, which may contribute to protection against oxidative damage and inflammation. Similarly, peppermint tea (*Mentha spp*.) has shown antioxidant capacity, being associated with the reduction of oxidative stress and potential benefits for digestive health [32, 33].

2. Material and methods

2.1. Sample preparation

5 g of each of the parts of the plants that were used to prepare each infusion were weighed: Cinnamon-bark, chamomile-flowers, tea-lemon leaves, mint-leaves, peppermint-leaves, orange-exocarp, Stevia-lemon leaves. -exocarp, Bugambilia-flowers and mandarin-exocarp (all samples were fresh except cinnamon).

For infusions sweetened with stevia, 2.5 g of leaves, flowers and/or bark were added. 300 mL of drinking water was placed in a flask and 3 boiling stones were added. It was heated on a heating plate until the water reached a temperature of 92° C. Once the water reached a temperature of 92 °C, the leaves were added to prepare the infusion, the heating plate was turned off and it was allowed to cool for 20 minutes.

2.2. Antioxidant activity using the ABTS radical

The generation of the ABTS cation [2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)], is the basis of a spectrophotometric method. The original ABTS assay was based on the activation of myoglobin with hydrogen peroxide in the presence of ABTS, to produce the cationic radical in the presence and absence of antioxidants.

The improvement of the technique for the generation of $ABTS^{\bullet+}$ involves the direct generation of the blue/greenish coloration, characteristic of the $ABTS^{\bullet+}$ chromophore, by a chemical reaction between ABTS and potassium persulfate. This chromophore has the following maximum absorption wavelengths: 415, 645, 734 and 815 nm, however; the most used is 415 nm.

The addition of antioxidants to the preformed cationic radical causes the reduction of the chromophore, this is dependent on the time, activity and concentration of the antioxidant. In this way, the discoloration as a percentage of inhibition of the cationic radical $ABTS^{\bullet+}$, is determined as a function of concentration and time, calculated with respect to the reactivity of Trolox (analogue of vitamin E, soluble in water) as a standard, under the same conditions.

The method is applicable for the study of water-soluble and lipid-soluble antioxidants, pure compounds and food extracts. To determine the sequestering capacity of the extracts, the procedure described by Kuskoski [34] was followed.

Produce the $ABTS^{\bullet+}$ radical using the 7 mM ABTS solution (2,2-azinobis-3-ethylbenzothiazolin-6-sulfonic acid, diammonium salt) with 2.45 mM potassium persulfate (final concentration). It was mixed and incubated in the dark for 12-16 h at room temperature. Once the radical was formed, it was diluted with ethanol until an absorbance value of 0.700 to .702 at 436 nm was obtained in a spectrophotometer. Subsequently, 10 µL of each infusion was taken and 1 mL of the diluted $ABTS^{\bullet+}$ solution was added. The sequestering effect was monitored every minute for 6 min.

2.3. Evaluation of antioxidant activity by the DPPH method

One of the most used techniques to know the activity and efficiency of antioxidants can be estimated using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). This method is based on the reduction by antioxidants of the absorbance measured at 515 nm of the DPPH• radical. DPPH at 133.33 µM is used as an inducer of free radicals. 50 µL of the sample was placed (infusions) plus 50 µL of the DPPH radical• 133.33 µM was added. It was incubated at 37 °C for 30 minutes under orbital shaking. Next it was read in the Elisa reader in 96-well plates at a wavelength of 515 nm. The percentage reduction in DPPH• was calculated with the following formula:

DPPH % reduction formula= [(C-E) / C] x 100

This procedure is done in triplicate.

2.4. Carbonylation (protein oxidation and generation of carbonyl groups

Obtaining a blood sample Inclusion criteria;

- Male
- Individuals from 18 to 25 years of age
- Individuals who fast from 8 to 12
- Clinically healthy

2.4.1. Exclusion criteria

- Individuals who have consumed alcoholic beverages
- Individuals who smoke
- Individuals who have eaten food in the previous 12 hours
- Individuals who are on any pharmacological treatment
- Patients had to fast for 10 to 12 hours

2.4.2. Carbonylation test

The sample was collected in 5 mL heparinized vacutainer tubes and placed on ice, it was centrifuged for 10 minutes at 12000 RPM. Then the supernatant was extracted to start working with it. Proteins were quantified by the Lowry method.

2.5. Determination of carbonylated proteins

The protein was incubated for 18 hours at 37 °C with phosphate buffer (pH 7.2, 50 mM) and ferrous sulfate, 1 mg/mL proteins. The final volume was 10 mL (Mesquita [35]). 500 µL of the sample solution was taken and 500 µL of 10 mM DNPH was added in 0.5 M H_3PO_4 was mixed and incubated for 15 min in the dark at 25°C.

Subsequently, 250 µL of 50% TCA (trichloroacetic acid) was added and incubated at 20 °C for 15 min with orbital shaking. It was centrifuged at 12,000 rpm for 5 min and the supernatant was discarded without removing the pellet; It was then washed three times with 1 mL of ethanol/ethyl acetate (v/v, 1:1), leaving it to rest for 10 min and centrifuging at 12,000 rpm for 5 min. The supernatant was discarded again without removing the sediment. This is suspended in 1 mL of 6 M guanidine hydrochloride, and the absorbance is measured at 370 nm. 6 M guanidine hydrochloride was used as a blank. This is done in triplicate and for each individual. The final result will be obtained using the following formula.

$$carbonyls\ \frac{\mu mol}{mg} = \frac{Absorbance\ 370nm}{0.022}$$

2.6. TBARS method in human erythrocytes

This technique was carried out having as a background that used with rat and rabbit brains in previous research [36].

2.6.1. Blood Collection

Samples were collected in 4 mL EDTA tubes. The puncture area was sterilized with alcohol and samples of approximately 20 mL were obtained. The patient presented fasting. Blood collection was carried out from 8-9 am. For erythrocyte ghost cells; the blood was placed directly into the EDTA tubes and gently shaken for 10 seconds. The tubes were centrifuged at 3000 rpm for 3 minutes. The plasma fraction was separated from the erythrocytes and the white cells were removed. They were washed by adding PBS (pH 7.4 ratio 1:1) to the erythrocytes, gently shaken and centrifuged at 3000 rpm for 3 minutes (2 times), in each wash the top part (supernatant) was removed to give way to the next wash. In the 3rd wash it was now washed with PBS (pH 8.0 - 4 times) to ensure that the greatest amount of hemoglobin had been extracted from the erythrocytes and it was left with this PBS for 2 hours to ensure the removal of all the hemoglobin from the erythrocytes. It was then washed again with PBS (pH7.4 - 2 times). A sac of erythrocytes was formed from which all the liquid was removed so that it was subsequently brought to the original volume of erythrocytes that was obtained at the beginning to dilute this sac, the liquid was gently recirculated.

2.6.2. Protein count by the Lowry method and adjust to the desired concentration

The counting was carried out to adjust the protein content of the samples obtained from erythrocytes (with hemoglobin or ghost cells) to a concentration of 2,666 mg/mL, since it is known that the amount of protein present is proportional to the amount of lipids in blood [37], this allows us to ensure the amount of lipids to which it will be exposed to lipoperoxidation. To 5µL of the supernatant was taken and placed on an Elisa plate. Subsequently, 20µL of distilled water and 150µL of the Lowry mixture were added. It was incubated for 10 minutes, Follin's reagent was then added and incubated for 30 min, and PBS was added to another well of the Elisa plate, which was the target, finally it was read at 540 nm to obtain the mg of protein.

Measure the absorbance at 540 nm. And substitute the values into the formula to obtain the protein content in the homogenate.

$$Protein\ (^{\mu g}/_{ml}) = \left[\frac{A_{540nm-}\ 0.05012}{0.0028}\right] 40$$

Adjust the protein content to 2.666 mg/mL with PBS (9.5 mM, pH=7.4).

$$\mathbf{V_2 = \frac{C_1 V_1}{C_2}}$$

The erythrocytes are diluted with the standardization that was done for protein counting, where 1.7 mL of ghost cells are diluted in 29,633 mL of PBS pH 7.4 to bring it to the concentration of 2,666 mg/mL.

2.7. Induction of oxidant stress with AAPH at 600 mM

Stress was induced since we want to quantify the main product of lipid oxidation, malondialdehyde (MDA). Which can be detected and quantified by reacting with thiobarbituric acid (TBA). The measurement of these "Thiobarbituric Acid Reactive Substances" (TBARS) is a good method to determine the capacity of different antioxidants to inhibit lipid peroxidation. Everything was worked in an ice bath. 375 µL of erythrocytes (2,666 mg prot./mL) were placed in triplicate in 1.5 mL Eppendorf tubes. 50 µL of 10 µM EDTA dissolved in PBS was added (final concentration 1 µM). 25 µL of the sample (20 times more concentrated) was added. It was incubated for 30 minutes at 37ºC with orbital shaking. 50 µL AAPH was added to a concentration of 600 mM (prepare fresh). Subsequently, it was incubated for 1 hour at 37ºC with orbital shaking. Then 500 µL of TBA reagent (thiobarbituric acid-trichloroacetic acid 1:1) was added. It was incubated for 10 minutes at room temperature. For 50 minutes, it was incubated in a water bath at 90 ºC. Cover and secure the tubes well since pressure is generated in this step. The tubes were allowed to cool to room temperature and were uncapped to release the pressure. They were centrifuged for 5 minutes at 12,000 rpm at room temperature. (in this technique the button is so compact that suspension is difficult). 200 µL of supernatant was taken from each tube and placed in a well of the 96-well plate. Finally, the absorbance was measured at 540 nm . To obtain the inhibition percentage, the following formula was used.

$$\text{TBARS}\ (\mu M) = \frac{A_{540nm} + 0.07386}{0.09042}$$

Calculate the % inhibition (Calculated with the µM concentration) Equation (C- E/ C) x 100

Where C (Control) is the average optical density of the AAPH + vehicle.

2.8. TBARS Inhibition % Calculation

Interpolate the values obtained into the Malondialdehyde standard curve (Figure 1) generated from Tetramethoxypropane, to obtain the TBARS content in solution. This allows us to obtain the % inhibition that tells us if the extracts protect erythrocytes from lipoperoxidation and therefore know if this antioxidant activity is relevant in the experiment.

Volume of the solutions to fill the tubes that are prepared in triplicate for each experiment

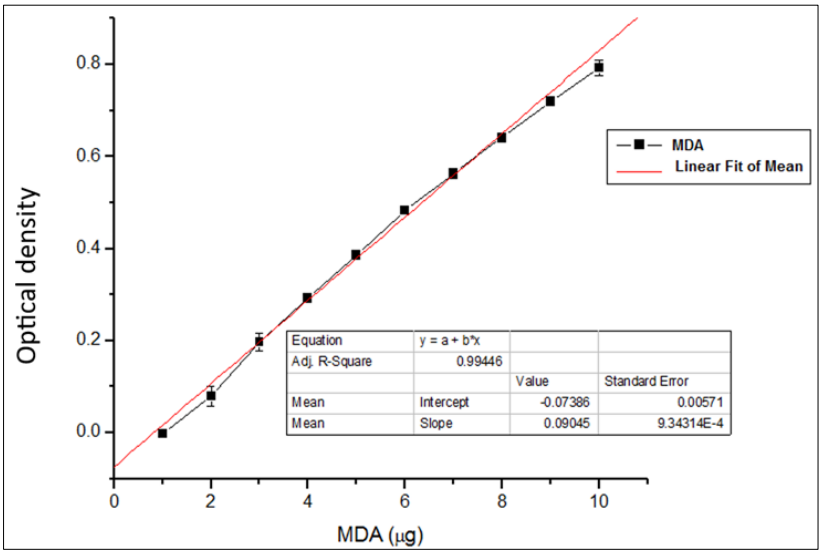

Figure 1 Malondialdehyde standard curve generated from Tetramethoxypropane

3. Results

All the infusions that were used in this project presented antioxidant activity from those that are classified as low activity to those that are considered as high activity.

3.1. Antioxidant activity using the ABTS radical

In a study carried out by Méndez [38], categorized the antioxidant activity (high 80-100%, moderate 50-79%, low 10-49% and null <10%) as can be seen, the infusion that had the highest percentage of inhibition was cinnamon with Stevia reaching 95.2% which is considered a high activity, an infusion that draws attention is that of peppermint since when Stevia is added it increases up to 47.4% going from low to moderate activity, other infusions. The ones found with activity were lemon tea, mandarin exocarp, and mint.

All infusions were significantly different (Tukey $p<0.05$), except Bugambilia-chamomile, tangerine-mint, mandarin-telimón, chamomile-orange and mint-lemon tea (Tukey $p>0.05$).

So it can be said that Stevia enhances the antioxidant activity of the infusions, figure 2.

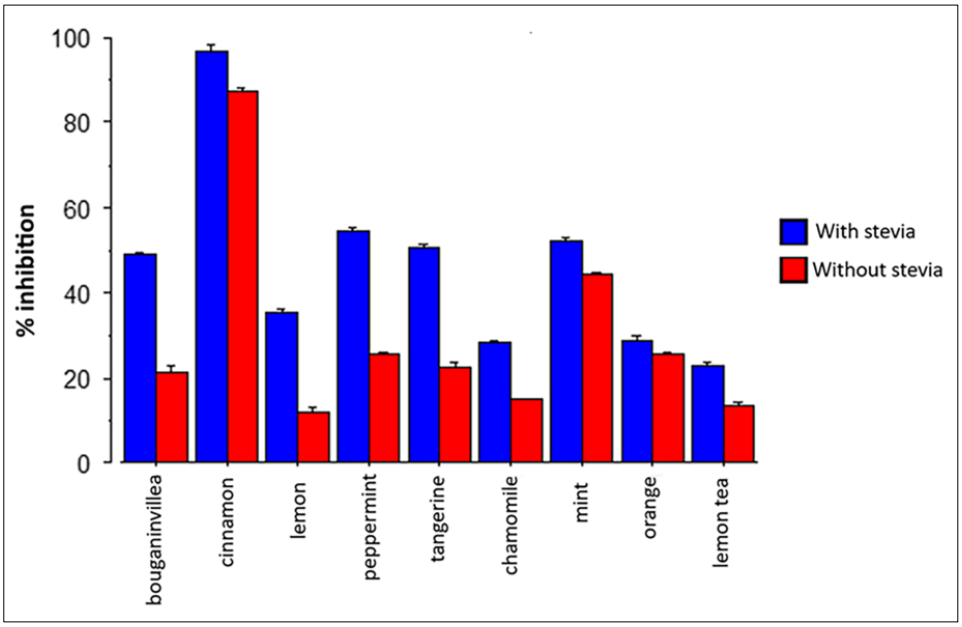

Figure 2 Percentage of inhibition of the ABTS radical

An ANOVA was carried out to find out if there were significant differences between the infusions that were sweetened with Stevia and those to which it was not added and the following can be observed

We found significant differences between the following factors infusion ($F_{8,36} = 920.702$, $p< 0.001$) treatment ($F_{1,36} = 226.190$, $p<0.001$) interaction ($F_{8,36} = 24.134$, $p<0.001$).

3.2. Evaluation of antioxidant activity by the DPPH method

In the DPPH (Figure 3) it is observed that cinnamon similarly presents the highest antioxidant activity both without Stevia and with Stevia, reaching 97.5%, classifying it as having high antioxidant activity, on the other hand, peppermint, as seen Its activity increases when stevia is added up to double, going from a low activity to a moderate activity, the same thing happens with mandarin and Bugambilia exocarp infusions, going from low to moderate activity when Stevia is added [38].

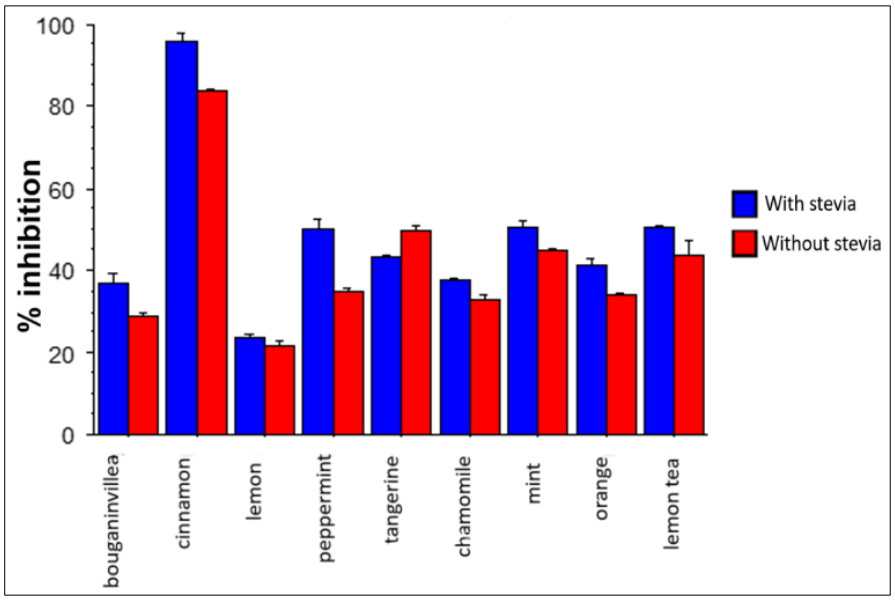

Figure 3 Percentage of DPPH inhibition

To find out if there were significant differences, an ANOVA was also applied.

We found significant differences between the following factors infusion ($F_{8,36} = 3834.17$, $p < 0.001$) treatment ($F_{1,36} = 4828.89$, $p<0.001$) interaction ($F_{8,36} = 197.76$, $p<0.001$).

All infusions were significantly different (Tukey $p<0.05$), except bougainvillea-tangerine (Tukey $p>0.05$). These results indicate that there are also significant differences when adding Stevia to infusions, causing them to enhance their low to moderate activity, as is the case of peppermint, Bugambilia and orange exocarp.

3.2.1. Determination of carbonylated proteins

In Figure 4, the protection of stevia in the carbonylation of proteins in human plasma is observed.

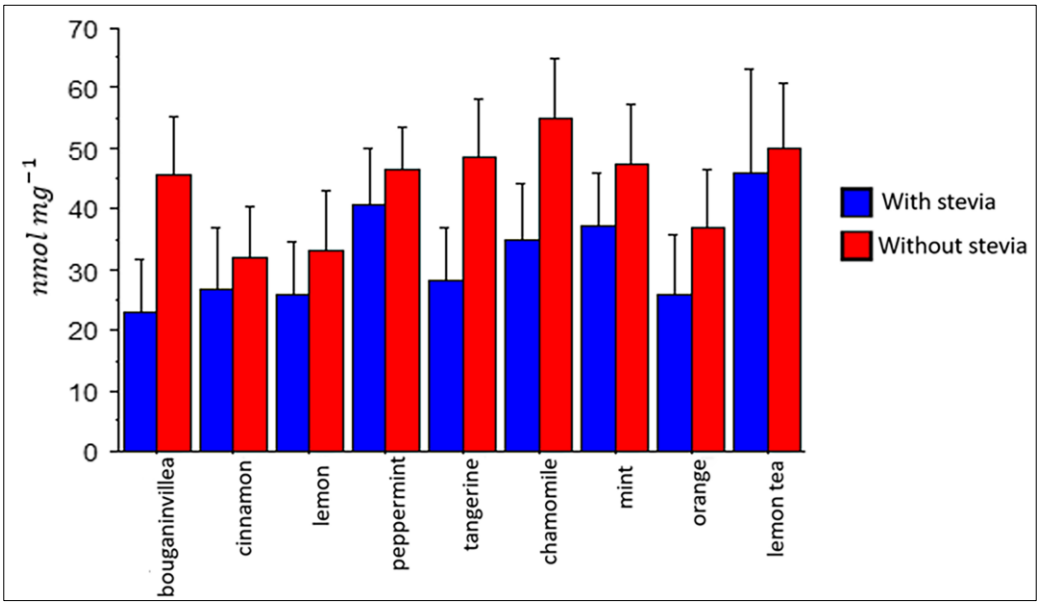

Figure 4 Concentration of carbonyl groups

To corroborate whether Stevia significantly increased the inhibition of carbonyl groups, an ANOVA was applied, showing the following results.

We found significant differences between the following factors infusion ($F_{8,252} = 14.49$, $p < 0.001$) treatment ($F_{1,252} = 78.23$, $p<0.001$) interaction ($F_{8,252} = 6.35$, $p<0.001$)

All infusions were significantly different (Tukey $p<0.05$), except bugambilia-cinnamon, bugambilia-lemon, bugambilia-peppermint, bugambilia-chamomile, Bugambilia-mint, cinnamon-lemon, cinnamon-mint, lemon-mint, and orange-peppermint, e.orange-chamomile, e.orange-mandarin, e.orange-lemon tea, peppermint-mandarin, peppermint-chamomile mint-mint, e.mandarin-chamomile, e.mandarin-lemon and chamomile – lemon tea (Tukey $p>0.05$)

3.3. TBARS inhibition percentage

In this test, what is measured is the final product of the oxidation of lipids in human plasma (malondialdehyde), as we can see in figure 5, the infusion that has the greatest antioxidant activity is the infusion of cinnamon with stevia 62%, followed of the infusion of orange exocarp with stevia 55%, peppermint with Stevia 50% and chamomile with Stevia 50%, classifying them as having moderate antioxidant activity.

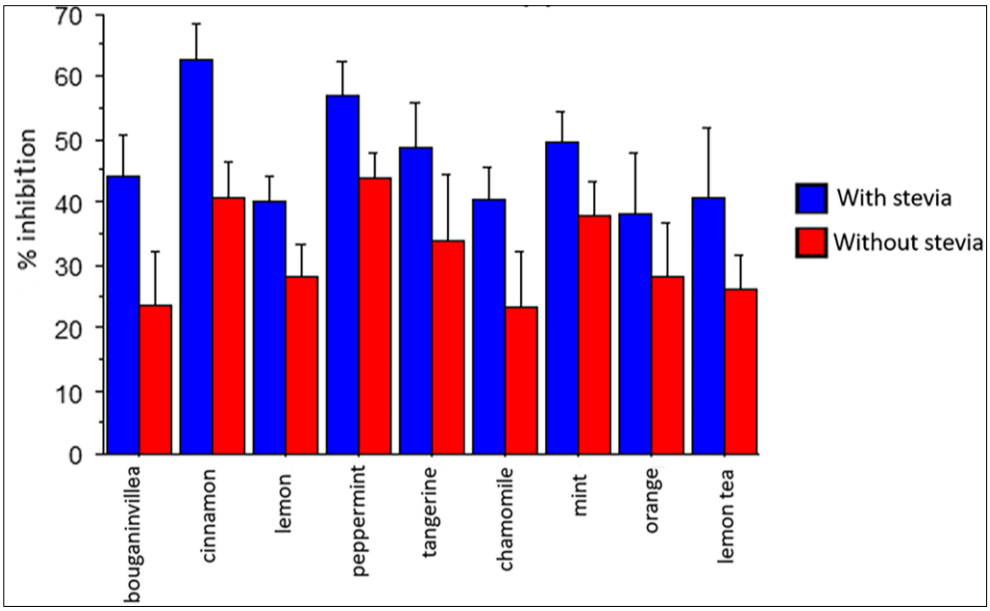

Figure 5 Percentage inhibition of TBARS

To find out if there were significant differences between those that were sweetened with Stevia and those that were not sweetened, an ANOVA was performed and the following was obtained:

We found significant differences between the following factors infusion ($F_{8,252} = 14.49$, $p < 0.001$) treatment ($F_{1,252} = 78.23$, $p<0.001$) All infusions were significantly different (Tukey $p<0.05$), except those of bougainvillea-e .tangerine, bugambilia-e.lemon, bugambilia-mint, bugambilia-télimón, cinnamon-e.orange, e.lemon-mandarin, e.lemon-mint, e.lemon-télimón, chamomile spearmint.

Which indicates that Stevia, in the same way as in previous tests, in some infusions can potentiate antioxidant activity to help inhibit some oxidation processes, such as lipoperoxidation.

4. Discussion

One of the contributions that this research makes is to verify that each of the infusions used presents antioxidant activity. In the background table we can see that the same species that we used presented antioxidant activity but in extracts from different solvents, so now it was done in the form of infusions since it is an easy, accessible and low-cost way for the population to be able to consume these infusions in addition to corroborating that they maintain this antioxidant activity. Each of the species is described below.

It has been shown in different studies that cinnamon has various health benefits such as acting in the prevention of oxidative stress. It is known that cinnamon is rich in phenolic compounds, which could give it the characteristic of acting as an excellent source of antioxidants as well. that it is mainly used as a treatment for diarrhea, stomach upset,

respiratory problems and as a skin antiseptic (Aguilar [39]; González [40]; Linares [41]; Rakshit and Ramalingam [42]). In this study it was demonstrated that the cinnamon infusion had the greatest antioxidant activity which could prevent processes such as lipoperoxidation and carbonylation as well as inhibit the synthetic radicals ABTS and DPPH. It is the first study where infusions of various flowers are prepared., leaves and bark. There are several articles where they only use extracts and where the antioxidant power of cinnamon has also been demonstrated, such is the case of where the results of this study show that cinnamon leaf extract can be used as an Easily accessible source of natural antioxidants or in the pharmaceutical industry. It can also be used to stabilize foods against oxidative deterioration, and as results they obtained that cinnamon leaves can inhibit the radical DPPH 85%, ABTS 65% and TBARS 18.6% which are not far from these results obtained and that despite Because the cinnamon was subjected to a high temperature, it was able to maintain its compounds.

Another study carried out by Mathews and Abraham [43] made methanol extracts with cinnamon leaves, they tested it against ABTS and it was able to inhibit it by 90%, against the DPPH radical 91%, these results are still comparable and similar to ours, although we use the bark, it maintains that antioxidant activity and neither does the temperature to which it is subjected decomposes the metabolites that act as antioxidants.

Currently, carbonylation is a new topic that has not been studied enough. This could be one of the first investigations of how infusions, mainly cinnamon, can inhibit this process that causes irreversible damage to proteins, generating loss of function. of proteins in addition to proteolysis, which can trigger various diseases such as cancer, diabetes, Alzheimer's, among others.

As observed in the results of the TBARS test, cinnamon could also inhibit lipid oxidation since it was the most effective in this test.

4.1. Peppermint

Rameshwar in 2012 carried out a study in which the antioxidant activity of the methanolic extract of peppermint was evaluated and they observed that this extract was able to inhibit the DPPH radical by 54.84%, which if we compare it with our results 43.3 and 50.9% are similar, probably In our study we obtained a lower result since to prepare the infusions they are subjected to high temperatures which could be affecting the antioxidant properties that this plant has.

4.2. Lemon tea

In our research we were able to observe that when preparing an infusion using 5 g of leaves in 300 mL of water it was able to inhibit the DPPH radical by 13.25% and when adding Stevia its activity increased up to 23.5% and that if we compare it with the study carried out by Villalobos [44] where they prepared a tea with lemon tea leaves obtained an inhibition of 79.9%, if we compare the percentages of both studies they are completely different, it could be associated with the fact that in Villalobos' study the leaves were allowed to be at the boiling point for 30 minutes which could have collaborated in the extraction of more antioxidant compounds

4.3. Lemon exocarp

The results obtained in this sample demonstrated a low antioxidant activity in the DPPH tests (without Stevia 11.9 and with Stevia 36.3%), TBARS (without Stevia 29% and 40% with Stevia) and ABTS (without Stevia 21.92% and with Stevia 23.6 %), even adding Stevia it could not be potentiated. In a 2016 study carried out by Otang and Afoloyan [45], they made methanolic extracts with the lemon exocarp to measure its antioxidant activity through the DPPH test, resulting in an inhibition percentage of 80%, which could be classified as high according to Méndez [38]. This could be because as they made the extract they could have obtained greater metabolites with antioxidant capacity. Another factor that could have influenced would be that, for the infusion, it was subjected to elevated temperatures which could have affected the composition of the antioxidant compounds.

4.4. Chamomile

Chamomile is a perennial flowering herb that grows in widespread regions, including Europe, Africa and Asia. This flower is widely distributed in Iran and is used in traditional Iranian medicine for various conditions. One of the most important flavonoids in chamomile is apigenin, which has mild relaxing properties in addition to antioxidant activity.

This sample of chamomile flowers presented a moderate antioxidant activity only in the TBARS test, reaching 40% inhibition and when adding Stevia, it increased to 50%, concluding that this flower has antioxidant activity that could inhibit the lipoperoxidation process considerably. Alibabaei [46] tested an ethanolic extract of chamomile flower to see

if it could inhibit lipoperoxidation and measure its antioxidant activity, and observed that this extract could inhibit the DPPH radical by up to 70%, and reduce the generation of malondialdehyde. in brain and blood plasma of rats. Which is similar to the results we obtained since in all the tests in which it presented the most antioxidant activity was in the TBARS test.

4.5. Bugambilia

The Bougainvillea genus is a widespread group throughout the world; some species of bougainvillea have emerged as sources of traditional medicine for human health, such as infusions. In this study, Bougainvillea spectabilis was used and it could be seen that it was one of the best infusions to inhibit the carbonylation process (with Stevia 24 nmol mg-1 and without Stevia 45 nmol mg-1), probably associated with the fact that it has a significant amount of phytochemicals and antioxidant activity *in vitro* and to the significant linear relationship between antioxidant activity and phytochemicals, which are responsible for the antioxidant property in vitro according to the study conducted by Chauhan [47].

4.6. Mandarin exocarp

Citrus foods are an important crop used mainly in food industries for juice production and the exocarp of these fruits are the main byproduct of these processes.

In general, the skin of the fruit contains a higher concentration of antioxidant substances than the flesh of the fruit. Citrus exocarp is a rich source of bioactive compounds that include natural antioxidants such as phenolic acids and flavonoids.

According to the results of this study, the mandarin exocarp behaved similarly in the ABTS, DPPH and TBARS tests, obtaining moderate antioxidant activity.

4.7. Mint

Elmastas [48] made an ethanol extract with mint leaves and this was tested against the DPPH radical, being able to inhibit it by 92%, while in our study it could only inhibit the DPPH radical by 45.3 and 48.7%, and the antioxidant compounds when subjected to boiling point, in addition to the fact that an extract contains a greater amount of antioxidant components, on the other hand in the carbonylation test it was one of the infusions that performed best and that could inhibit the formation of these groups.

5. Conclusions

All infusions presented antioxidant activity. Infusions without Stevia only obtained moderate antioxidant activity in all tests except cinnamon. In the ABTS test, the infusions of mandarin, Bugambilia and peppermint alone only obtained moderate antioxidant activity, but adding Stevia boosted its activity to almost double.

- Infusions sweetened with Stevia presented a significant difference compared to those that were not added.
- Stevia can act as a natural sweetener, enhancing the antioxidant activity of infusions.
- The cinnamon infusion was the one that presented the highest antioxidant activity in the DPPH and ABTS and TBARS tests.

Compliance with ethical standards

Disclosure of conflict of interest

The authors and all co-authors declare that they have no conflicts of interest in connection with this document, and the material described is not in the process of being published nor is it intended for publication elsewhere.

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