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# Effect of infusions based on fig leaves (*Ficus carica*) and stevia (*Stevia rebaudiana*) on antioxidant capacity and inhibition of the α-glucosidase enzyme *in vitro*

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## Abstract

*Ficus carica* and *Stevia rebaudiana* leaves exhibited biological activity, specifically antioxidant activity and α-glucosidase enzyme inhibition. This research aimed to evaluate the antioxidant activity through the DPPH and ABTS methods, as well as the in vitro α-glucosidase enzyme inhibition of fresh and dried leaf infusions at different concentrations. Results showed that infusions made with dried leaves demonstrated higher biological activity than those made with fresh leaves. When evaluated separately, the leaves showed low antioxidant activity percentages in the DPPH and ABTS tests. For fresh leaves, *Ficus carica* presented (41.1% ± 1.9), and *Stevia rebaudiana* (46.1% ± 1.1), while for dried leaves, Ficus showed (75% ± 1.8) and Stevia (87% ± 2.1). In enzyme inhibition, *Ficus carica* showed (7.9% ± 1.2), and Stevia (37.9% ± 1.3). When the dried leaves were combined, antioxidant capacity and α-glucosidase enzyme inhibition increased. In the DPPH neutralization test, the best results were achieved with an infusion containing a 2.5:1.5 ratio of *Ficus carica* and *Stevia rebaudiana*, showing 59% neutralization. In the ABTS radical neutralization test, the infusion with a 1:1 ratio of fresh leaves achieved a reduction of (88.5%). Lastly, in the α-glucosidase enzyme inhibition test, the highest percentage was obtained with the 2:1 infusion of *Ficus carica* and *Stevia rebaudiana*, achieving (67%), and with the 1:1 treatment of both fresh leaves, achieving (62.1%). This demonstrates that the combination of these leaves exhibits a synergistic effect, enhancing the biological activity of these plants.

**Keywords:** Antioxidants; Free Radicals; Hydrolases; Polyphenols; Synergism

## 1. Introduction

Over the years, plants or their parts have been used for medicinal purposes. Currently, hundreds of plants have been evaluated using validated experimental methods to determine qualitatively and quantitatively if they exhibit biological activity that could benefit health. Recently, research lines have emerged to identify chemical compounds such as metabolites that can assist in the treatment of Type 2 Diabetes Mellitus (T2DM), aiming to inhibit digestive enzymes like α-glucosidases, which are responsible for breaking down oligosaccharides into glucose molecules. Various plants and seeds, such as the leaves of *Juglans neotropicalis* [1], *Salvia hispanica* [2], and *Carica papaya* [3], have shown inhibitory effects on α-glucosidase enzymes, suggesting that this biological activity may be attributed to the phenolic compounds these plants contain. Some Ficus species have been used in traditional medicine to treat diabetes, with evidence suggesting that the biological activity of the Ficus genus may be linked to its antioxidant activity and the phenolic compounds in *Stevia rebaudiana* leaves [4]. Based on this information, this research proposed the evaluation

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of the *in vitro* biological activity of *Ficus carica* and *Stevia rebaudiana* and their potential synergism for inhibiting the α-glucosidase enzyme.

## 2. Material and methods

### 2.1. Plant materials

*Ficus carica* and *Stevia rebaudiana* leaves were obtained from San Matías Tepetomatitlán, in the municipality of Apetatitlán, Tlaxcala, Mexico. A total of 500 g of each type of leaf was collected and selected based on their physical appearance.

### 2.2. Sample preparation

For the preparation of infusions with fresh and dried leaves, the same sequence was followed: 320 mL of water was poured into Erlenmeyer flasks, heated to 92 °C, then the leaves of each plant were added and allowed to cool. The infusions were left to stand for 2 hours, followed by filtration. The quantities of leaves for each treatment are shown in Table 1.

**Table 1** Treatments of the evaluated infusions

| **Treatment** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
|---|---|---|---|---|---|---|---|---|---|
| *Ficus carica* Leaf | 4g | --- | 3.5 g | 3.0 g | 2.5 g | 2.0 g | 1.5 g | 1.0 g | 0.5 g |
| *Stevia rebaudiana* Leaf | --- | 4 g | 0.5 g | 1.0 g | 1.5 g | 2.0 g | 2.5 g | 3.0 g | 3.5 g |

### 2.3. DPPH radical neutralization

This widely used technique measures antioxidant capacity based on the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical [5], which is unstable due to its unpaired electron. When it interacts with an antioxidant agent, it becomes stable. The percentage of radical inhibition was calculated using the formula: % Inhibition = (C-E/C) x100

C is the average absorbance of the radical control and E is the average absorbance of the sample control minus the sample absorbance.

The procedure is performed in triplicate:

50 µL of the samples (infusions) were placed in wells. Then, 150 µL of DPPH• 133.33 µM mixed with ethanol was added. The samples were incubated at 37°C for 30 minutes with orbital shaking. The absorbance was read in an ELISA plate reader at 515 nm.

### 2.4. ABTS radical capture

The methodology developed by Delgado [6] was used, in which the radical 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) is generated by myoglobin activation with hydrogen peroxide in the presence of $ABTS^{\cdot+}$. To measure the % radical reduction and its Trolox equivalent, the following formulas were applied:

$$\% = \frac{\text{Initial Abs} - \text{Absorbance per minute)} \times 100}{\text{Initial Abs}} \qquad \text{TEAC} = \frac{\% \text{ reduction} - 3.09777}{4.76498}$$

To generate the ABTS radical, ABTS 7 mM was mixed with $K_2S_2O_8$ 2.45 mM at final concentration, incubated in darkness for 16 hours under normal conditions. The radical was diluted with 7 mL ethanol to obtain an absorbance range of 0.700-0.702 at 436 nm. Then, 10 µL of the sample was mixed with 990 µL of the diluted $ABTS^{\bullet+}$ solution to monitor the scavenging effect every minute for six minutes in a spectrophotometer.

### 2.5. Determination of *α*-glucosidase enzyme inhibition

*α*-glucosidase (EC 3.2.1.20 from Saccharomyces cerevisiae obtained from Sigma-Aldrich Co., St. Louis, USA) inhibition was evaluated using an adapted method from Tong-Zhou [7] and Xiao-Ping [8]. A 25 µL solution of test samples in DMSO-$H_2O$ (1:1) was added to 150 µL of phosphate buffer solution (PBS, 67 mM, pH 6.8) and incubated at 37°C for 10 minutes with 25 µL of reduced glutathione (3 mM in PBS) and 25 µL of 0.2 U/mL *α*-glucosidase type I in PBS solution.

Then, 25 µL of the substrate solution (23.2 mM *p*-nitrophenyl-*α*-D-glucopyranoside in PBS) was added and incubated for an additional 15 minutes at 37°C with shaking. The reaction was stopped with 50 µL $CaCO_3$ 1M, and after 5 minutes of agitation, optical density was measured at 405 nm. Quercetin was used as a positive control. The inhibition percentage was calculated by equation:

Inhibition (%) = [(A control- A sample) / A control]x100.

Were A is the absorbance at 405 nm of sample and control.

## 3. Results

### 3.1. Evaluation of antioxidant capacity via DPPH

To evaluate the antioxidant capacity of the infusions using the DPPH test, differences among the factors (treatment and type of leaf) were analyzed using a two-way ANOVA. Using the statistical package Statview.

The treatments were evaluated in triplicate through spectrophotometric techniques with the synthetic DPPH radical, as shown in Figure 1.

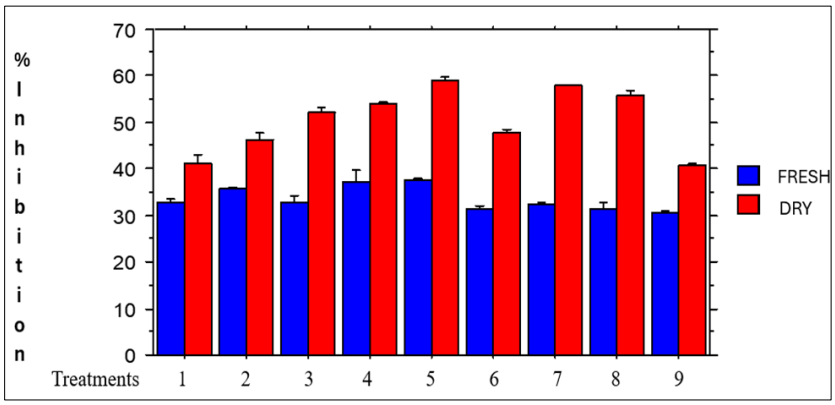


**Figure 1** Average ± S.E. of the % inhibition with the DPPH test. Factors: treatment of the 9 infusions, of fresh and dry leaves. n= 3 replicates

An average percentage of DPPH radical reduction was found to differ among the treatments (9 infusions) ($F_{8, 36}$ = 27.29, $p < 0.0001$). Differences were also found between types of leaves (fresh and dried) ($F_{1, 36}$ = 1028, $p < 0.0001$). Additionally, differences were noted in the interaction between treatments and types of leaves ($F_{8, 36}$ = 14.95, $p < 0.0001$). A Tukey's test was performed to determine which treatments were different, showing that most treatments were significantly different (Tukey $p < 0.05$).

Among the three treatments with a high percentage of radical inhibition (Treatments 4, 5, 7), it was observed that combining *Ficus carica* and *Stevia rebaudiana leaves* increased the DPPH radical inhibition capacity. Optimal concentrations were achieved with 1–1.5 g of *Stevia rebaudiana* and 2.5–2 g of *Ficus carica.* In comparison, Treatments 1 and 2, which evaluated these leaves independently, showed lower inhibition percentages, with 41% and 46%, respectively.

### 3.2. Antioxidant activity via the ABTS method

In analyzing the average percentages of ABTS radical inhibition, significant differences among treatments (9 infusions) were observed ($F_{8, 36}$ = 16.69, $p < 0.0001$). Similarly, differences were found between types of leaves (fresh-dried) ($F_{1, 36}$ = 3894, $p < 0.0001$), and differences were also observed in the interaction between treatments and leaf types ($F_{8, 36}$ = 15.71, $p < 0.0001$).

A Tukey's test revealed significantly different treatments, including T1-T7, T1-T9, T2-T4, T2-T7, T2-T9, T3-T4, T3-T5, T3-T7, T3-T9, T4-T8, T4-T9, T5-T9, T6-T9, T7-T8, T7-T9, T8-T9 (Tukey $p < 0.05$), while no significant differences were found in the other treatments (Tukey $p > 0.05$). This suggests that *Stevia rebaudiana* leaves potentiate the antioxidant property of the infusions when combined.

In Figure 2, data from the treatments represent the average of three repetitions (mean ± SE). All treatments with dried leaves showed high radical inhibition percentages (>70%, according to Argüelles [9], while fresh leaves exhibited moderate to low inhibition (<70%, Argüelles).

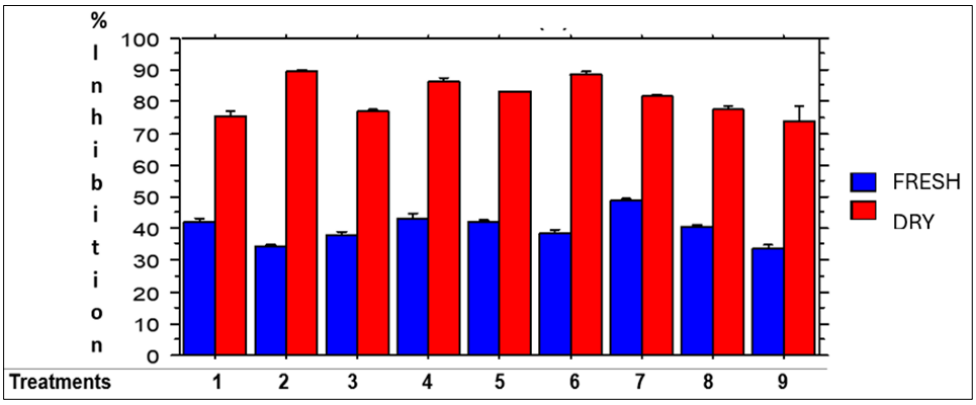


**Figure 2** Average ± S.E. of the % inhibition with the ABTS test. Factors: treatment of the 9 infusions, type of fresh and dry leaf. n= 3 replicates

When dried leaves of *Ficus carica* and *Stevia rebaudiana* were evaluated separately, inhibition percentages of 75.5% and 87.3% were reported, respectively. When combined, Treatment 6 achieved a high inhibition percentage of 88.5%, surpassing that of the individual leaves. Treatment 4 followed closely with 86% inhibition, using optimal concentrations of *Stevia rebaudiana* (1–1.5 g) and *Ficus carica* (2.5–2 g), which were lower than the concentrations used in Treatments 1 and 2 (4 g each), suggesting potential synergism between these plants that enhances the radical inhibition activity even with reduced leaf concentrations.

### 3.3. $\alpha$-Glucosidase enzyme inhibition test

A two-way ANOVA was conducted to assess significant differences among treatments, leaf types, and their interactions regarding α-glucosidase enzyme inhibition. Significant differences were found among treatments (9 infusions) ($F_{8,\ 36}$ = 115.8, $p < 0.0001$), among leaf types (fresh-dried) ($F_{1,\ 36}$ = 1667.5, $p < 0.0001$), and among interactions of treatments and leaf types ($F_{1,\ 36}$ = 43.3, $p < 0.0001$), as shown in Figure 3.

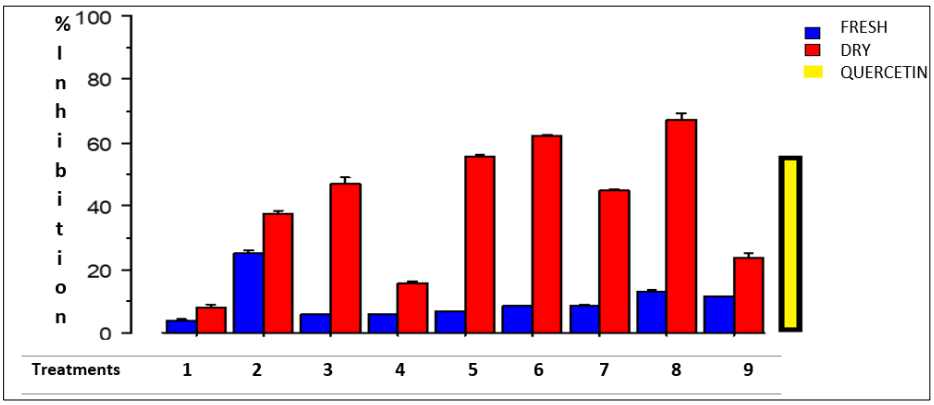


**Figure 3** Average ± S.E. of % inhibition of the enzyme $\alpha$-glucosidase and $IC_{50}$ (21.6 µM) of quercetin: treatments (9 infusions), leaf type (fresh and dry). n= 3 replicates

## 4. Discussion

Treatments for controlling type 2 diabetes mellitus (T2DM) present a wide variety of adverse effects reported over the years, which depend on each individual's physiological response [10], as well as the clinical complications that can arise from poor disease management. Choosing the right treatment, ensuring proper clinical laboratory testing, and controlling the condition are key factors for the successful management of T2DM. Some non-natural drugs pose a higher

risk of adverse effects for individuals with this disease [11], which is why there is great interest in seeking natural alternatives for treating diseases such as T2DM.

This research proposed the use of aqueous infusions made from *Ficus carica* and *Stevia rebaudiana* leaves, both as individual infusions of each plant species and in combination of the two species at different concentrations. It should be noted that previous studies on these plant species have utilized extracts [12]; however, in this study, we aimed to analyze infusions, or commonly known as teas, rather than extracts prepared with various solvents, as well as to study the interaction between these two plant species when combined and at different concentrations. The analyses involved both fresh and dried leaves using various *in vitro* methods, specifically DPPH, ABTS, and α-glucosidase enzyme inhibition.

### 4.1. Antioxidant method by DPPH

When evaluating the antioxidant capacity through the aforementioned method, we found that infusions made with dried leaves exhibited greater antioxidant capacity than those made with fresh leaves in all treatments.

In the results obtained, it is noteworthy that *Ficus carica* leaves alone had an antioxidant capacity of 32.6% in the infusion made with fresh leaves and 41% in the dried leaf infusion, using the same amount of leaves (4 g). This value is comparable to that obtained by Chunying [13], who tested various extracts from *Ficus carica* residues, one of which was an aqueous extract that achieved 40% radical inhibition at a concentration of 2.5 mg/mL.

Additionally, it was observed that *Stevia rebaudiana* leaves alone had an antioxidant capacity of 35.6% in the fresh leaf infusion and 46% in the dried leaf infusion, using the same amount of leaves (4 g). In a comparable study, a dried *Stevia rebaudiana* leaf infusion achieved 60% radical inhibition at a concentration of 10 mg/mL, which is higher than the values reported here. Other authors [14] report that the drying conditions applied to stevia leaves have a significant effect on steviol glycosides, with an increase in antioxidant capacity and a decrease in stevioside concentration.

When combining the two leaves, Treatment 5 achieved an antioxidant capacity of 37.3% with fresh leaves and 59% with dried leaves, using 2.5 g of *Ficus carica* and 1.5 g of *Stevia rebaudiana*, which was higher than the results obtained in Treatments 1 and 2 where the leaves were evaluated separately. This suggests a possible synergistic effect between the leaves that enhances their biological activity.

### 4.2. Antioxidant method by ABTS

The reduction of the ABTS radical, which is reduced by interaction with electron-donating molecules, was quantified. This radical is measured at a wavelength of 734 nm and is generated by an oxidation-reduction reaction with potassium persulfate.

In the results obtained, it is noteworthy that the infusion made with *Ficus carica* leaves alone had an antioxidant capacity of 42% in the fresh leaf infusion and 75% in the dried leaf infusion, using the same amount of leaves (4 g). Ergul and Mustafa [15] used a concentrated aqueous extract of dried *Ficus carica* leaves (10 g in 50 mL of water) and reported 50% radical inhibition at a concentration of 1000 μg/mL, which is lower than the values reported in our study.

Similarly, it was observed that *Stevia rebaudiana* leaves alone had an antioxidant capacity of 43% in the fresh leaf infusion and 89% in the dried leaf infusion, using the same amount of leaves (4 g). This result differs from that reported by Singh [16], who found 68% inhibition in dried leaves at a concentration of 40 mg/mL.

When combining the two leaves, Treatment 6 exhibited a higher antioxidant capacity with 40% in fresh leaves and 88.5% in dried leaves, using 2 g of *Ficus carica* and 2 g of *Stevia rebaudiana.* Treatments 5, 7, and 8 also showed higher antioxidant percentages than the other treatments, with similar proportions of *Ficus carica* and *Stevia rebaudiana* leaves ranging from 1.5 to 2 g in combination. This suggests a possible synergism between these leaves, resulting in enhanced biological activity that is not observed when the leaves are evaluated separately.

### 4.3. α-Glucosidase enzyme inhibition

α-Glucosidase is a key enzyme in carbohydrate digestion, catalyzing the hydrolytic cleavage of the α-glycosidic bond in oligosaccharides to release monosaccharides (glucose). This enzyme is therefore a target for modulating postprandial hyperglycemia, the earliest metabolic abnormality in T2DM. Extracts with α-glucosidase inhibitory effects could thus be beneficial in treatment or as bioactive ingredients in antidiabetic supplements. The evaluation of α-glucosidase inhibition is based on the spectrophotometric monitoring of *p*-nitrophenol release by the enzymatic action of *p*-

nitrophenyl-*α*-D-glucopyranoside (pNPG) used as a substrate [17]. Inhibitors present in the extracts deactivate the enzyme, preventing substrate hydrolysis and p-nitrophenol formation. All tested treatments exhibited inhibitory activity.

When assessing enzyme inhibition using the aforementioned method, we found that infusions made with dried leaves showed higher inhibition than those made with fresh leaves.

In the results obtained, it was observed that *Ficus carica* leaves alone had an enzymatic inhibition of 3.8% in the fresh leaf infusion and 7.9% in the dried leaf infusion, using the same amount of leaves (4 g). Quercetin was used as a positive control, where the IC50 was 21.6 μM, indicating 50% inhibition of the α-glucosidase enzyme. These data suggest a possible synergistic effect between these leaves, potentially enhancing the biological activity related to α-glucosidase inhibition.

In similar studies evaluating enzyme inhibition *in vitro*, [18] found 50% inhibition using 100 g of leaf in 500 mL of water at a concentration of 500 μg/mL in an aqueous *Ficus carica* extract.

Additionally, it was observed that *Stevia rebaudiana* leaves alone had an enzymatic inhibition of 25.1% in the fresh leaf infusion and 37.4% in the dried leaf infusion, using the same amount of leaves (4 g).

A study by Uswatun [19] evaluated an aqueous extract of *Stevia rebaudiana* leaves and reported no enzyme inhibition at any concentration, differing from our findings, which did show enzyme inhibitory activity. Amhad [20] evaluated an aqueous extract prepared with dried stevia leaves for α-glucosidase inhibition, reporting 18.3% inhibition using 5 g in 50 mL of water at a concentration of 1 mg/mL, which was lower than the values reported here.

When the two leaves, *Ficus carica* and *Stevia rebaudiana*, were combined, an increase in α-glucosidase enzyme inhibition was observed, with Treatments 5, 6, and 7 showing inhibition percentages of 55%, 51%, and 67%, respectively, using dried leaves in these treatments. Overall, analyzing all three tests (DPPH, ABTS, α-glucosidase inhibition), high percentages of antioxidant capacity and enzyme inhibition were achieved in Treatments 5, 6, and 7, all of which used combined leaves of Ficus and Stevia at concentrations of (T5: 2.5 g/1.5 g), (T6: 2 g/2 g), and (T7: 2 g/1.5 g). Optimal leaf concentrations were found in these treatments, while treatments with leaves evaluated separately at 4 g per treatment showed lower percentages than Treatments 5, 6, and 7, where the leaf combination was applied. This indicates the possibility of a synergistic effect where phenolic compounds in these leaves interact to enhance their biological activity in the tests conducted.

## 5. Conclusion

In this study, treatments were evaluated with *Ficus carica* and *Stevia rebaudiana* leaves separately, as well as in combination, using both fresh and dried leaves. It was found that antioxidant capacity against DPPH and ABTS radicals was higher in dried leaves than in fresh leaves. The same pattern was observed in the α-glucosidase enzyme inhibition test. Furthermore, treatments where Ficus and Stevia leaves were evaluated separately showed lower antioxidant capacity and α-glucosidase enzyme inhibition than those performed with the combined leaves, suggesting a possible synergism between the secondary metabolites in these plants, which enhances their biological activity in antioxidant capacity and α-glucosidase enzyme inhibition.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors declare that there is no conflict of interests.

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