Inhibition of Urease, Elastase, and β-Glucuronidase Enzymatic Activity by Applying Aqueous Extracts of Opuntia oligacantha C.F. Först Acid Fruits: In Vitro Essay under Simulated Digestive Conditions

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Abstract: Non-communicable diseases such as gastric inflammatory diseases and the hepatic pathologies are mainly related to bad lifestyle habits such as recurrent consumption of non-steroidal anti-inflammatory drugs (NSAIDs), excessive intake of alcohol, tobacco, steroids (high doses), alkaline agents, strong acid foods, and high-fat food, and Helicobacter pylori infections, among others. The fruit of Opuntia oligacantha C.F. Först var. Ulapa (xoconostle) is currently being studied due its nutritional and functional properties. The objective of the present study was to evaluate gastroprotective, anti-inflammatory, and hepatoprotective activities of different parts of xoconostle fruit by establishing in vitro simulated gastrointestinal conditions. Four treatments were established to test aqueous extracts (pericarp (P), mesocarp (M), endocarp (E) and whole fruit (W)). The quantified bioactive compounds were the total phenols, flavonoids, tannins, and betalains. The enzymatic assays were: urease, elastase, and β-glucuronidase. Significant differences (p < 0.05) of bioactive compounds content were measured in xoconostle extracts, the highest concentration was found in W (phenols 313 mg GAE/100 g, flavonoids 189 mg QE/100 g, tannins 71 mg CATE/100 g). The betalains content was higher in E; 17 mg/100 g significant differences were observed (p < 0.05) in the enzymatic inhibitions test (urease, elastase and β-glucuronidase), where W presented the highest inhibition activity (86%, 79%, and 84%), respectively. Bioactive compounds after in vitro gastrointestinal tests were maintained above 60% enzymatic inhibition activity.

Keywords: Opuntia sp.; bioactive compounds; enzymatic inhibition; betalains; flavonoids; phenols; tannins; in vitro
1. Introduction

Chronic gastric-inflammatory diseases are mainly related to lifestyle, such as excessive consumption of strong acids, alkaline agents, the presence of the bacterium *Helicobacter pylori*, abuse in the consumption of fatty foods, and the irresponsible use of non-steroidal anti-inflammatory drugs (NSAIDs) [1]; the last two factors also cause liver damage [2]. Ureases have been shown to catalyze the hydrolysis of urea to form ammonia and carbon dioxide. Ammonia allows the growth of *H. pylori* bacteria in the presence of extra-cellular gastric acid, responsible for the negative effects [3]. In previous studies where oregano (*Origanum vulgare*) and blueberry (*Vaccinium corymbosum*) extracts were applied, the enzymatic activity of ureases was inhibited by 40 and 9%, respectively. The authors established that this inhibitory activity could be related to the content of polyphenols, which can chelate the active site of enzymes [4]. Elastase is a proteolytic enzyme related to inflammatory diseases of epithelial tissue [5]. Neutrophils and macrophages (blood cells) secrete elastase in the presence of toxic agents and pathogens such as fungi, bacteria, and viruses, with the ability to degrade components of the extracellular matrix that amplify inflammation [6,7]. B-glucuronidase is associated with liver damage and occurs with elevated levels of this enzyme in the blood [8]. This enzyme catalyzes the hydrolysis of β-D-glucuronic acid [9], which, at abnormal levels, limits the process of xenobiotic biotransformation of the conjugation of toxins with glucuronic acid; thus, it could reduce the capacity of the system to deactivate toxic compounds and eliminate them from the body [10].

*Opuntia robusta* (nopal taponochtle) and *Opuntia streptacantha* (nopal cardon) prickly pears contain a wide variety of functional compounds that promote liver health [11,12]. The leaves of *Tithonia tubaeformis* and *Sylbum marinum* have an anti-inflammatory effect attributed to their bioactive compounds such as phenols [13,14].

The fruits of *Opuntia oligacantha* c.f. först (xoconostle) contains bioactive compounds such as phenols and flavonoids that have attributed the ability to inhibit biological enzymes such as α-amylase and α-glucosidase [15]. Furthermore, it has been attributed antioxidant and antimicrobial activity [16]. It has been observed that during stomach digestion there is absorption of some phenols [17], having a bioavailability close to 50% [18] and an absorption of 5–10% of the total intake thanks to different transporters present in the epithelial cells of the small intestine [10]. The exposure of phenolic compounds to different pH conditions and inhibitors or enzymes of the gastrointestinal tract represent an influential factor in the use of their anti-oxidant properties [17]. The objective of the present study was to evaluate the inhibition of the enzymes urease, elastase, and β-glucuronidase related to gastroprotective, hepatoprotective, and anti-inflammatory effect, respectively, by different structures of acid prickly pear by applying in vitro tests and gastrointestinal simulated conditions.

2. Materials and Methods

2.1. Plant Material

Extracts from cactus pear (*Opuntia oligacantha* C.F. Först var. Ulapa) were obtained from physiological ripe fruits harvested in the municipality of Melchor Ocampo, Tetepango Hidalgo, Mexico (altitude 2040 latitude 20°80’41.7” N longitude 99°09’59” W) during the summer season (July) of 2020. The intact fruits were stored in a refrigerator at 4 °C in dark containers until use.

2.2. Preparation of the Extracts

Fruits were first washed with tap water, and then disinfected by immersion in sodium hypochlorite (commercial bleach 2.5% (v/v) for 5 min), and finally rinsed four times with sterile water. This procedure was followed before the processing of fruit to obtain the four extracts: pericarp (P), mesocarp (M), endocarp (E), and whole fruit (W); (see Figure 1). The fruit fractions were extracted separately following the method described by Cenobio-Galindo et al. [19] with some modifications; fruit fractions were mixed with distilled water to a proportion 1:2 and homogenized in an industrial blender HGBSS (Waring Commercial,
Torrioting, CT, USA) for approximately 3 min then the product was vacuum filtered using Whatman paper number 1 to obtain the extracts, with a final concentration 4% of total solids. The mixture was centrifuged at 10,000 rpm for 20 min; the supernatant was placed into a 10 mL tube, then the extracts were stored at −70 °C until use.

Figure 1. Xoconostle Ulapa (Opuntia oligacantha C.F. Först).

2.3. Quantification of Total Phenols

The polyphenol test was based on Singleton [20] with modifications. An aliquot of 0.5 mL of the extract and 2.5 mL of Folin–Ciocalteau (F9252, Sigma-Aldrich, St. Louis, MO, USA) were mixed for 7 min in the dark, then 2 mL of 7.5% sodium carbonate (Na2CO3) (FQ 17881 Fermont, Monterrey, Mexico) were mixed and left unmoved for 10 min and the samples were read at 720 nm with a Gallic acid stock solution (G7384 Sigma-Aldrich, St. Louis, MO, USA) dissolved in distilled water to a proportion 1:2 and homogenized in an industrial blender HGBSS (Waring Commercial, Torrington, CT, USA) for approximately 3 min then the product was vacuum filtered using Whatman paper number 1 to obtain the extracts, with a final concentration 4% of total solids. The mixture was centrifuged at 10,000 rpm for 20 min; the supernatant was placed into a 10 mL tube, then the extracts were stored at −70 °C until use.

The tannin content was determined using the methodology proposed by Prince et al. [22] with some variations. An aliquot of 200 µL of each sample and 600 µL of 0.1 M FeCl3 were placed in test tubes and then allowed to react for 5 min in the dark. Then, 600 µL of 0.008 M K3Fe(CN)6 was added and left unmoved for 10 min and the samples were read at 720 nm in a spectrophotometer (JENWAY Model 6705 Dunmow, UK). Distilled water was used to prepare the blank. The standard calibration curve was made based on a catechin (43412 Sigma-Aldrich, St. Louis, MO, USA) dissolved in distilled water 0, 10, 20, 30, 40, 50, 60, 90 ppm of catechin (R2 0.9781). The absorbance of samples was interpolated on a quercetin standard curve (EQ).

2.4. Quantification of Flavonoids

The method described by Espinosa-Muñoz et al. [21] with some modifications was followed; 0.5 mL of the samples, 75 µL of sodium nitrite (NaNO2) at 5% (Meyer, Mexico City, Mexico) was mixed and left to rest for 5 min at room temperature. Subsequently, 150 µL of 10% aluminum trichloride (AlCl3) (449598 Sigma-Aldrich St. Louis, MO, USA) was added and it was kept at rest for 6 min, then 500 µL of 1M sodium hydroxide (NaOH) (Meyer, Mexico City, Mexico) and 275 µL of distilled water were added. Absorbance was read at 415 nm in a spectrophotometer (JENWAY, Model 6705 Dunmow, UK); distilled water was used as a blank. A standard curve was made with quercetin (1592409, Sigma-Aldrich, St. Louis, MO, USA) at 0, 15, 30, 60, 90, and 120 ppm with distilled water as solvent (R2 0.9978).

2.5. Tannin Quantification

The tannin content was determined using the methodology proposed by Prince et al. [22] with some variations. An aliquot of 200 µL of each sample and 600 µL of 0.1 M FeCl3 were placed in test tubes and then allowed to react for 5 min in the dark. Then, 600 µL of 0.008 M K3Fe(CN)6 was added and left unmoved for 10 min and the samples were read at 720 nm in a spectrophotometer (JENWAY Model 6705 Dunmow, UK). Distilled water was used to prepare the blank. The standard calibration curve was made based on a catechin (43412 Sigma-Aldrich, St. Louis, MO, USA) dissolved in distilled water 0, 10, 20, 30, 40, 50, 60, 90 ppm of catechin (R2 0.9781). The absorbance of samples was interpolated on a quercetin standard curve (EQ).
2.6. Determination of Betalains

To quantify total betalains, the protocol of Cenobio-Galindo et al. [19] was followed. One gram of each structure was blended in 10 mL of distilled water (1:2) in the dark, then the product was centrifuged (HERMLE Z 232 HKII, Labortechnik GmbH, Wehingen, Germany) at 10,000 rpm for 20 min. Absorbance readings were read on a spectrophotometer (JENWAY Model 6705 Dunmow, United Kingdom) at a wavelength of 483 nm (betaxanthines) and 538 nm (betacyanins). The results were expressed in mg/g of extract.

\[
\text{Betanins} = \left(\frac{a}{1.129}\right) \times \text{DF} \times 100 \quad (1)
\]

\[
\text{Vulgaxantins} = \left(\frac{y}{750}\right) \times \text{DF} \times 100 \quad (2)
\]

where:
\[
a = 1.095 \times (A_{538} - A_{600})
\]
\[
y = A_{476} - (A_{538} - a) - (a/3.1)
\]
\[
\text{DF} = \text{Dilution factor}
\]

2.7. Evaluation of the Percentage of Inhibition of the Enzyme Urease as an Indicator of Gastroprotective Effect

The method described by Toledo et al. [3] with some modifications was followed. The reaction was started by placing 100 µL of urease solution (Jack Bean urease 5 U / mL Sigma-Aldrich, St. Louis, MO, USA), and 100 µL of inhibition extract incubated at 37 °C in a bath with a horizontal shaker (Scorpion Scientific, CDMX, Mexico) for 10 min; later, 200 µL of phosphate buffer (pH 6.8) with urea (U5128 Sigma-Aldrich, St. Louis, MO, USA) were added (100 mM) and incubated at 37 °C for 30 min. Subsequently, 500 µL of phenol reagent or reagent A (1% w/v phenol (P1037 Sigma-Aldrich, St. Louis, MO, USA) and 0.005% w/v sodium nitroprusside (Meyer, Mexico City, Mexico)) and 500 µL of alkaline reagent or reagent B (0.5 % w/v NaOH and 0.1 % NaOCl) was incubated for 30 min at 37 °C. The reaction was stopped with 600 µL of 4% H₂SO₄ (Meyer, Mexico City, Mexico). The absorbance was read at 630 nm using a spectrometer (JENWAY, Model 6705, Dunmow, UK). Thiourea (T8656 Sigma-Aldrich, St. Louis, MO, USA) was used as a standard urease inhibitor. Urease activity was determined by measuring ammonia production using the indophenol method. Indophenol is an intense blue compound that is formed by the reaction of ammonium, hypochlorite, and phenol catalyzed by sodium nitroprusside. An ammonium chloride calibration curve was made to determine the amount of ammonium produced during the tests and thus evaluate the enzymatic activity performed by urease.

2.8. Evaluation of Percentage of Inhibition of the Elastase Enzyme as an Indicator of Anti-Inflammatory Effect

The spectrophotometric technique using porcine pancreatic elastase (E1250 Sigma-Aldrich, St. Louis, MO, USA) was applied according to the modified method of Lee et al. [23] cited by Kim et al. [24]. N-Succinyl-Ala-Ala-Ala-p-nitroanilide (PPE type IV, Sigma-Aldrich, St. Louis, MO, USA) was used at a concentration of 1015 M, dissolved in 0.1 M Tris-HCl (PHG0002 Sigma-Aldrich, St. Louis, MO, USA) at pH 8 as substrate and 0.01 mg of elastase was dissolved with 0.2 M Tris-HCl buffer (pH 8). In order to carry out the reaction, 10 µL of each of the extracts with the different concentrations, 10 µL of elastase and 130 µL of the previously prepared substrate were added to these to obtain a final volume of 150 µL and incubated in a horizontal shaker bath (Scorpion Scientific, CDMX, Mexico) for 40 min. Finally, a reading was taken at 410 nm and the calculations corresponding to the formula were carried out:

\[
\% \text{ Inhibition} = \left(\frac{\text{Abs Control} - \text{Abs Sample}}{\text{abs Control}}\right) \times 100 \quad (3)
\]
2.9. Evaluation of the Percentage of Inhibition of the Enzyme β-Glucuronidase as an Indicator of Hepatoprotective Effect

β-Glucuronidase inhibition assay was carried out as per the method of Karak et al. [10] with modifications. A measure of 100 µL of β-glucuronidase (G7396 Sigma-Aldrich, St. Louis, MO, USA) (986.4 units/mL in 0.1 M phosphate buffer, pH 7.0) and 340 µL of test solution were pre-incubated at 37 °C for 15 min. Following the pre-incubation, 60 µL of p-nitrophenyl-β-D-glucuronide (N1627 Sigma-Aldrich, St. Louis, MO, USA) (3.15 mg/mL in 0.1 M phosphate buffer, pH 7.0) was added and incubated at 37 °C for 50 min. The color developed was read at 405 nm in spectrophotometer. Saccharide acid 1,4 lactone (Sigma-Aldrich, St. Louis, MO, USA) was used as the control. Controls were devoid of test samples. The percent inhibition was calculated as follows:

\[
\text{% Inhibition} = \left[ \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \right] \times 100
\]

(4)

2.10. Gastrointestinal Simulation Test Evaluation

The gastrointestinal simulation was carried out with the different extracts at a concentration of 24 mg/mL, as it was the treatment with the greatest inhibition effect on the enzymes urease, elastase, and β-glucuronidase. The in vitro test was carried out in two phases. In the first, the extract was diluted (1:5) with distilled water and adjusted to pH 2 by adding 6 N HCl and 20 mL of gastric fluid (16% pepsin (P7000 Sigma-Aldrich, St. Louis, MO, USA) and 10% NaCl in 0.1M HCl); subsequently, the mixture was incubated at 37 °C for 2 h in a water bath with horizontal shaking (Scorpion Scientific, CDMX, Mexico). At the end of the test, 2 aliquots were taken for subsequent analysis in phase 2. In this phase, the pH of the samples from the first test was adjusted to 7 with sodium bicarbonate (S6014 Sigma-Aldrich, St. Louis, MO, USA) (0.5 M), then 1.25 mL of pancreatin-bile mixture (Sigma-Aldrich, St. Louis, MO, USA) prepared (0.4 g of pancreatin and 2.5 g of bile salts in 100 mL of 0.1 M NaHCO₃ (pancreatic fluid). The sample was then incubated at 37 °C for 2 h in a shaking water bath. At the end of the test, an aliquot was taken for later analysis. At the end of the gastrointestinal digestion, the samples were heated in a boiling bath for 4 min to inactivate the enzymes and immediately centrifuged in a centrifuge (HERMLE Z 232 HKII, Labortechnik GmbH, Wehingen, Germany) at 12,000 rpm for 10 min at 4 °C. The followed method was Rufian-Henares et al. [25] with some modifications.

2.11. Statistical Analysis

A completely randomized design was carried out with three repetitions (n = 3) of the bioactive compounds and the enzymatic activities. The results were analyzed with an analysis of variance (ANOVA); when significant differences (p < 0.05) were found between the treatments, a comparison of means was used using the Tukey test. The IBM SPSS Statistics 20 software was used. For the analysis of percentage of enzymatic inhibition of urease, elastase and β-glucuronidase after the simulated digestion test in vitro, a Student’s t-test (p < 0.05) was used.

3. Results

3.1. Bioactive Compounds

In the different structures of the xoconostle, there are significant differences (p < 0.05) in all the bioactive compounds (Table 1). In phenols, flavonoids, and tannins, the highest concentration was found in whole fruit extracts (313.26 mg GAE/100 g, 189.53 mg QE/100 g and 71.067 mg CATE/100 g), respectively, and betalains with 17.45 mg/100 g; the highest concentration was found in the seed. In the betalain results of xoconostle Ulapa, variation was found in comparison with xoconostle Rosa since it has been reported values of 31 mg/100 g and 47 mg/100 g in the endocarp and pericarp, respectively, according to Morales et al. [26]. This difference could be mainly due to the species and the ripening of the fruits.
Table 1. Content of bioactive compounds in extract of xoconostle variety Ulapa (*Opuntia Oligacantha* C.F. Först).

| Extract     | Phenols mg GAE/100 g | Flavonoids mg QE/100 g | Tannins mg CAT/100 g | Betalains mg/100 g |
|-------------|----------------------|-------------------------|----------------------|-------------------|
| Endocarp    | 262.00 ± 2.42 a      | 151.80 ± 3.8 a          | 48.30 ± 4.75 a       | 17.45 ± 0.83 a    |
| Mesocarp    | 276.10 ± 5.15 b      | 83.57 ± 4.83 b          | 50.83 ± 1.01 ab      | 5.83 ± 0.35 b    |
| Pericarp    | 281.73 ± 8.69 bc     | 117.80 ± 1.60 c         | 27.10 ± 8.00 c       | 6.22 ± 0.09 c    |
| Whole Fruit | 313.27 ± 3.05 d      | 189.53 ± 6.02 d         | 71.07 ± 3.20 d       | 9.22 ± 0.19 d    |

The results were expressed as means ± standard deviation in triplicate (n = 3). The superscripts in upper case a, b, c, d in the same column represent significant differences (p < 0.05) between the different structures of the xoconostle (endocarp, mesocarp, pericarp, and whole fruit) structure using the Tukey mean comparison test.

3.2. Biological Activities in In Vitro Tests with Aqueous Extract of Xoconostle (*Opuntia oligacantha* C.F. Först)

As can be seen in Figure 2a, there were significant differences (p < 0.05) between the different extracts, the whole fruit extract being the one that presented the highest power against urease with 86.1% in the highest concentration of 24 mg/mL.

![Figure 2](image-url)
In Figure 2b, regarding the enzymatic inhibition capacity of the xoconostle extract on elastase, significant differences ($p < 0.05$) were identified between the extracts, with the effect of the endocarp and pericarp and that of the whole fruit being similar, with the highest potential (79%). Figure 2c shows the inhibitions of the enzyme $\beta$-glucuronidase, where it is indicated that there are significant differences ($p < 0.05$) between the three doses. There are no significant differences between the mesocarp and pericarp ($p > 0.05$). The whole fruit was the one with the highest inhibition, with 84%.

3.3. Bioactive Compounds and Biological Activities in Gastrointestinal Conditions In Vitro

Figure 3a shows the content of total phenols in the aqueous extracts of the different parts of the xoconostle was conserved on average 51% during the gastric phase and 30% in the intestinal phase. Figure 3b shows the similar behavior in flavonoids, 42% in the gastric phase, and 28% in the intestinal phase. The tannins were 43% and 24%, respectively (Figure 3c). Finally, as can be seen, the content of betalains in the xoconostle extracts did not have significant differences ($p < 0.05$) between the gastric and intestinal phase and there was an average of 32% (Figure 3d).

**Figure 3.** Total phenol content (mg GAE/g) (a), flavonoid content (mg QE/g) (b), tannin content (mg CATE/g) (c), and betalain content (mg/g) (d) before and after a simulated in vitro digestion. The capital letters A, B, C, D indicate significant differences ($p < 0.05$) between the three treatments (undigested, gastric phase, intestinal phase) and the lower-case letters a, b, c, d represent significant differences ($p < 0.05$) between the extracts (endocarp, mesocarp, pericarp, and whole fruit) during the same treatment, using the Tukey test.
In Figure 4a it can be seen that the percentage of urease inhibition by the different structures of xoconostle decreases by approximately 8% when going through the digestion process; however, there were no significant differences ($p > 0.05$) between the gastric phase and intestinal with the exception of the endocarp. In Figure 4b, the elastase enzymatic activity dropped an average of 14% from the undigested phase to the gastric digestion phase, and in the passage from this to intestinal conditions, there were no significant differences ($p > 0.05$) in the endocarp extracts and complete fruit, the opposite being observed in the mesocarp and pericarp. The enzymatic activity of $\beta$-glucuronidase decreased its effect on average by 7% when passing from the undigested phase to the gastric one, in intestinal conditions the four extracts had a slight decrease on average of 4%, finding significant differences ($p > 0.05$) between these (Figure 4c).

![Graphs showing urease inhibition, elastase inhibition, and $\beta$-glucuronidase inhibition](image)

**Figure 4.** Gastroprotective (a), anti-inflammatory (b), and hepatoprotective (c) bioactivity of the aqueous extract of xoconostle after an in vitro gastrointestinal. The lowercase letters a, b, c, in the columns indicate that there are significant differences ($p < 0.05$) between the samples in the undigested phase, gastric phase, and intestinal phase of the simulated digestion.
4. Discussion

The content of total phenols in the extracts (endocarp, mesocarp, pericarp, and whole fruit) of xoconostle Ulapa are higher than those reported by Monroy-Gutiérrez et al. [27], where the white Lent and Red Lent xoconostle were studied, which presented 29.61 mg EAG/100 g and 44.61 mg EAG/100 g, respectively, and those reported by Cenobio-Galindo et al. [28]. The differences between the species of these fruits can be influenced by environmental conditions (temperature, relative humidity, and light), as well as pre- and post-harvest handling, where the nutrition of the crop, the harvest index, among other factors can be included [27].

On the other hand, compared with Morales et al. [26], it was found that the endocarp extract of xoconostle Ulapa (Opuntia oligacantha CF Först) has a higher content of flavonoids (151.80 mg QE/100 g) than the endocarp extract of xoconostle (Opuntia matudae Scheinvar cv. Rosa), where a content of 5 mg QE/100 g was reported; however, the flavonoid content is higher in the pericarp of xoconostle (Opuntia matudae Scheinvar cv. Rosa) than in the pericarp of xoconostle Ulapa.

Currently, no studies on tannins have been reported in xoconostle, but research has been carried out on other plants; for example, Zapata et al. [29] carried out a study on antioxidant activity and polyphenol content in sour Guava (Psidium araca) where it reports a tannin content of 5.02 mg CATE/g. Rojano et al. [30] found a content of condensed tannins in Passiflora mollissima curuba equivalent to 492.4 mg of CATE/100 g of dry pulp. On the other hand, Fructoso et al. [31], in an investigation on the antioxidant capacity and content of tannins in pomegranate (Punica granatum), obtained 0.0061 mg equivalent of tannic acid per gram. In a study by Camones et al. [32] applying tangarana (Triplaris americana L.) endemic species of Latin America, a myrmecophilic tree, 0.1185 g of tannic acid/g were reported. As can be compared, the tannins in xoconostle Ulapa turn out to be lower and higher compared to those reported in other plant species; however, its content is also important and significant to provide beneficial health effects. Since these are considered bioactive compounds to which antioxidant, anti-inflammatory, anti-diarrheal, and wound-healing properties have been attributed due to their free radical inhibition activity, so they can stop some of the anti-inflammatory processes in addition to having the ability to inhibit specific enzymes [31].

González-Ponce et al. [11] explain that the variability in the presence or absence of many bioactive compounds in leaves, fruits, roots, seeds, and other natural by-products could be mainly due to different abiotic factors such as the genotype of both the species and of the crops, as well as the conditions of growth of the fruits, the degree of maturity in the harvest, the climate, composition of the soil, humidity, temperature, and season mainly.

A study by Biglar et al. [3] determined the inhibitory effect of different species on urease, which turned out to present high inhibition. Among these studied plants are Camellia sinensis, a plant native to China, which is currently cultivated throughout the world, whose leaves are used for the preparation of infusions of black tea and green tea; this species presented 95.4%; on the other hand Citrus aurantifolia, also called lime or lemon tree, had a higher inhibition value with 97.6% and another species to highlight its high gastroprotective power is Nasturtium officinale, commonly called watercress, with 99.13% inhibition of activity of urease. Biglar et al. [3] attribute inhibitory activity to flavonoids due to the presence of hydroxyl groups in the compounds, which can easily chelate with nickel (Ni) in the active site of urease; in the same way, [33] explain that dihydroxyl residues (-OH) in the C ring of flavonoids can interact with residues near the nickel atoms in the active site of urease in a similar way to thiourea.

Xoconostle presented higher elastase inhibitions than the leaves of Tithonia tubaeformis, known as acahual, and Sylbum marimum, wild plants from the northern and southern region of Jalisco; the former had an inhibition percentage of 60% attributed to its bioactive compounds such as phenols, flavonoids, alkaloids, water-soluble tannins, steroids, and coumarins [13]. The xoconostle values are better than those found by Lee et al. [23] where Perilla frutescens and Selaginella tamariscina showed an inhibition on elastase 7 and 23%,
respectively. In another elastase inhibition study [34], it was reported that the percentage of inhibition of the elastase enzyme with the extract of *Garcinia indica*, known as kokum, was 81%, which is similar to that obtained with the extract of xoconostle variety Ulapa.

The inhibition that xoconostle turned out to have on elastase is probably part of the action of the tannins contained in it, since these are considered bioactive compounds with anti-oxidant and anti-inflammatory properties due to their ability to inhibit specific enzymes and their destructive activity of free radicals, which is why they can stop some of the anti-inflammatory processes [31]. In a study carried out by Arroyo et al. [35], it was found that the methanolic and ethanolic extract of the leaves of matico (*Piper aduncum*) in animal models achieved a decrease in inflammation of more than 66% ($p < 0.05$) due to the presence of phenolic compounds, flavonoids, tannins, saponins, and alkaloids, since these (mainly flavonoids) can inactivate cellular enzymes that cause changes in the permeability of the membrane. As elastase is a proteolytic enzyme related to inflammatory diseases of the epithelial tissue, [5,6] explain that inflammatory cells such as macrophages and neutrophils, in the presence of toxic agents, can secrete various protease enzymes such as tryptase, chymase, and elastase, capable of degrading components of the extracellular matrix that amplify inflammation and free radicals. This means that xoconostle can be considered a preventive alternative as a functional food with anti-inflammatory properties.

Cartaya and Reynaldo [36] reported that *Swertia bimaculata* presented 30% inhibition of β-glucuronidase from a concentration of 500 µg/mL, while *Swertia chirayita* had 95% inhibition. This author mentions that xanthones (carbonyl antioxidant compounds) are the main inhibitor of β-glucuronidase, which is used in scientific tests as indicative of tumor and pathogenic activity on the liver. Flavonoids have the capacity to inhibit the enzyme β-glucuronidase and can suppress the formation of free radicals by bonding with heavy metal ions [37,38].

Regarding bioactive compounds and biological activities in gastrointestinal conditions in vitro, the results obtained were similar to those obtained in a study carried out in tropical fruits (papaya, mango, and pineapple), where approximately 50% of the total phenolic compounds were released in all the fruits in the gastric phase, increasing to 60% in the intestinal phase in mango and pineapple. Therefore, the presence of dietary fiber did not affect phenolic compounds according to in vitro digestion [39]. Calvo-Lerma et al. [40] evaluated the profile of total phenols in chia seeds (*Salvia hispanica*) and in whole chia flour after in vitro digestion and reported an expression of 83% and 64% of phenols, respectively. On the other hand, Vallejo et al. [41] found an 85% reduction in flavonoids in broccoli extract after in vitro digestion, the bioavailability of the compounds is highly variable, since this effect depends on the plant matter and other factors [42,43]. Some research on enzyme inhibition after in vitro digestion has been reported, Lavermicocca et al. [44] evaluated the activity of probiotic lactobacilli (*Lactobacillus plantarum*) on urease inhibition, where an effect of 60% was reported and verifies the survival of these strains after an in vitro digestion where there was an effect of $11 \log_{10} \text{CFU/mL}$ to $2.5 \log_{10} \text{CFU/mL}$. Prze-myslaw et al. [45] analyzed the biological activity and nutritional value of a gluten-free bread enriched with cricket flour, where the reduction of phenolic compounds under simulated digestion had an impact on the activity of β-glucuronidase with an effect of 65.9%. In another study in which changes in the phenolic compositions of the olive tree (*Elaeagnus umbellata*) were analyzed after gastric digestion, an approximate 77% decrease in the inhibition of aldose reductase was found [42]. Medina-Pérez et al. [15] evaluated the antidiabetic effect of xoconostle extract by inhibiting α-amylase and α-glucosidase, establishing that after gastrointestinal conditions, xoconostle presented 63% and 46%, respectively.

There are few studies in which it has been observed that the absorption of some flavonoids can occur during stomach digestion and during intestinal phase: quercetin, daidzein, or anthocyanins [17,43]. Some phenolic compounds can be absorbed thanks to different transporters present in the epithelial cells of the intestine. Low-molecular-weight polyphenols, such as monomeric and dimeric structures, can be absorbed by the small intestine [46].
With respect of the non-bioaccessible compounds, it is known that these are susceptible to degradation by the intestinal microbiota in the large intestine [43]; said representation was made in the centrifugation stage, where in the supernatant there was surely a small fraction of compounds. The presence of dietary fiber in the dietary matrix of some fruits plays an important role in the release and absorption of their bioactive compounds, such as phenolic compounds, of which a part reaches the large intestine where they are found to be fermented [47]. Although the mechanisms have not yet been clarified, it has been shown that chemical interactions may exist between the hydroxyl groups of phenolic compounds and the polar groups of the polysaccharides that make up dietary fiber [48].

It has also been reported that betacyanins are more stable than betaxanthins; both at room temperature and when heated, the glycosylated structures are more stable than aglycones, probably due to the greater oxidation-reduction potentials of the former. Similarly, it was reported that the optimal pH for the enzymatic degradation of betacyanins and betaxan-thinning was around 3.4, so in the in vitro simulation test during the gastric phase, it could be that some part of these compounds was lost; this was reflected in the decrease in the percentage of inhibition that the extract had after a simulated digestion [49].

5. Conclusions

The aqueous extract of the complete fruit of xoconostle variety Ulapa (Opuntia oligacantha CF Först) presented the highest content of bioactive compounds (phenols, flavonoids, tannins, betalains) and a greater anti-inflammatory effect was found due to its inhibitory capacity on the elastase enzyme (75%) and in hepatoprotective effect to its inhibition value on the enzymatic activity of β-glucuronidase (84%); after the in vitro test of the digestion simulation, the anti-inflammatory and hepatoprotective effect in the in vitro simulated digestion on the enzyme 62% elastase and 72% β-glucuronidase. This fruit could be a preventive alternative to suffering from inflammatory and liver diseases as it is an easy-to-obtain fruit. Further studies on isolation and characterization of the bioactive compounds from this fruit that could be responsible for these important activities and also clinical trials need to be carried out to ascertain the effect of these extracts as a good alternative to conventional drugs in the treatment of gastrointestinal diseases.

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