RESEARCH ARTICLE

Nutritional characterization and *in vitro* anti-inflammatory activity of guava-*Aloe vera* puree and its histopathological effect in gastric lesion-induced rats

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ABSTRACT

The prevalence of gastritis requires the consumption of functional foods to counteract this disease. The objective of this work was to characterize guava-*Aloe vera* purees, evaluate their *in vitro* anti-inflammatory effect and demonstrate the gastroprotective effect in a murine model induced to the gastric lesion. The nutritional composition, physicochemical parameters, and bioactive compounds were determined in fresh guava-*Aloe vera* puree (FP) as well as in guava-*Aloe vera* puree stored at 4 °C for six months (RP). The *in vitro* anti-inflammatory activity of polyphenolic extracts from purees and the effect of FP intake on gastric lesion-induced rats were also evaluated. FP was rich in dietary fibre (10%), vitamin C (238.81 mg/100 g), phenolic compounds (1969.70 mg/100 g), and minerals such as Cu, Fe, and K; while RP decreased in 80%, 64%, and 69% of vitamin C, minerals, and phenolic compounds, respectively. The polyphenolic extracts from the purees showed high *in vitro* anti-inflammatory activity (~60-80%). The purees did not cause significant changes ($p > 0.05$) in the plasma biochemical parameters. The activity of the enzyme lactate dehydrogenase decreased in the gastric lesion-induced rats after treatment with the puree. The intake of FP by gastric lesion-induced rats accelerated the cell regeneration of the gastric tissue, showing a fast recovery. Guava-*Aloe vera* puree is a healthy alternative and serves as a gastroprotective.

Keywords: *Aloe vera*; Guava; Nutrition; Anti-inflammatory; Gastric lesion; Histopathology

INTRODUCTION

The consumption of fresh fruits and vegetables is essential for health. However, processed functional foods, such as fruit purees, are increasingly consumed for potential health benefits (Becerra-Verdin et al., 2019; Morales-Avila et al., 2020a, 2020b). The food industries have formulated fruit purees principally for children, but also there are for adults, such as guava purees added with strawberry, passion fruit, soursop fruit, and blackberry or plant extracts (*Hibiscus sabdariffa*) and prebiotics (agave fructans). The purees above mentioned have been nutritionally characterized, demonstrating the presence of bioactive compounds and nutrients such as carotenoids, dietary fiber, vitamins,

phenolic compounds, and minerals (Ullah et al., 2016; Pérez-Beltrán et al., 2017). In addition, they are functional foods due to their reported anti-obesity, antidiabetic and hepatoprotective activities (Pérez-Beltrán et al., 2017; Becerra-Verdin et al., 2019; Morales-Avila et al., 2020a; 2020b).

The Purees and Derivates of Nayarit (PDN) company (Nayarit, Mexico) formulated a new puree for treating gastric lesions since, in recent years, various gastrointestinal diseases, especially gastritis, have increased due to poor dietary and hygienic habits. These diseases can be silent and induce the formation of gastric ulcers or gastric cancer (Huang et al., 2021; Rodríguez-Nogales et al., 2017).

Medical treatments for gastritis are available, including proton pump inhibitors (omeprazole, pantoprazole), H_2 receptor antagonists (ranitidine, cimetidine), and mucoprotective agents (misoprostol, sucralfate); however, there are serious side effects of these drugs, and if the diet is inadequate the disease can become complicated (Valdivia-Roldán, 2011). Therefore, processed foods that may provide medical or health benefits, including disease prevention, are being developed (Pechey et al., 2021).

Diets rich in antioxidants have a beneficial effect against gastric diseases because they favor the protection of the gastric mucosa (de Oliveira et al., 2021). Antioxidants reduce oxidative damage to gastric mucosa because they protect the cell membrane fatty acids of free radicals and attenuate the inflammatory cascade induced by gastritis (Haixin and Hongbin, 2018). Other bioactive compounds with an anti-inflammatory effect in gastric lesions include different polyphenolic compounds (Alabi et al., 2018). Quercetin (50 mg/kg) reduced the malondialdehyde levels on ethanol-induced gastric damage in Wistar rats and significantly increased the activity of antioxidant enzymes (Coşkun et al., 2004).

Aloe vera and guava (*Psidium guajava*) are widely used in traditional medicine. It has been demonstrated that they have a beneficial effect against gastrointestinal diseases such as gastric ulcers, gastritis, and irritable bowel syndrome (Xu et al., 2016; Kim et al., 2019). The ulcer index from ulcer-induced rats treated with *Psidium guajava* extract (200 mg/kg/day orally for 30 min) significantly decrease compared to the control group (Jayakumari et al., 2012). Uduak et al. (2012) investigated the antiulcer activity of the methanolic extract from *P. guajava* leaves on gastric ulceration-induced rats. The authors reported a significant dependent dose against the ulceration decreasing in groups treated with *P. guajava* extract compared to the control groups. Phromnoi et al. (2019) demonstrated that the ethanol extract from *P. guajava* leaves significantly inhibited tumor necrosis factor-a, interleukin-1, and interleukin-6. In addition, these extracts showed to contain the highest content of polyphenols. The authors concluded that *P. guajava* has the most significant anti-inflammatory and antioxidant activities and polyphenols.

The glucogalactomannan polysaccharide (APS-F1) from *Aloe vera* protected against oxidative stress-induced gastric mucosal damage. It induced cell apoptosis and decreased superoxide dismutase, catalase, and glutathione peroxidase activities of human gastric epithelial GE-1 cells, and APS-F1 improved ethanol-induced gastric damage in rats (Xu et al., 2016; Hussein et al., 2021). Furthermore, Park et al. (2017) mentioned that *Aloe vera* (150 mg/kg body weight) exhibited drastically few ulcer lesions in gastric lesion-induced mice;

also, the protein expression levels of metalloproteinase (MMP)-9 was significantly alleviated. Thus, this enzyme is closely associated with gastroprotection. However, studies using fruit purees against gastric lesions were not found; therefore, the objective of this work was to characterize nutritionally guava-*Aloe vera* puree, to evaluate the *in vitro* anti-inflammatory effect and gastroprotective effect in gastric lesion-induced rats.

MATERIALS AND METHODS

Raw material
The commercial guava (*Psidium guajava*) -*Aloe vera* purees were generously donated by the PDN company (Nayarit, Mexico). The samples included freshly prepared puree (FP) and refrigerated puree at 4 $\pm$2 °C for six months (RP).

The general diagram of the experiment is shown in Fig. 1. The work was carried out in two stages. In the first stage, the physicochemical and nutritional parameters, the content of bioactive compounds, antioxidant capacity, and the *in vitro* anti-inflammatory activity of the purees were evaluated. In the second stage, the effects of the intake of the FP by gastric-lesion induced rats were determined.

Physicochemical parameters
Total soluble solids (TSS) in the purees were determined with a refractometer (Abbe, BOE32400, Buenos Aires, Argentina) (Method 932.12, AOAC, 2005). The pH values were measured directly in homogenate purees with a pH meter (Hanna Instrumental, 221 PH/MV, Padovana, Italia), and titratable acidity (TA) was measured according to the method 942.15 (AOAC, 2005). Finally, the color was measured using a Minolta Colorimeter (Konica Minolta CR-300, Osaka, Japan).

Nutritional parameters
Moisture (Method 934.06), fat (Method 950.54), ash (Method 940.,26), and protein (Method 978.04) contents in the purees were determined by using the mentioned official AOAC methods (AOAC, 2005). Soluble carbohydrates (SC) were quantified by the phenol-sulphuric method (Dubois et al., 1956). Soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF) were analyzed by the AOAC enzymatic-gravimetric method (Method 991.42) modified by Mañas and Saura-Calixto (1993). The data were reported as grams per 100 g of fresh weight (g/100 g FW or %).

Vitamin C (ascorbic acid) and vitamin E were determined using the HPLC method reported by Barbosa-Gámez et al. (2017). The samples (20 µL) were injected into an HPLC (Agilent Technologies 1260 infinity, Waldbronn, Germany)

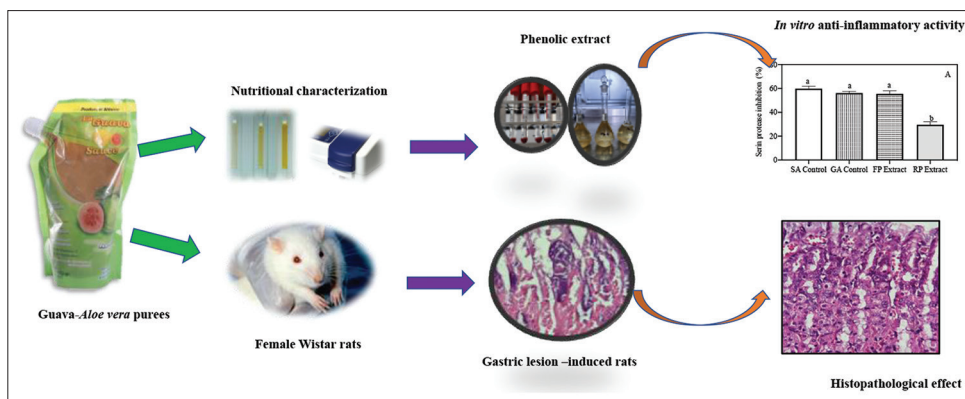Emir. J. Food Agric ● Vol 33 ● Issue 9 ● 2021

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Fig 1. General diagram of experiment.

equipped with a C18 column (4.6x100 mm ZORBAX Eclipse plus, Santa Clara, USA). The results were expressed as milligram per 100 g of fresh weight (mg/100 g FW).

Minerals content
Puree samples were digested with nitric (HNO$_3$) and perchloric (HClO$_4$) acids at a ratio of 2:1 (HNO$_3$: HClO$_4$). K, Ca, Mg, Na, Fe, Zn, Mn, and Cu were measured using atomic absorption spectrophotometer (Perkin Elmer, AAnalyst400, Singapore). Phosphorus (P), boron (B), and sulfur (S) were determined using spectrophotometry (Benton et al., 1991). The results were reported as mg or μg/100 g FW.

Content of bioactive compounds and antioxidant capacity
Total carotenoids (TC) content in the purees was determined using a spectrophotometric method (Cano and De Ancos, 1994). The extracted carotenoids were measured at 448 nm in a spectrophotometer (Jenway, model 6705, Felsted, United Kingdom). The quantification was performed using a calibration curve of β-carotene standard, and results were expressed as mg/100 g FW.

Total soluble phenols (TSP) in the purees were extracted with an organic aqueous extraction (Pérez-Jiménez et al., 2008). The TSP content was determined in the extracts using the methodology reported by Montreau (1972). The hydrolyzable polyphenols (HP) content was obtained based on the method described by Hartzfeld et al. (2002). The released phenolic compounds were quantified with the Folin–Ciocalteu´s reagent (Montreau, 1972). Condensed tannins (CT) were also determined in the residues, according to Reed et al. (2001). Absorbance was measured at 555 nm. CT were calculated from a standard curve of tannins from *Ceratonia siliqua* L. All phenolic compounds were reported in mg/100 g FW.

Two methods performed antioxidant capacity. The 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

diammonium salt (ABTS) radical assay was performed in the puree samples according to the method of Re et al. (1999), and the ferric ion reduction method (FRAP) was performed according to the method of Benzie and Strain (1996). The antioxidant capacity was reported in millimole equivalent of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per gram of fresh weight (mmol/g FW).

The profile of phenolic acids was determined in the purees extracts from TSP and HP. The samples (20 μL) were injected into the mentioned HPLC-DAD system with a C18 column (4.6 x 250 mm, 5 μm). The mobile phase was acidified water with 2% acetic acid (eluent A) and acidified water (0.5% acetic acid): methanol (10:90, eluent B). The elution gradient was 0% B-100% B at a flow rate of 0.4 mL/min. The components were followed at 280 and 320 nm, and calibration curves of polyphenol standards were performed to identify and quantify the phenolic compounds (Aguilar-Hernández et al., 2019).

***In vitro* anti-inflammatory activity**
Two assays were performed, including the percentage of inhibition of serine protease activity (SPA) and the percentage of cell membrane stabilization (CMS), according to Ruiz-Ruiz et al. (2017), with some modifications. The TPS extracts were used, and the concentrations in each extract from FP and RP samples were 793.25 μg/mL and 527.60 μg/mL, respectively. Acetylsalicylic acid (50 μg/mL) and gallic acid standards (50 μg/mL) were used as positive controls.

Percentage of SPA Inhibition. The extracts or positive controls (700 μL), 25 μL of proteinase (Alcalase E.C. 3.4.21.62) and 700 μL of Tris-HCl buffer at pH 7.4 were mixed and incubated at 37 °C for 5 min. Then, 60 μL of an ovalbumin solution (0.8% w/v) was added and incubated at 37 °C for 1 min, followed by 1400 μL of trichloroacetic acid solution (10%, v/v) to stop the reaction. The mixture was

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centrifuged at 5320 *xg* for 10 min, and the absorbance of the supernatant was measured at 210 nm. The mixture without extracts or positive control was used as the blank.

Percentage of CMS. The human blood (10 mL) contained in heparinized tubes was used, and washing with a saline solution (0.9%) was done to obtain clean erythrocytes. The blood (500 μL) was diluted in 4 mL of saline solution, the mixture was centrifuged at 479 *xg* for 3 min at 4 °C, the supernatant was discarded, and the erythrocytes were re-suspended with saline solution to obtain a concentration of 10% (v/v) of erythrocytes. The extracts or positive controls (1 mL) and 1 mL of erythrocytes (10%) were mixed, and instead of the sample, only a saline solution was added to the blank test tube. The mixture was incubated at 56 °C for 1 min, followed by cooling. The samples were centrifuged at 8990 *xg* for 5 min, and the absorbance was measured at 560 nm in the spectrophotometer.

The percentages were calculated with the following formula: percentages (%) = (absorbance blank−absorbance sample)×100/absorbance blank.

Animals and experimental design

Female rats (*Rattus norvegicus* Wistar), with an average weight of 220 ± 60 g, were obtained from the Universidad Autónoma de Nayarit. The State Bioethics Committee of Nayarit, Mexico, approved the protocol (No. CENB/03/2017) and the protocols of the Institutional Animal Care and Use Committee according to Mexican standards (NOM-062-ZOO-1999) were followed. A group of pilot rats (n=6) were induced to the gastric lesion (Pilot-LG) with a single dose of 0.5 mL of ethanol and 0.15 M HCl (60:40 v/v), which was administered through an orogastric tube (Suchecka et al., 2017). After induction (24 h), the rats were euthanized, and the lesions were evaluated with histological sections of the stomach wall. Lactate dehydrogenase (LDH) activity was measured in plasma blood.

The experimental design was initiated once the induced gastric lesion was verified. A total of 18 rats were divided into three groups (n=6). A healthy control (HC) group consisted of healthy rats fed during the whole experiment with a balanced feed or standard rodent diet Nutricubo Purina® (16 g/day) and water *ad libitum*. The gastric lesion-induced rats (as it was described before) were divided into two groups: 1) the gastric lesion group (LG) without treatment, which was fed with a standard diet (16 g/day) and water *ad libitum* for three weeks, and 2) the gastric lesion group with treatment (LG+Puree) that they were fed with 2.5 g of fresh guava-*Aloe vera* puree per kg of animal weight (0.5 g/220 g of animal weight), standard diet (16 g/day), and water *ad libitum* for three weeks. The

nutritional composition of the diets can be observed in Table S1 (Supplementary material). All animals were maintained under standard biothermal conditions of 12 h light/dark cycles at 25 ± 3 °C during the whole duration of the experiments.

The treatments lasted for three weeks, and the animals had fasted for 12 h. The euthanasia protocol was followed according to the Manual on the Use and Care of Experimental Animals (NOM-062-ZOO-1999). Blood samples were extracted from each animal by a puncture in the inferior vena cava and placed in a sterile red tube without anticoagulant BD® brand (New Jersey, USA). They were then centrifuged for 5 min at 652 *xg* (Beckman Coulter, Allegra X-5, California, USA) to obtain the serum used in the biochemical tests.

Biochemical parameters

Glucose, triacylglycerols (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined according to the methods of the International Federation of Clinical Chemistry using enzymatic colorimetric kits (Biosystems Reagents and Instruments, Barcelona, Spain) using a calibrated Biosystem Auto-analyzer (BTS-350, Barcelona, Spain). In addition, the low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) fractions were determined according to the Friedewald equations (Siri-Tarino and Krauss, 2016).

Histopathological analysis

A representative portion of the stomach tissue was taken from rats of each treatment, fixed in a formalin solution at 10% (pH 6.8), and then embedded in kerosene. The tissues were cut in sections (5 μm) and stained with Hematoxylin and Eosin (H and E) (Morales-Avila et al., 2020a), and histopathological changes were observed under a microscope (Leica, DME, Model 1359, USA) with a 40X objective.

Statistical analysis

Physicochemical and nutritional data are expressed as the mean of three replicates ± standard deviation (SE). The *in vivo* data are expressed as the mean of six replicates ± standard error (SE). The data obtained were analyzed statistically using analysis of variance (ANOVA), as well as the test of means (Fisher's LSD, $p < 0.05$) using the statistical software STATISTICA (v.10 StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Physicochemical parameters

Table 1 shows the results of the physicochemical parameters (TSS, TA, pH, and color). They were maintained (except

Table 1: Nutritional composition of puree of guava-*Aloe vera* fresh and stored for six months at 4 ± 2 ºC

Physicochemical parameters	Fresh puree	Puree stored at 4 ºC for six months
Total soluble solids (ºBrix)	12.10 ± 0.45[a]	12.36 ± 0.67[a]
Titratable acidity (% citric acid)	1.23 ± 0.01[a]	1.32 ± 0.01[a]
pH	3.32 ± 0.05[a]	3.26 ± 0.01[a]
Hue (h) value	95.50 ± 0.26[a]	91.37 ± 0.21[b]
Nutritional composition (g/100 FW)		
Moisture	83.51 ± 1.20[a]	83.45 ± 1.29[a]
Fat	0.21 ± 0.06[a]	0.20 ± 0.09[a]
Ash	0.54 ± 0.09[a]	0.46 ± 0.03[a]
Protein	1.08 ± 0.05[a]	1.02 ± 0.02[a]
Soluble carbohydrates	9.49 ± 2.21[a]	9.01 ± 0.07[a]
Soluble dietary fiber	1.22 ± 0.02[a]	1.23 ± 0.01[a]
Insoluble dietary fiber	9.19 ± 1.08[a]	9.06 ± 0.81[a]
Total dietary fiber	10.41± 1.07[a]	10.29 ± 0.80[a]
Vitamins		
Vitamin C (mg/100g FW)	238.81 ± 2.32[a]	190.0 ± 3.51[b]
Vitamin E (µg/100g FW)	398.32 ± 4.28[a]	235.62 ± 26.07[b]
Macro minerals (mg/100 g FW)		
Phosphorus (P)	26.80 ± 2.15[a]	17.45 ± 0.78[b]
Potassium (K)	292.34 ± 2.71[a]	222.90 ± 4.33[b]
Calcium (Ca)	29.01 ± 3.18[a]	18.60 ± 0.37[b]
Magnesium (Mg)	13.66 ± 0.66[a]	11.50 ± 0.63[a]
Sulfur (S)	1.16 ± 0.90[a]	1.37 ± 0.07[a]
Microminerals (µg/100 g FW)		
Iron (Fe)	193.35 ± 9.22[a]	69.80± 18.84[b]
Zinc (Zn)	170.74 ± 5.47[a]	180.50 ± 3.40[a]
Manganese (Mn)	171.90 ± 0.87[a]	120.00 ± 0.65[b]
Copper (Cu)	190.65 ± 2.94[a]	95.55 ± 7.11[b]

Values indicate mean± standard deviation (n ≥ 3). Means within the same line with different superscript letters indicate a significant difference using the LSD test (p < 0.05). FW: Fresh weight

color) without significant differences (p> 0.05) during six months of storage. The stability of these parameters during the same time of storage has already been reported in guava purees (Chávez-Tapia et al, 2016; García-López et al, 2017). The puree had an initial ºHue value of 95.50, which corresponds to a yellow color, but after storage, the ºHue value gradually decreased to 91.37 (yellow-brown). The color change is due to the oxidation of ascorbic acid, phenolic compounds, and photodegradation of pigments such as carotenoids (Ioannou et al., 2012).

Nutritional parameters

The nutritional parameters (moisture, soluble carbohydrates, fat, protein, ash, and dietary fiber) did not show significant changes (p> 0.05) during storage (Table 1). The results are similar to those reported in guava purees by García-López et al. (2017). However, dietary fiber values were from 9.06 to 9.19%, which indicates that the puree contributes 25% of the recommended daily intake (WHO, 2021). Therefore, it is an excellent alternative to include in the diet since

dietary fiber has a beneficial effect on the gastrointestinal content, decreases gastric emptying, and serves as a prebiotic (Lunn and Buttriss, 2007).

In FP, Vitamin C and vitamin E contents were 238.81 mg/100 g FW and 398.32 µg/100g FW, respectively, but they decreased in RP. The decrease in the vitamin content is probably due to oxygen, light, and temperature effects, factors that cause their oxidation during storage (Chávez-Tapia et al., 2016). However, after six months of storage, 80% of vitamin C and 59% of vitamin E contents were retained, and thus refrigeration partially decreased their losses (García-López et al., 2017). These results indicate that the puree has a high antioxidant potential if consumed fresh or stored for less than six months at 4 ºC.

The puree of guava-*Aloe vera* was rich in iron (193. 35 µg/100 g), copper (190.65 µg/100 g), and potassium (292.34 mg/100 g). Guava and *Aloe vera* have been reported to be rich in these minerals (Chiveu et al., 2019; Hęś et al., 2019). However, a significant decrease (p<0.05) in the content of P, K, Ca, Fe, Mn, and B was detected in RP. It may attribute to the formation of chemical complexes through the chelation of metallic ions by vitamins, organic acids, phenolic compounds, or iron oxidation, and therefore the possibility of losses during calcination (Paredes-Salido and Clemente-Fernández, 2005; Peréz-Jiménez and Saura-Calixto, 2018).

Content of bioactive compounds and antioxidant capacity

The initial carotenoids content in the FP was 19.25 mg/100 g FW but decreased during storage (RP) to 6.56 mg/100 g FW (Table 2), which coincided with the change of color. Nora et al. (2014) reported that the stability of carotenoids depends on the dissolved oxygen in the food, the light, and the storage temperature, and therefore the pigments were quickly oxidizable in the puree.

The FP was rich in phenolic compounds (1969.70 mg/100 g FW), while RP had 66%, 28%, and 46% of TSP, PH, and CT, respectively, were maintained compared to FP. The decrease of phenolic compounds is probably due to their use as antioxidants to neutralize free radicals produced during the oxidation reactions of lipids, pigments, and vitamin C and their oxidation by the presence of metals, dissolved oxygen, and light (Deng et al., 2018). Fresh guava contained 276-497 mg/100 g FW of TSP (Van de Velde et al., 2013), and *A. vera* leaves contained 138.13 mg/g TSP from an ethanolic extraction (Jyoti et al., 2015). Thus, it indicates that the FP is an excellent source of important bioactive compounds.

Table 2: Content of bioactive compounds, antioxidant capacity (CAOX), and profile of soluble and hydrolyzable phenolic compounds in guava-*Aloe vera* puree fresh and stored for six months at 4 ± 2 ºC

Bioactive compounds	Fresh puree	Puree stored at 4 ºC for six months
Total carotenoids (mg/100g FW)	19.25 ± 1.56[a]	6.56.96 ± 0.95[b]
Phenolic compounds (mg/100 g FW)		
Total soluble phenols	793.25 ± 89.71[a]	527.60 ± 60.31[b]
Hydrolyzable polyphenols	587.05 ± 28.87[a]	167.69 ± 3.62[b]
Condensed tannins	589.40 ± 18.21[a]	270.77 ± 8.19[b]
Total phenolic compounds	1969.70 ± 38.57[a]	966.06 ± 31.49[b]
Antioxidant capacity (mM/g FW)		
ABTS assay	55.28 ± 0.38[a]	51.83 ± 4.42[a]
FRAP assay	45.01 ± 0.50[a]	39.20 ± 1.59[b]
Profile of soluble phenolic compounds (µg/g FW)		
Gallic acid	561.37 ± 47.29[a]	14.67 ± 3.88[b]
Protocatechuic acid	817.08 ± 11.75[a]	25.77 ± 3.49[b]
Neochlorogenic acid	21.60 ± 0.33[a]	16.31 ± 4.97[a]
Chlorogenic acid	1419.29 ± 5.45	ND
Caffeic acid	36.36 ± 2.24[a]	36.07 ± 2.23[a]
ρ-Coumaric acid	172.11 ± 0.26[a]	170.69 ± 0.29[b]
Ferulic acid	5786.74 ± 4.97	ND
Kaempferol	1696.62 ± 1.49[a]	1481 ± 5.70[a]
Profile of phenolic compounds released of hydrolyzable polyphenols (µg/g FW)		
Gallic acid	0.68 ± 0.09[a]	0.40 ± 0.02[b]
Protocatechuic acid	0.05 ± 0.01[a]	traces
Neochlorogenic acid	1.66 ± 0.54[a]	0.37 ± 0.03[b]
4-Hydroxybenzoic acid	14.36 ± 0.36[a]	11.71 ± 0.21[b]
Chlorogenic acid	212.24 ± 0.15[a]	1.44 ± 0.25[b]
Caffeic acid	119.02 ± 3.23[a]	0.47 ± 0.08[b]
Syringic acid	2338.19 ± 1.74[a]	228.21 ± 23.17[b]
p-Coumaric acid	0.68 ± 0.09[a]	0.40 ± 0.02[b]
Ferulic acid	35.36 ± 2.03[a]	29.62 ± 3.19[b]
Kaempferol	32.45 ± 0.10[a]	15.65 ± 1.03[b]

The values indicate the mean ± standard deviation (n ≥ 3). Means within the same line with different superscript letters indicate a significant difference using the LSD test (p < 0.05). FW: Fresh weight. ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt. FRAP: Ferric reducing antioxidant power. ND: Not detected

The antioxidant capacity determined by the ABTS assay was not significantly different (p> 0.05) between the purees (55.28 mmol/g FW). Then, it indicates that the content of vitamin E, carotenoids, vitamin C, and phenolic compounds present in FP and RP was sufficient to neutralize the ABTS radical.

On the other hand, a significant decrease (p <0.05) of antioxidant capacity was observed using the FRAP assay in RP. The response was directly related to the decrease in vitamin C (R^2 = 0.9962), since it has been demonstrated that this vitamin has a high chelating capacity (Martínez et al., 2012; Pérez-Jiménez and Saura-Calixto, 2018).

The principal phenolic acids found in FP were gallic acid (561.37 µg/g), protocatechuic acid (817.08 µg/g), chlorogenic acid (1419.29 µg/g), ρ-Coumaric acid (172.11 µg/g), ferulic acid (5786.74 µg/g) and kaempferol (1696.62 µg/g). These compounds have already been reported in fresh guava (Irondi et al., 2016; dos Santos et al., 2017), as well as in *Aloe vera* (López-Cervantes et al., 2018). In the PH extracts, the same phenolic compounds were detected but in a smaller proportion, in addition to 4-hydroxybenzoic acid and syringic acid. The acid hydrolysis breaks the bonds between the dietary fiber and phenolic compounds and releases them (Andreasen et al., 2001). The released phenolic compounds can be hydroxycinnamic acids (Benoit et al., 2006) and hydroxybenzoic acids (Arranz et al., 2009), as they were detected in this experiment. The non-extractable phenolic compounds (HP and TC) present in the purees also have the antioxidant capacity and potential health benefits (Arranz et al., 2009).

The concentration of specific phenolic compounds was higher in FP than in RP. The decrease of each type of phenol during storage is dependent on its chemical structure (Cao et al., 2021). Phenolic acids such as hydroxycinnamic acids are more susceptible to degradation by a_w (water activity), light, and oxygen during storage than some flavanones and flavonoids (Klimczak et al., 2007; Cao et al., 2021).

In vitro anti-inflammatory activity

Fig. 2A shows that the positive controls and the FP phenolic extract had a similar inhibitory effect of a serine protease (55.47-59.90%) (p> 0.05), while RP maintained an enzyme inhibition of less than 30%. In case of severe tissue damage, as gastric damage, lysosome proteases are released. Proteases play an essential role in tissue damage in response to inflammation. In gastritis, these enzymes disintegrate the polymeric mucus structure and weaken its function as a barrier due to the gradual loss of its viscosity (Piñol-Jiménez and Paniagua-Estévez, 1999). Leelaprakash and Mohan-Dass, (2011) reported that neutrophils are found in lysosomes and are rich in serine proteases. Their release into the cytoplasm causes significant tissue damage during inflammatory reactions (da Silva Barros et al., 2019; Paredes-Salido and Clemente-Fernández, 2005). The serine protease inhibition assay indicated that the phenolic compounds extracted from the puree inhibited a serin-protease.

The concentration of phenolic compounds was significant for enzyme inhibition. The highest concentration of phenolic compounds in the extract caused the highest percentage of enzyme inhibition but with no significant differences (p>0.05) with acetylsalicylic acid, which is considered anti-inflammatory (Mohamed et al., 2019). Phenolic compounds can inhibit the enzymes by binding

to active sites, but their effectiveness depends on the type of polyphenolic compounds and the administered dose (da Silva Barros et al., 2019). Different phytochemicals such as carotenoids, vitamins, and polyphenols, can inhibit pro-inflammatory inductors (Corrales-Bernal et al., 2016), cyclooxygenases, proteases, and kinases and are mediators of the inflammatory process such as C-reactive protein or adhesion molecules (Paredes-Salido and Clemente-Fernández, 2005).

The positive control of gallic acid and the phenolic extract from FP presented up to 83.44 and 77.86% of CMS, respectively, with no significant differences (p> 0.05) between treatments. In contrast, the positive control of acetylsalicylic acid and the RP extract presented 48.98-51.02% of CMS, respectively (Fig. 2B). These differences may be due to the highest amount of phenolic compounds in the FP, which could protect the erythrocyte membrane (Ruiz-Ruiz et al., 2017). The mode of action of phenolic compounds or acetylsalicylic acid as anti-inflammatories could be related to their hydroxyl groups (-OH) or acylated sugar that could bind to the polar charges of the erythrocyte membranes with the consequent protection, avoiding their degradation (Ruiz-Ruiz et al., 2017; Oyeleke et al., 2018). Furthermore, flavonoids exerted a stabilizing effect on the lysosomal membrane, both *in vivo* and *in vitro* (Oyeleke et al., 2018). Stabilization of lysosomes *in vivo* is essential to limit the inflammatory response because if they are damaged, the released lysosomal enzymes may promote the inflammation signaling cascade. In addition, extracellular enzyme activity is related to acute or chronic inflammation (Leelaprakash and Mohan Dass, 2011). Thus, the phenolic compounds present in the purees have anti-inflammatory activity and, therefore, the potential to protect damaged tissues.

Biochemical parameters and histopathological analysis
The results of blood glucose and lipid profile are shown in Table 3. There was no significant effect (p> 0.05) of the induction to gastric-lesion on the biochemical markers after treatment, which is attributed to the fact that the rats were healthy.

LDH activity (Fig. 3) increased dramatically in the blood plasma once the gastric injury was induced (Pilot-LG). It confirmed an excellent induction of the gastric lesion (Cai et al., 2019).

On the other hand, LDH activity had normal values (1123.17 U/L) in the blood plasma from the HC group after the three weeks of the experiment, while in the LG group, the LDH activity was higher than in the HC group but lower than in the Pilot-LG group (Fig 3). However, it was observed that the LDH activity in the LG+Puree group (720.83 U/L) was statistically the lowest (p<0.05) compared to the HC and LG groups. Therefore, it was clear that adding the puree to the diet accelerated the recovery from gastric injury.

It has been reported that phenolic compounds, especially flavonoids, reduce the free radicals and interfere with the metabolism of prostaglandins, which inhibit the secretion of hydrochloric acid (De Lira Mota et al., 2009). These compounds were evaluated in gastric lesion-induced rats and treated with grape seed extracts. The results showed a percentage of inhibition of the gastric ulceration in which, when increasing the dose, a higher percentage of inhibition was achieved (Rahman et al., 2018).

Gastric glands with necrosis were observed in the stomach tissue from Pilot-LG rats (Fig. 4A). Gastric glands were affected in the fold cusps, in practically all cells of the damaged region (without the presence of cell nuclei), as well as the total loss of the architecture of the gastric gland, due to the solution used for its induction (Suchecka et al., 2017). On the other hand, the histology of the HC group (Fig. 4B) showed a healthy stomach in the apical part of the gastric gland of the straight tubular type, with its different types of cells, an integral architecture, and normal functions (Suchecka et al., 2017). On the other hand, Fig. 4C shows the histology from the LG group after three weeks of treatment, demonstrating that the damaged tissue recovered without the puree treatment because there were cells with an integrated architecture, which indicates that proper diet may heal damaged gastric tissue.

Fig. 4D shows the stomach histological sections from the LG+Puree group. Similar histology to the HC group can be observed, which shows a more significant number of cells, blood vessels, and different cell types than the LG group. For

Table 3: Biochemical parameters (mg/dL) in the blood plasma of a healthy control group (HC), a gastric lesion-induced group without treatment (GL group), a gastric lesion-induced group with guava-*Aloe vera* puree treatment (GL+Puree group), for three weeks.

Groups	Glucose	Cholesterol	Triglycerides	HDL-C	LDL-C	VLDL-C
HC	136.94±16.67[a]	52.24±10.01[a]	58.69±13.12[a]	63.02±10.01[a]	127.27±20.66[a]	25.692±8.70[a]
GL	131.59±17.24[a]	58.88±16.59[a]	69.36±14.28[a]	70.19±8.89[a]	124.76±13.16[a]	21.792±8.93[a]
GL+Puree	136.46±22.58[a]	63.92±11.96[a]	72.38±12.66[a]	65.24±16.69[a]	123.56±12.95[a]	29.018±8.02[a]

The values indicate the mean ± standard deviation (n≥6). Means within the same line with different superscript letters indicate a significant difference using the LSD test (p < 0.05). High-density lipoprotein-cholesterol (HDL-C); Low-density lipoprotein-cholesterol (LDL-C); Very low-density lipoprotein-cholesterol (VLDL-C)
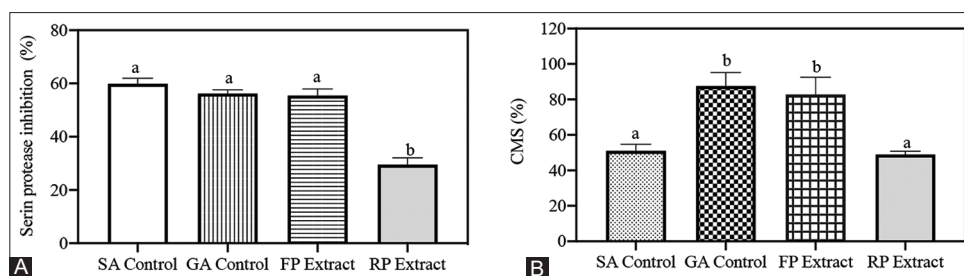
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Fig 2. (A) Serin protease inhibition (%) and (B) cell membrane stabilization (CMS) (%). Positive control of acetylsalicylic acid (SA control), positive control of gallic acid (GA control), phenolic extract from fresh puree (FP Extract), phenolic extract from puree stored at 4 ºC for six months (RP Extract).
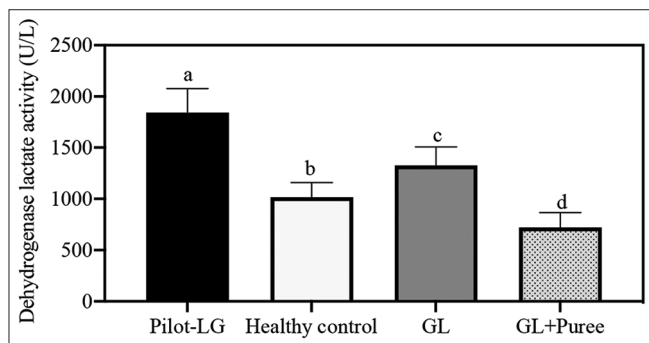

Fig 3. Lactate dehydrogenase (LDH) activity in pilot gastric lesion-induced rats (Pilot-LG), healthy control rats fed a standard diet (Healthy control), gastric lesion-induced rats fed the standard diet (GL), and gastric lesion-induced rats fed the standard diet and fresh guava-Aloe vera puree (GL+puree).
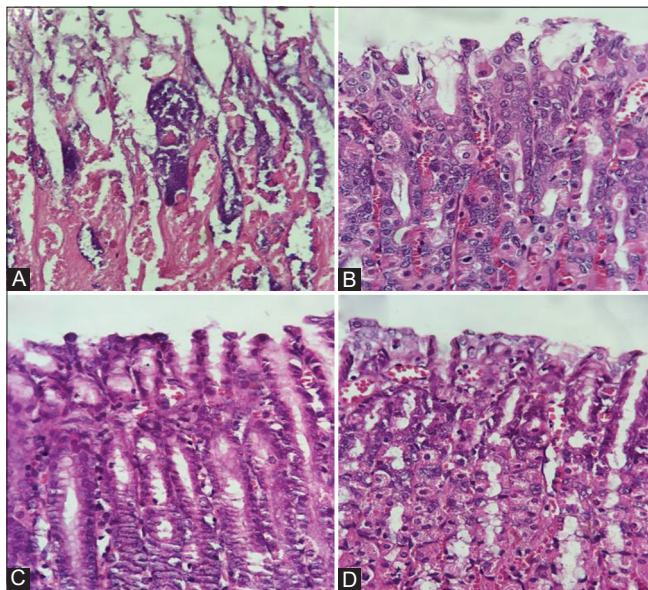

Fig 4. Hematoxylin and eosin staining in stomach sections (40X Zoom). Histological photomicrographs of fundic region stomach sections showing the apical part of the gastric glands of rats with an induced gastric lesion (6 µm). At the beginning (A), and after three weeks of treatment: healthy rats fed a standard diet (B), gastric lesion-induced rats fed the standard diet without treatment (C), and gastric lesion-induced rats fed freshly prepared guava-*Aloe vera* puree and standard diet (D).

this reason, it is considered that the inclusion of guava-*Aloe vera* puree in the diet induced a more accelerated recovery of the gastric lesion, as long as proper diet is followed.

Lee et al. (2012) reported that the intake of Bojungikki-tang extract (herbal extract) by gastric injury-induced rats caused a decrease in the injury and regeneration of the cells. The authors attributed this result to bioactive compounds (phenolic compounds) and their antioxidant capacity. *Aloe vera* and *Psidium guajava* have gastro-protective effects against gastrointestinal ulcers, attributed to the antioxidant compounds that neutralize free radicals that cause significant damage to the stomach and other organs. Likewise, they can cause inhibition of proteases and inhibition of pro-inflammatory enzymes, which can inhibit gastric acid production, stimulating pepsin and mucosal secretions, and promote healing (Werawatganon et al., 2014; Hęś et al., 2019).

CONCLUSIONS

Fresh guava-*Aloe vera* purees are highly nutritious, low in calories, and have a good source of minerals, dietary fiber, vitamin C, vitamin E, and other bioactive compounds. Refrigerated storage conserved the physicochemical characteristics of the guava-*Aloe vera* purees for six months. However, some nutrients and bioactive compounds decreased during the storage, maintaining 80% of vitamin C, 59% of vitamin E, 34% of provitamin A, 64-100% of some minerals, and 69% of phenolic compounds. It was demonstrated that phenolic extracts from purees had *in vitro* anti-inflammatory activity, and therefore, they have potential anti-inflammatory activity *in vivo*. The *in vivo* histopathological study concluded that the intake of guava-*Aloe vera* puree by gastric lesion-induced rats caused a gastroprotective effect.

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AUTHOR´S CONTRIBUTIONS

All authors contributed substantially to the writing and revising of the manuscript. Veronica Aida Machuca-Gollaz, Eduardo Mendeleev Becerra-Berdín, and Efigenia Montalvo-González designed the work, acquired, analyzed, and interpreted the data. Irma Julieta González-Acuña measured and obtained the minerals data. Veronica Aida Machuca-Gollaz, Eduardo Mendeleev Becerra-Berdín, and Efigenia Montalvo-González developed and standardized the *in vivo* assay. María de Lourdes García-Magaña, Veronica Aida Machuca-Gollaz, and Efigenia Montalvo-González conducted the statistical analysis. Gustavo Adolfo González-Aguilar, Efigenia Montalvo-González and Elhadi M. Yahia critically reviewed, corrected, and edited the manuscript.

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