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Research Article

POTENTIAL USES OF THE PEEL AND SEED OF *Passiflora edulis* f. *edulis* Sims (GULUPA) FROM ITS CHEMICAL CHARACTERIZATION, ANTIOXIDANT AND ANTIHYPERTENSIVE FUNCTIONALITIES

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ABSTRACT

Objective: Assess the performance of a crude ethanolic extract, a dichloromethane fraction and a hydroalcoholic residue, which are the basis for chemically and biologically characterizing the husk and seed of *Passiflora edulis* f. *edulis*, collected in the region and Colombia with a view to determining potential uses.

Methods: Agroindustrial residues of gulupa (peel and seed) were analyzed through a bromatological study; subsequently, they were macerated with ethanol (96%). The crude ethanolic extract was partitioned with dichloromethane, leaving a hydroalcoholic residue. The content of total phenols, the composition of phytophenols (high-performance liquid chromatography-mass spectrometry), the total antioxidant capacity using 3-ethyl benzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-pyridyl hydrazyl ($DPPH^{\bullet}$), the oxygen radical absorbance capacity (ORAC), and the ferric reduction power (FRAP) were determined to the extract, the fraction, and the residue. The evaluation of the inhibitory activity of the angiotensin-converting enzyme inhibitor (ACEI) and the cell viability assay with diphenyl bromide 3- (4,5-dimethylthiazole-2-) il) -2,5-tetrazolium on human leukocytes complemented the characterization.

Results: Agroindustrial waste of *P. edulis* f. *edulis*, peel and seed, contains as main constituents: Protein (8.49 and 7.29%), fiber (34.2 and 55.7%), phosphorus (1.67 and 3.09), and boron (53.3 and 58.4 mg/kg), respectively. The seed showed 25.5% oil. The crude seed extract exhibited a higher phenolic content (15.34 gEAG/100 g). Likewise, it presented the highest antiradical capacity against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ (706.17 and 82.81 trolox equivalent antioxidant capacity [TEAC], respectively) and antioxidant in ORAC and FRAP (142.79 TEAC and 103.63 equivalent ascorbic acid EAA, respectively). The ACEI activity (50% inhibitory concentration 17.62 mg/L) of the crude seed extract was higher than the other samples. No toxicity was found in the samples evaluated at concentrations higher than those of the biological activities manifested.

Conclusion: The agroindustrial residues of *P. edulis* f. *edulis* (peel and seed) are rich in nutrients, which propose them for use in food matrices. The ethanolic extract from seed showed the highest antioxidant, antiradical, and inhibitory biological activity of the ACEI so that it could be proposed the gulupa seed as a promising phytotherapeutic product associated with its phenolic content, especially its flavonoids. The results obtained allow an added value to the fruit, reducing the chances that its waste contributes to environmental pollution.

Keywords: Gulupa, *Passiflora*, Antioxidant, Antihypertensive activity, Agroindustrial residues.

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INTRODUCTION

Fruit market in Colombia is particularly important contributions made around the production, marketing and use of agroindustrial byproducts derived from the processing of these natural products, especially when it comes to the so-called exotic fruits, understood as those striking fruits by its shapes, colors, unusual characteristics, and taste and pleasant aroma, in addition to its excellent nutritional value. The interest for them is more and more growing, the most demanding palates appeal to the natural, in simple or concentrated juices, or in different preparations. The most important thing is that a good number of them is produced in Colombia, given their climatic diversity and high availability of soils with agricultural vocation.

An obvious example of this is the so-called "passion fruit" market, of which 170 species of *Passiflora*, between wild and cultivated, are reported for Colombia; with the warning that the Passifloraceae family has almost 700 species [1]. It is estimated that in 2018 the Colombian market produced more than 241,000 tons of yellow passion fruit (*Passiflora edulis* f. *flavicarpa*), granadilla (*Passiflora ligularis*), gulupa or purple passion fruit (*P. edulis* f. *edulis*), banana passion fruit (*Passiflora mollisima*), cholupa (*Passiflora maliformis*), and badea (*Passiflora quadrangularis*) [2]. Of these, yellow passion fruit, granadilla, gulupa, and banana passion fruit occupy the highest order of importance for the country, taking into account the number of related producers, the areas cultivated in the different regions, and the priority interest of the national fruit plan.

In particular, the name of the gulupa is especially associated with the department of Tolima as the third national producer, with a contribution of more than 2300 tons in the aforementioned year; figures that will be constantly increasing until 2020 due to the interest in these export products by the government. It should be recognized that gulupa has attractive market prospects and reaches 40% of Colombian exports; this merits it currently occupies the third line within the fruits exported to the European market, after the banana (*Musa paradisiaca*) and the cape gooseberry (*Physalis peruviana*) [3]; however, its consumer culture is still incipient in the interior of the country.

Gulupa is cultivated between 1100 and 2750 masl [4]; its fruit is of a unique, sweet, and slightly acid taste [5], its nutritional, organoleptic characteristics of flavor and aroma have procured great use in the food industry for the preparation of juices, pulps, sweets, jellies, jams, and cocktails, among others [6,7]. Being part of the passionflower, this fruit provides essential Vitamins such as A, B12, and C, it is also a source of

calcium, fiber, phosphorus, iron, proteins, magnesium, potassium, and carbohydrates [8,9]. This passionflower has a high presence of alkaloids such as Harmanol and harmol, recognized for their natural sedative effect, lowering blood pressure and acting as tranquilizers [6]. In addition, the leaves contain passiflorine, a compound used as a sedative and antispasmodic [10], seeds are a source of oil for haute cuisine [1], the beauty of its flowers makes it desirable as a plant ornamental, the pulp of the fruit possesses flavonoid glycosides [11], alkaloids [12], triterpenes and saponins, cyanogenic glycosides, phenols, carotenoids, anthocyanins, and free amino acids, among others [13]. The fruit has also been found uses in cosmetology and perfumery, derived from the presence of essential oils [10]. The nutraceutical industry finds application as a complement to multivitamin products as a source of minerals and Vitamin C [1]. Many of the aforementioned compounds are closely related to antioxidant, anticonvulsant, anti-inflammatory, and antihypertensive and even have been suggested as a possible alternative treatment for congestive heart failure [14].

With all this in its favor, this abundant and diverse field of industrial application is, in turn, a generator of adverse effects due to the poor disposition of agroindustrial waste, which would be associated with the ignorance of the potentialities they offer. It is important to point out that the fruit of the gulupa is made up of 50–60% of the skin, 30–40% of the juice, and 10–15% of the seeds [15]. It is then noted that between the peel and the seed makeup 70–80% of the total weight of the fruit are in unused biomass [16], with the consequent high costs of final disposal and generation of negative environmental impact. Hence, the need to implement strategies in search of the integral use of the fruit, decrease of environmental damage and generation of extra-economic benefits in the productive stage, by taking advantage of this agroindustrial waste [16].

With this thought in mind, the present study was interested to investigate some of the chemical and bioactive properties of the agroindustrial residues of gulupa for its potential phytopharmacological use. The information obtained would be of great support to the passiflora chain of Tolima, to the Colombian fruit agroindustry and to the flourishing export market of exotic national fruits.

METHODS

Chemical reagents

All the reagents used in the present study, including 2,2-diphenyl-1-pyridyl hydrazyl (DPPH$^{\bullet}$), 3-ethyl benzothiazoline-6-sulfonic acid (ABTS$^{\bullet+}$), diphenyl bromide 3- (4,5-dimethylthiazole-2-) il) -2,5-tetrazolium (MTT), hippuryl histidyl leucine (HHL), and angiotensin-converting enzyme (ACE) (EC 3.4.15.1, 5.1 U/mg) were purchased from SIGMA-ALDRICH® (St. Louis, MO, USA). The chromatographic reagents used were high-performance liquid chromatography (HPLC) grade supplied by J.T. Baker (Deventer, The Netherlands). Other chemical products used were of analytical quality (Merck, USA). The permission to use human samples was authorized by the Ethics Committee of the University of Tolima.

Collection area

Fruits in optimum phytosanitary status were collected in 2016 in crops of the municipality of Cajamarca (4 ° 26 '27 "N 75 ° 25' 40" W), to the west of the department of Tolima Colombia. The territory of the municipality is generally mountainous with soils of acidic pH, located in the eastern part of the central mountain range, with very steep slopes which characterizes it as broken relief, with high runoff [17]. A complete specimen of the plant (leaves, flower, and fruit) was prepared and determined at the National Herbarium of Colombia to confirm the species of interest.

Preparation of plant material

Peel and seed were manually separated from the pulp and subjected to a drying process (45±2°C, 24 h). The plant material thus prepared was crushed and degreased with n-hexane by the Soxhlet extraction method. The degreased product was macerated with ethanol (96%), renewing the solvent every 2 h. A crude ethanol extract of peel (CEP) and seeds (CES) was obtained, which was subjected to liquid-liquid fractionation with dichloromethane, obtaining dichloromethane fraction of peel (DFP), and seed (DFS) fraction, in addition to, the hydroalcoholic residues of peel (HRP) and seed (HRS). To these products, the solvent was removed under vacuum (45±2°C, rotavapor R-114, Büchi, Flawil, Sweden) and stored (−85°C, Freezer Kaltis 390) until its use in the analysis.

Bromatological and mineral analysis

The moisture content, ash, crude fat, crude protein, and raw fiber were established in a portion of the dry plant material, independently for peel and seed. Similarly, the content of major elements (K, Na, Mg, and Ca) and minor (Fe, Mn, Cu, and Zn) was determined by atomic absorption spectrophotometry (Thermo Scientific iCE 3000 Series AA), while elements P, B, and S were quantified using ultraviolet (UV)-vis spectrophotometry (Evolution 260 BIO). All determinations were developed under the standard indications of the AOAC [18].

Phytochemical analysis of crude extracts and fractions

A preliminary phytochemical analysis was carried out according to the indications of Harborne [19]. In addition, in the products obtained from the plant material the total content of phenolic compounds, tannins (only for CEP and CES) were measured, applying the Folin–Ciocalteu method with some modifications [20]. The flavonoid content for CEP and CES was quantified based on what was reported by Palomino *et al*. [21].

Chromatographic analysis

For CEP and CES, the chemical profile was carried out by HPLC analysis in a Waters Alliance 2695 separation module system, coupled to a dual-channel λ absorbance detector (280 and 320 nm). The compounds were separated with a Waters Atlantis dC18 column (5 µm, 2.1 mm, and 150 mm), using a gradient system with a mobile phase consisting of H_2O (solvent A, formic acid 0.1% v/v) and 100% methanol (solvent B, acetic acid 0.1% v/v). The flow rate was 0.500 mL/min. Linear gradient elution was performed varying the ratio of solvent A to solvent B, according to Delpino-Rius *et al*. [22]. The data were acquired using the chromatographic behavior and the UV-vis absorption spectra, together with the published information on the main phenolic compounds in the samples. To quantify, calibration curves were constructed by tracking the areas of chromatographic peaks measured at the specific wavelengths of the chemical standards.

Antiradical and antioxidant capacity

The ability of the compounds present in the samples to interact with free radicals was evaluated using DPPH$^{\bullet}$ [23] and ABTS$^{\bullet+}$ [24]. The 50% inhibitory concentration (IC_{50}) (IC_{50} concentration of the sample stabilizing 50% to the ABTS$^{\bullet+}$ radical) and the antioxidant capacity in trolox equivalent antioxidant capacity (TEAC) for both radicals were calculated. The antioxidant capacity of the analyzed samples was evaluated by means of the oxygen radical absorbance capacity (ORAC) techniques, expressed as TEAC and ferric reduction power (FRAP), expressed in g equivalents of ascorbic acid per 100 g of sample (g ascorbic acid equivalents/100 g). All spectrophotometric measurements were made with a 96 wells microplate UV-vis reader (Multiskan® GO Thermo scientific).

Inhibition of the ACE

HHL prepared in sodium borate buffer was used as the substrate for the ACE. To 100 µL of the HHL substrate was added 40 µL of each sample. Subsequently, 2 mU of ACE (EC 3.4.15.1, 5.1 U/mg) dissolved in 50% glycerol was added. The reaction was carried out at 37°C for 30 min. The enzyme was inactivated by lowering the pH, adding 150 µL of 1 N HCl. The hippuric acid formed was extracted with 1000 mL of ethyl acetate. After stirring and subsequent centrifugation (4000 gravities, 10 min, room temperature), the organic phase was taken, which was evaporated by heating (95°C, 15 min). The hippuric acid residue was dissolved in 800 µL of distilled water, and after stirring, the absorbance was measured at λ228 nm. The ACE inhibitory (ACEI) activity was calculated as the sample concentration needed to inhibit 50% of the

enzyme (IC_{50}). The activity of each sample was determined in triplicate and the inhibitory activity of each sample by the following formula:

$$\%IECA = \frac{ABScontrol - ABSsample}{ABScontrol - ABSblank} \times 100$$

Where: ABScontrol: Absorbance of hippuric acid formed after the action of ACE without inhibitor; ABSblank: Absorbance of HHL that has not reacted and that has been extracted with ethyl acetate; ABSsample: Absorbance of hippuric acid formed after the action of ACE in the presence of inhibitory substances.

Toxicity on human leukocytes

Initially, the blood cells were isolated to which the toxicity protocol of the Laboratorio de genómica viral y humana de la Facultad de Medicina-Universidad Autonoma de San Luis Potosi [25] was later applied, with some modifications. With the isolated leukocytes, a 1:1 dilution was made with phosphate-buffered saline (pH 7.4), homogenizing vigorously by inversion. The percentage of cell viability was measured by the MTT colorimetric method [26], where microplates were used to which 25 µL of the different concentrations of the samples, 25 µL of the leukocytes were added to each treatment, and finally 50 µL of MTT were added. Plates were incubated at 37°C for 2 h. Finally, 50 µL of dimethyl sulfoxide was added to dissolve the formazan crystals. The plates were read on a 96 wells microplate UV-vis reader (Multiskan® GO Thermo scientific) at λ570 nm. The viability percentage was calculated [27].

Statistic analysis

The reported values represent the analysis of at least three separate replicates for each sample ± standard deviation. The statistical analysis of the data was based on a one-way analysis of variance (ANOVA). The values of $p<0.05$ were considered statistically significant, for which the assumptions of normality and homogeneity of the variance were verified ($p>0.01$). The IC_{50} values were calculated using linear regression. All data processing was done with the statistical package Statgraphics Centurion XV.II. P.

RESULTS AND DISCUSSION

Bromatological analysis and mineral content

The results of the nutritional analysis of the plant materials used in this study appear in Table 1. Considering that the volume of biomass contributed by the peel is 6 times higher than that of *P. edulis* f. *edulis seeds*, the ashes result is not surprising. The value of this parameter in the peel is noted 5 times higher than the seeds (9.03% and 1.76%, respectively). The results found with the gulupa differ from that reported for the yellow passion fruit peel (1.33±0.028%) by Adeyeye *et al*. [28]. Although it is stated that the ashes represent approximately 5% of the dry matter, it seems acceptable that in the peels a higher level is obtained, since in this part of the fruit there is recalcitrant material (silicates), in charge of giving resistance to the surface [29]. The discrepancies in the thickness and firmness of the shell of each species of *Passiflora* could respond to the values obtained in each case.

The ashes of a food are the inorganic waste resulting from calcining the organic matter, but they differ from the original contents in the food product as a result of losses due to volatilization or some interaction between the constituents; in addition, the calcination causes them to become oxides and salts (sulfates, phosphates, silicates, and chlorides). The main contribution of this nutritional assessment is related to the level of minerals in the sample, in vegetables, the potassium derivatives predominate, and the sodium in the products of animal origin. The ashes can also be taken as a parameter of quality control (purity of some ingredients or type of refinement and grinding, among others) [30].

It should be remembered that the size of the seeds among different plant species varies greatly. For a large number of passionflowers, the fruits contain small seeds of dimensions ranging between 1.4 and 9.1 mm in length, 1.6–6.9 mm in width, and 1.1–2.9 mm in thickness, the cover is hard, leathery in texture and the surface of the central zone is ornamented [31]. Adeyeye *et al*. [28] found that the yellow passion fruit seed (*P. edulis* f. *flavicarpa*) has 2.26±0.014 ash; this value and that of the gulupa here determined (1.76%) are evidence of the differences between these two species. Something very important to bear in mind is that the characteristics of the seed surface are little affected by environmental conditions, their ontogenetic origin is constant with a well-defined function; consequently, they can reflect the genome of the plant and the phylogenetic relationships between species, which, in turn, are associated with phytoconstituents [32].

The importance of the seeds does not lie only in the function of multiplying and perpetuating the species to which they belong; they are also a true reservoir of nutrients [33]. Then, the information reported in Table 1 is valuable. A slightly lower value in protein, a higher percentage in fiber and the fat content five times higher, in relation to the peel, is evident in the seed. However, the percentage of protein found in the peel meal of *P. edulis* f. *edulis* surpasses what was reported by Carvajal-De pabón *et al*. [34] in *P. ligularis* (4.37%), but it is comparable to that found by Salgado *et al*. [35] in yellow passion fruit peel (9.8%). Carvajal-De pabón *et al*. [34] described a protein content of 12.52% in gulupa seeds.

Despite the significant differences in fiber content shown in Table 1, the level can be considered as abundant in peel and seed. Regarding the oil content, *P. edulis* f. *edulis* is recognized by different authors as an oily species [38-41], with values similar to those found in fruits of greater commercial impact such as yellow passion fruit [38,42].

The content of major elements, both for the peel meal and for the seed meal, is also shown in Table 1. Among the major elements, phosphorus, followed by potassium and calcium were the highest presence, both in peel and in seed, while the minor elements varied in their content in the two by-products of the fruit. Thus, boron was more abundant, followed by iron, copper, and manganese in the shell. In the seed they were presented in the following order: boron> Cu> Zn> Fe> Mn. Sulfur was the element with the lowest content and phosphorus was found with the highest levels, in the two vegetable parts.

Phosphorus is one of the main essential nutrients for plants, its functions cannot be carried out by any other element, making it necessary for optimal growth and reproduction, and therefore, it is required by the plant in greater quantity than any other [43]. Similarly, phosphorus is an essential nutrient for humans and animals; its deficiency is the most widespread of all the mineral deficiencies suffered by livestock and meat-producing animals, severely affecting growth, reproduction, pregnancy, and lactation [44]. The presence of this mineral in the agroindustrial waste analyzed makes it possible to propose it as a raw material in the preparation of concentrates and nutritional supplements for agricultural purposes [36].

Boron as one of the most abundant minor elements in the analyzed samples is not a coincidence, since this element plays a fundamental role during the synthesis of pectins, recognized for their presence in fruits of the genus *Passiflora* [45,46]. The greater presence of Cu with respect to Fe in the seed may be due to the fact that it is a transient element and shares similarities with iron in the formation of stable complexes and easy transfer of electrons [47]. The highest concentration in the peel, although not very distant from the seed, shows its effect on the formation and chemical composition of the cell wall. Copper, moreover, functions as a cofactor of polyphenol oxidases, a group of Cu-containing enzymes that catalyze the oxidation of phenolic precursors of lignin, which gives hardness to plant tissues such as fruit peels [48]. The results of the bromatological and mineral analysis suggest that both the flour from gulupa peel and from seeds could be used as a complementary source of nutrients or for their incorporation into food matrices.

Phytochemical characterization of crude extracts and fractions

Medicinal plants are a valuable resource of phytotherapeutics as a source of bioactive compounds. When applying different extraction

methods, the components of pharmaceutical and/or nutritional interest can be obtained as extracts; these, in turn, being partitioned with solvents of different polarities that allow fractions to be obtained where the constituents are found in a greater degree of concentration, more free of impurities and, it is expected, that their bioactivity increases [49,50]. Through a sequence of steps, it is pertinent to know the type and variability of compounds (secondary metabolites) that are part of extracts and fractions. The applied qualitative phytochemical analysis revealed the presence of reductive carbohydrates, terpenes, and phenolic compounds of tannic nature, as well as flavonoids for extracts and fractions of gulupa peels and seeds (Table 2). The phenolic constituents in extracts obtained from *P. edulis* f. *edulis* have already been reported by different authors [51,52], it is stated that these metabolites are responsible for the multiple activities biological attributed to the fruits of passion, among others: Antihypertensive, anxiolytic, and antidiabetic activities [53-55].

It was also possible to verify the presence of anthraquinones in the seeds crude extract (CES) and in the polar fraction derived from them (DFS). The presence of alkaloids was detected only in the extract and fractions coming from the peel (CEP, DFP, and HRP). Other species of the genus *Passiflora* have been recognized for their content of indole alkaloids (harmine, *harmaline*, and *harmalol*) [56]. Some researchers have reported that *P. edulis* contains harmonic alkaloids in different tissues [57]. The detection of saponins and alkaloids in the extract and fraction from the shell suggests that these two types of secondary metabolites are part of the plant's defenses against predators that seek nutrients from the edible part of the fruit [58].

On the other hand, indole alkaloids are related to the neurotransmitter serotonin, a molecule widely implicated in brain function and cognition as an endogenous receptor agonist, this property has deserved to find to its application in the treatment of neurological disorders [59]. The evidence of alkaloids in gulupa peel opens perspectives of use to this agroindustrial waste and the productive chain of the fruit. Several studies have indicated that *Passiflora incarnata* has a sedative action, attributed to a synergism between indole alkaloids, maltol, and flavonoids [60].

Phenolic compounds are important constituents in various plants, so their quantification can generate valuable information regarding the antioxidant function, food quality, and various health benefits of plant extracts. The quantification of total phenols of the crude extracts and fractions is shown in Table 2. The same table shows the evaluation of the content of total tannins, total flavonoids, and the compounds quercitrin, cumárico acid, epicatechin, chlorogenic acid, and rutin, found in the raw extracts of shell and seed. It should be noted that these determinations were not made to the fractions.

The crude seed extract (CES) revealed a phenolic content almost 5 times higher than that of the peel (CEP), but the fractionation distributed them in similar proportions between DFS and HRS, without statistically significant differences between them ($p>0.05$). In peel, the level of phenols can be categorized as follows: HRP> DFP> CEP, with a significant difference between the first two ($p<0.5$). On the other hand, the tannins and flavonoids showed the seeds and the shell as their best reservoirs, respectively.

In crude extracts derived from the pulp of gulupa (*P. edulis* f. *edulis*), phenol contents have been reported close to 0.3 gEAG/100 g [61], while in a methanol extract of pulp from yellow passion fruit (*P. edulis* f. *flavicarpa*) Marroquín *et al*. [62] obtained values of 0.001±0.001 gEAG/100 g. The fruit juice of yellow passion fruit harvested in different areas of India showed contents between 0.018

Table 1: Bromatological and mineral analysis

Parameters	Sample		Ranges for the edible part of *passion flower**
	Peel	Seed	
Percent content of nutrients			
Ash	9.03±0.41[a]	1.76±0.13[b]	NR
Crude protein	8.49±0.63[a]	7.29±0.58[b]	0.6–2.8
Crude fat	5.6±0.24[a]	25.5±2.1[b]	NR
Crude fiber	34.2±2.49[a]	55.7±3.19[b]	4.4–15.9
Mineral content			
Major elements (%)	0.57±0.04[a]	0.18±0.00[b]	0.20–0.33
Potassium	0.04±0.00[a]	0.02±0.00[b]	NR
Sodium	0.40±0.02[a]	0.16±0.00[b]	0.003–0.014
Calcium	0.04±0.00[a]	0.07±0.00[b]	0.009–0.018
Magnesium	1.67±0.11[a]	3.09±0.24[b]	0.03–0.08
Phosphorus	0.11±0.00[a]	<0.01	235.84–377.35
Minor elements (mg/kg)			
Iron	48.5±3.01[a]	10.1±1.12[b]	23.58–28.30
Manganese	11.5±0.95[a]	5.7±0.48[b]	NR
Boron	53.3±4.12[a]	58.4±5.47[b]	NR
Copper	20.1±1.25[a]	17.9±1.69[b]	NR
Zinc	2.7±0.14[a]	12.5±1.52[b]	4.71–9.43

*Taken from Deshmukh *et al*., [37]; a, b: Different letters are equivalent to statistical significance

Table 2: Quantification of phenolic compounds

Quantification		Sample					
		Peel			Seed		
		CEP	DFP	HRP	CES	DFS	HRS
Total phenols	gEAG	3.77±0.13[a]	3.99±0.04[a]	4.68±0.18[b]	15,34±0,78[c]	11.77±0.48[d]	10.56±0.35[d]
Tannins		0.18±0.00[a]	NA	NA	1.20±0.01[b]	NA	NA
Flavonoids	gEQ	5.56±0.11[a]	NA	NA	5.32±0.09[b]	NA	NA

CEP Crude ethanol extract of peel, DFP: Dichloromethane fraction of peel, HRP: Hydroalcoholic residues of the peel, CES: Crude ethanol extract of seed, DFS: Dichloromethane fraction of seed, HRS: Hydroalcoholic residues of seed, gEQ: Equivalent grams of quercetin per 100 g of sample, gEAG: Equivalent grams of gallic acid per 100 g of sample, NA: Not applied, a, b, c, d: Different letters equate to statistical significance

and 0.027 gEAG/100 g [63]. In the leaves, a content between 1.39 and 1.87 mg/100 g of phenols was found from hydroalcoholic extracts [64]. It is evident that the species, the part of the vegetable, and the abiotic factors (collection site, among others) are determinant in the values that can be found of these phytoconstituents.

To establish a chemical footprint of the extract obtained from agroindustrial residues of *P. edulis* f. *edulis*, authentic samples of phenolic compounds were used as reference compounds. Based on the results for the shell indicated in Table 2, the contents can be ordered as follows: Chlorogenic acid (28.45 mg/100 g), routine (19.29 mg/100 g), quercitrin (10.40 mg/100 g), epicatechin (8.69 mg/100 g), and cumaric acid (0.84 mg/100 g). In the seed: Only quercitrin (9.96 mg/100 g) and cumaric acid (5.40 mg/100 g) were found. Many of the above-mentioned compounds are recognized for their antioxidant and on coprotective activities [65], which extends the bioprospecting of the by-products of the gulupa fruit.

Antiradical and antioxidant capacity

Due to the complexity and chemical variety of the components found in the samples of interest, antiradical activity data were homogenized to be expressed as IC_{50} (concentration necessary to reduce *in vitro* 50% of the radical species) and TEAC, while the antioxidant activity was expressed as TEAC and EAA (equivalent antioxidant capacity of ascorbic acid). The data appear in Table 3.

The ANOVA indicated that the IC_{50} obtained in the peel, CEP and DFP, as well as in the seed, CES and HRS, do not show significant differences against $ABTS^{\bullet+}$. The values expressed as TEAC imply low activity in any of the samples. Some researchers have found that the radical $ABTS^{\bullet+}$ reacts in the presence of a hydroxylated aromatic compound, regardless of its actual antioxidant potential, this has led to it being recognized as having very low selectivity [21]. Comparing TEAC values corresponding to crude extracts, CEP and CES, against to DPPH•, it is noted that they are considerably lower than those corresponding to $ABTS^{\bullet+}$, particularly in CES.

If one thinks of the phenolic compounds (flavonoids and tannins, among others) as the main actors of the antioxidant activity, it should be taken into account that $DPPH^{\cdot}$ does not react with those flavonoids lacking hydroxyl groups in the B ring and that the steric hindrance produced by the molecular structure of this radical limits access to the radical site by compounds with high molecular weight or whose molecular organization is complex [21,66]; the above would partially justify the lower values obtained against $DPPH^{\cdot}$. Our results are similar to those found by Carvajal *et al*. [67] who worked with the edible part of the gulupa fruit, finding values against $DPPH^{\cdot}$ (20.07 TEAC) and $ABTS^{\bullet+}$ (42.34 TEAC), although the values determined in this study for peel are higher, not for seed. These same authors reported 8.58 and 27.78 TEAC, for yellow passion fruit pulp against DPPH•and $ABTS^{\bullet+}$, respectively, data that resemble those obtained in this research for gulupa peel.

In order to have a greater knowledge about the antioxidant potential of the set of compounds present in the extracts and fractions from the peel and the pulp of gulupa, two complementary methodologies were applied to the antiradical activity: the test FRAP, based on the Fenton reaction, and ORAC, which reflects the synergistic or potentiation interaction of all the antioxidants present in the sample to inactivate the peroxyl radicals (ROO•).

In Table 3, it is clearly noted that the ORAC value of the seed extract significantly exceeds that of the peel ($p<0.05$), which agrees with the phenolic content (15.3 and 3.77 for CES and CEP, respectively, Table 2). In the dried pulp of *Passiflora mollissima*, Rojano *et al*. [68] found an ORAC value equal to 108164.9 mmol of trolox/100 g, considered by the same authors as a higher result than that obtained with the majority of fruits and vegetables. Although the ORAC of the gulupa seed extract is lower (142.79 mmol TEAC/100 g of sample), it should not be forgotten that it is a by-product of a smaller size than the pulp.

In the FRAP trial, the results ranged from 2.89 g equivalents of ascorbic acid/100 g sample, for CEP, to 103.63 g equivalents of ascorbic acid/100 g sample, for CES. Both the fraction derived from the seed extract, DFS, and the hydroalcoholic residue, HRS, showed greater reducing power than the corresponding products derived from the peel. The phenolic content of these samples showed the same behavior. Furthermore, Navarro *et al*. [69] observed a correlation between phenols and reducing power (FRAP).

The values obtained with FRAP and ORAC reflect the overall capacity or antioxidant activity of the samples evaluated. These are two methodologies that have been implemented in recent years due to their great sensitivity and precision [70,71], which makes it possible to post the agroindustrial residues of gulupa as a source of antioxidant components. Phenolic compounds and alkaloids are some of the metabolites recognized for their antioxidant action against these evaluations [72].

Inhibitory activity of the ACEI

The data evidencing the ACEI by crude ethanolic extracts, dichloromethane fractions and the respective HRP and seed of *P. edulis* f. *edulis* are presented in Table 4. The activity of the samples was tested at concentrations between 13 and 213 mg/L; those that showed greater effectiveness were CES, on the part of the seed, and HRP derived from the peel, not finding significant differences between these two ($p<0.05$). DFS and HRS were less effective. Nonetheless, CEP and DFP succeeded in inhibiting the enzyme by more than 80%; therefore, the relatively low yields of these samples should not obscure their biological potential. Although the greater ACEI activity was dependent on the level of concentration applied in the trial, it should not be forgotten that extracts and fractions contain a diversity of compounds, which can have an antagonistic effect on each other.

Comparing the action of all the samples, again, the lowest IC_{50} was found in the crude extract from seed, (CES 17.62 mg/L). On the other hand, DFP, CEP, and DFS showed the highest values (36.23, 29.17, and 26.31, respectively). In the peel, significant differences were found between DFP and HRP ($p<0.05$), but, the opposite happened in the samples from the seed ($p>0.05$).

Table 3: Potenciales antirradical y antioxidante

Test		Sample					
		Peel			Seed		
		CEP	DFP	HRP	CES	DFS	HRS
$ABTS^{\bullet+}$	CI_{50}	62.07±5.13[a]	65.72±0.94[a]	101.58±15.84[b]	2.69±0.21[c]	8.45±0.09[d]	3.03±0.25[c]
	TEAC	30.61±2.68[a]	28.74±0.41[a]	18.88±2.79[b]	706.17±59.53[c]	223.61±2.46[d]	627.20±51.8[c]
$DPPH^{\bullet}$		10.41±0.17[a]	NA	NA	82.81±3.31[b]	NA	NA
ORAC		49.4±2.21[a]	NA	NA	142.79±3.03[b]	NA	NA
FRAP	EAA	2.89±0.19[a]	3.07±0.03[a]	3.51±0.13[b]	103.63±6.17[c]	57.86±3.04[d]	69.65±3.07[e]

CEP: Crude ethanol extract of peel, DFP: Dichloromethane fraction of peel, HRP: Hydroalcoholic residues of the peel, CES: Crude ethanol extract of seed, DFS: Dichloromethane fraction of seed, HRS: Hydroalcoholic residues of seed, TEAC: mmol equivalent trolox/100 g sample, AAE: g ascorbic acid equivalent/100 g sample, CI_{50}: Concentration needed to reduce *in vitro* 50% of radical species (mg/L), NA: Not applied, a, b, c, d: Different letters equate to statistical significance

In a study carried out by Salazar-Aranda *et al*. [73], with herbal products derived from different plant species, including *P. incarnata*, it was found that two products made with this *passion flower* produced a moderate inhibition (72.9%±1.8 and 66.1%±0.5) of the ACE. In the opinion of the authors, this plant is traditionally recommended as a tranquilizer or sedative, although until now they did not locate any report of the ACEI activity for the species. Then, taking into account that the crude ethanolic seed extract, CES, showed percentages of inhibition between 42.37 and 95.51, it could be said that the seed of *P. edulis* f. *edulis* (gulupa) has constituents with potential antihypertensive use. In addition, so far this biological functionality has not been reported in the byproducts of this plant.

Hypertension as such is a syndrome of a set of metabolic and structural abnormalities [74], which are believed to be associated with reactive oxygen species contributing to their generation and/or maintenance. It is stated that several mechanisms intervene in the appearance of this pathology. In the case of the inhibition of the ACE, it occurs through the subtraction or blockade of the zinc atom in its active center [75]. This mode of modulating action has been reported for compounds such as flavonoids and quinones [76]. It could then be thought that the flavonoids detected in all the extracts were responsible, at least in part, for the ACEI activity reported here.

Flavonoids have been widely described for the genus *Passiflora* [11,62,77] so has the property of scavenging free radicals, in addition to the ability to inhibit multiple enzymes responsible for the synthesis of superoxide anion [78]. Likewise, the compounds quercitrin and cumaric acid found in both peel and seed have demonstrated antihypertensive activity [79-81]. All this suggests that the agroindustrial byproducts of *P. edulis* f. *edulis* have a significant ACEI activity derived from the presence to these compounds. The ACEI activity presented in the peel could be associated with the higher content of compounds identified in this plant part, among which is the rutin; this metabolite has been found to have antihypertensive properties *in vitro* and *in vivo* [81-83].

Cell viability assay

With the intention of knowing the possibility that extracts and fractions derived from peel and seed of *P. edulis* f. *edulis* can find therapeutic use, this work proved its toxicity on mononuclear cells (500–4000 mg/L) Table 5. The viability of leukocytes exposed to CEP was lower than that of the other samples, not exceeding 20% in any of the concentrations evaluated. Since it is a raw extract; it has all the metabolites that the subsequent fractions could distribute in the fractions, thus reducing the cytotoxic effect. It is possible that saponins and alkaloids are the major players in the cellular integrity of leukocytes [84].

It is important to show that at the lowest tested concentration (500 mg/L), the table shows, the bioactivity decreases when the concentration increases, although the significant differences between the samples are not known. On the other hand, the toxicity of samples from seed becomes more evident from 1000 mg/L. It should be noted that CEP shows the lowest cell viability among the samples. In contrast, HRS presented the highest viability in each of the concentrations evaluated, and significant differences were found between extracts and fractions derived from peel and seed.

It has been suggested that extracts that allow cell viability lower than 80% can be considered toxic [85]. In view of the above, the samples obtained from the peel are toxic on leukocytes from 500 mg/L, making it necessary to know this effect at lower concentrations, mainly in the range worked in the biological activity of the ACEI and antiradical/antioxidant type. As a point in favor of the agroindustrial residues of *P. edulis* f. *edulis*, the IC_{50} in the biological activities both antiradical and ACEI were found at concentrations 104 and 140 mg/L, respectively, these values are almost 20 times lower than those in which some degree of toxicity was found.

In comparison to the inedible parts of other fruits such as cholupa (*P. maliformis*), the by-products of gulupa have similar toxicity; however, extracts of yellow passion fruit (*P. edulis* f. *flavicarpa*) have lower toxicity (20–25%) [6], although at lower concentrations. In contrast, the shell of *P. foetida* has been reported to have the toxicity of more than 50%

Table 4: Inhibition of angiotensin-converting enzyme

Determination			Sample					
			Peel			Seed		
			CEP	DFP	HRP	CES	DFS	HRS
% ACEI	Concentration mg/L	213	96.26±2.80	89.09±1.09	93.24±1.02	95.51±8.22	72.00±4.58	94.20±9.24
		107	80.00±4.58	64.71±3.75	87.18±2.64	87.03±4.35	63.51±4.68	78.26±7.53
		53	60.00±4.58	54.29±7.47	76.32±7.89	74.03±7.38	57.45±4.88	64.50±4.13
		27	42.86±6.55	40.74±4.63	55.00±2.55	57.45±3.69	53.12±4.77	51.00±4.58
		13	37.08±2.60	36.84±3.29	38.00±3.00	42.37±5.08	40.91±3.15	40.83±6.61
CI_{50}			29.17±3.60^{ab}	36.23±4.15^{b}	20.07±2.55^{ac}	17.62±2.54^{c}	26,31±6.13^{abc}	23,76±3,51^{ac}

CEP: Crude ethanol extract of peel, DFP: Dichloromethane fraction of peel, HRP: Hydroalcoholic residues of the peel, CES: Crude ethanol extract of seed, DFS: Dichloromethane fraction of seed, HRS: Hydroalcoholic residues of seed, CI_{50}: Concentration needed to reduce *in vitro* 50% of radical species (mg/L), NA: Not applied, a, b, c, d: Different letters equate to statistical significance

Table 5: Percentage of cell viability on human leukocytes

Percentage of cell viability						
Concentration mg/L	Sample					
	Peel			Seed		
	CEP	DFP	HRP	CES	DFS	HRS
500	18.20±1.96^{a}	31.14±0.21^{b}	25.12±1.50^{c}	68.93±6.46^{d}	100.00±4.17^{e}	100.00±21.51^{e}
1000	16.12±0.63^{a}	24.50±0.90^{ab}	22.42±0.42^{a}	39.03±7.48^{b}	55.09±9.91^{c}	98.13±6.75^{d}
2000	15.71±1.56^{a}	18.27±0.55^{a}	16.89±1.02^{a}	38.96±3.84^{b}	UN	64.78±3.22^{c}
4000	15.09±0.48^{a}	15.05±1.03^{a}	13.98±0.86^{a}	35.09±11.10^{bc}	21.66±2.48^{b}	50.14±1.61^{c}

CEP: Crude ethanol extract of peel, DFP: Dichloromethane fraction of peel, HRP: Hydroalcoholic residues of the peel, CES: Crude ethanol extract of seed, DFS: Dichloromethane fraction of seed, HRS: Hydroalcoholic residues of seed, IC_{50}: Concentration needed to reduce *in vitro* 50% of radical species (mg/L), UN: Undetermined, a, b, c, d: Different letters equate to statistical significance

at concentrations well below those used in this study [86]. Conversely, Silva *et al*., 2012, found that an aqueous solution of polysaccharides obtained from *P. edulis*, increased the cell viability of leukocytes as the concentration increased [87].

CONCLUSION

The results show that the agroindustrial residues of *P. edulis* f. *edulis* (peel and seed) are rich in nutrients so that they can be used in biotechnological processes for inclusion in food matrices. The ethanolic extract from seed showed the highest content of phenols, the highest activity against the radicals ABTS•$^{+}$ and DPPH•, the highest reducing power (FRAP), and capacity of radical absorption of oxygen (ORAC) as well as a greater inhibitory activity of the converting enzyme of angiotensin so that it could be proposed to the gulupa seed as a promising herbal product with vasorelaxants, antihypertensive, and antioxidant potentials; all of which would be associated with the phenolic content, especially its flavonoids and hydroxycinnamic acids, such as quercitrin and coumaric acid, respectively. However, studies with greater technological strength and scientific depth are required to clearly know the type and chemical nature of the phenolics that are part of the agroindustrial residues of gulupa. In the same way, the obtained results allow giving an added value to the fruit, reducing the possibilities of its residues contributing to environmental contamination.

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AUTHORS' CONTRIBUTIONS

All the authors made substantial contributions to the conception, design, acquisition, analysis, and interpretation of data.

CONFLICTS OF INTEREST

All authors have none to declare.

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