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Chemical Study and Determination of the Antioxidant Activity of Three Varieties *Tropaeolum tuberosum* (Mashua)

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Abstract

Ecuador has among its foods a number of potatoes and tubers that are not only nutritious, but are also attributed properties as coadjutant in the treatment of different illnesses. The objective of this study was to conduct a comparative study of the mashua tuber (*Tropaeolum tuberosum spp tuberosum* Ruíz & Pavón, Kuntze), in its three pink, yellow and black varieties, by comparing the physicochemical characteristics and antioxidant activity. The comparative study was carried out on tubers obtained in the province of Pichincha, Quito-Ecuador. The physicochemical parameters were determined by the identification of the secondary metabolites present in the hydroalcoholic extracts, the quantification of phenols and flavonoids, and the characterization of the extracts by gas chromatography coupled to mass spectrometry and the antioxidant activity by three test methods. As a result, significant differences were found between the three varieties studied.

Keywords

Antioxidant Activity, Chemical Composition, Gas Chromatography-Mass Spectrometry

1. Introduction

Food and health at a global level are a growing concern between different gov-

ernments looking for a solution without altering local customs in every country. This has led to a search into traditional, natural resources, which may have not been properly distributed, being ignored among Ecuadorian populations [1].

Looking back at these traditional products, they noticed an Andean tuber and investigated its nutritional and pharmacological qualities of ancestral use [2].

Tropaeolum tuberosum Ruíz & Pavón, Kuntze (mashua), a Tropaeolaceae family tuber which grows between 2400 and 4300 m.a.s.l, was discovered in the Andes, covering the countries of Venezuela, Colombia, Ecuador, Perú, Bolivia, Chile and Argentina. It is ranked in fourth of nutritional tubers after potatoes, oca and olluco [3] [4]. On the other side, several studies have shed light upon its medicinal properties to relieve kidney and liver diseases [5], skin eczema, prostate disease and diabetes. These therapeutic properties would be related to the presence of phenolic antioxidants and these due to the high anthocyanins contents [6] [7].

Diversity among mashua tubers comes in differences in shape, color, pulp color, knob characteristics. Skin color varies from white to violet or dark purple, yellow, orange, red, pink, being possible to have one color only or different spots. In general, they have a high protein content, carbohydrates, fiber, ascorbic acid and calories [8].

A study from Campos *et al*. [9], to compare the antioxidant potential of some Andean crops, including colored potatoes, olluco and oca, showed that purple mashua tubers showed the highest antioxidant activity, eight or ten times higher than that of yellow mashua tubers. This superior activity was thought to be related to the high anthocyanin content. Afterwards, Chirinos *et al*. [6] [10] [11] [12], found that not only the mashua anthocyanin where contributing but also the phenols played an important role in the antioxidant power of the tubers. The main anthocyanins found in the different genotypes of mashua are delphinidin di- and acylated triglycosides with acetic acid. Cyanidins and pelargonidins are also present in minor quantities.

Despite these results, the different varieties lack pharmacognostic and phytochemical studies to support the ethnopharmacological uses against urinary, genital diseases, antibiotic, and depressor of the erectile function in men.

The yellow is the variety most commonly used, of better flavor and easiest to obtain, while the pink and black are available only from March to May, or directly from the owners of the crop.

The objective of this work was to determine and compare the physicochemical and antioxidant activity from the tuber *Tropaeolum tuberosum spp tuberosum* (Ruíz & Pavón, Kuntze) in its three varieties to learn more about their similarities and differences.

2. Materials and Methods

2.1. Collection, Drying and Grinding

The collection of different varieties took place at the Pichincha province,

Quito-Ecuador at 3360 m.a.s.l., with the following coordinates: 0˚13'47.5''S 78˚31'29.8''O, on April the 2018. The selection was done according to the size, color and weight, and was washed with a solution of sodium hypochlorite at 1%, then washed with potable water, drained, sliced and dried on a Teknolab SA stove at 50˚C ± 3˚C until the weight was constant. Afterwards, it was grinded in a PULVEX brand knife mill with 2 mm mesh.

2.2. Physical Chemical Parameters and Phytochemical Screening

For the powder of the three varieties, the physical chemical parameters were determined by triple (residual humidity, total ashes, ashes soluble in water and ashes not soluble in chlorhydric acid), by following the procedures described by WHO [13].

The phytochemical screening was done with dried tubers, according to the procedures described by Miranda and Cuellar [14]. An extraction system with a battery of solvents, from minor to major polarity, on the same vegetable material, to achieve that each metabolite was extracted correctly according to the selectivity of each solvent. The samples were extracted with ethyl ether, ethanol and water, to obtain the extracts which were used in the experiments.

2.3. Obtaining the Extracts

From the vegetable material we elaborated hydro alcoholic extracts at 30% of 20g of drug for each 100 mL of solvent, by maceration for seven days. We followed the procedure described by Miranda y Cuéllar [14].

2.4. Physical Chemical Parameters of the Quality of the Extracts

The quality of the extracts was determined by the procedure described by Miranda and Cuéllar [14]. Each experiment was repeated three times, evaluating the following parameters: organoleptic properties, total solids, refractive index, and relative density.

2.5. Quantification of Total Phenols

The total phenol content was determined by the method Folin-Ciocalteu [15] [16] [17] [18]. A dilution of 1:2 (extract: water, v:v) was done. As reference substance we used gallic acid at concentration levels of 10, 20, 30, 40 and 50 mg/100mL and we constructed an absorbance calibration curve against concentration of the patterned mentioned, and from this we determined the concentration of phenols in the extract on mg/mL [**Figure 1**], where a good correlation between the tested concentrations of the reference substance (gallic acid) and absorbances is shown, with a correlation coefficient ≥ 0.99.

2.6. Quantification of Total Flavonoids

The method used was the colorimetric method of aluminum trichloride [15] [19].

A dilution of 1:5 (extract: water, v:v) was performed. As reference substance

we prepared a mother solution of quercetin 10 mg/mL in ethanol at 96%, and from there we prepared solutions of concentrations of 5, 20, 50, 60 and 80 µg/mL. We constructed an absorbance calibration curve for the pattern and determined the flavonoid concentration for the extract expressed in mg/mL. A good correlation was achieved between the tested concentrations of the quercetin pattern and the absorbances, obtaining a correlation coefficient ≥ 0.99; this is a good indicative of the experimental data model [Figure 2].

2.7. Analysis of the Extracts by the Coupled Gas Chromatography-Mass Spectrometry System

Dried samples were mixed with N-Trimethylsilyl-N-methyl trifluoroacetamide (MSTFA), and heated in a water bath to 80˚C for 2 hours to permit the silylation of metabolites [20]. Next, GC-MS analysis was performed in a gas chromatography mass spectrometry equipment Agilent Technologies (7890A GC system and 5975C inert XL MSD with triple axis detector). A capillary column DB-5MS (30 m × 0.25 mm) with phenyl dimethylpolysiloxane was used as stationary phase (0.25-micron film thickness) and helium as the carrier gas (1.2 mL/min). The injection of 1 µL of derivatized sample was performed at 250˚C with splitless mode. The oven temperature was started at 70˚C for 2 minutes, then it was increased to 300˚C at 5˚C/min, and it was maintained at 300˚C for 6 minutes. The compounds identification was done by comparison of mass spectra based on Wiley 9th, and NIST 2011 MS Library. An electron ionization of 70 eV at

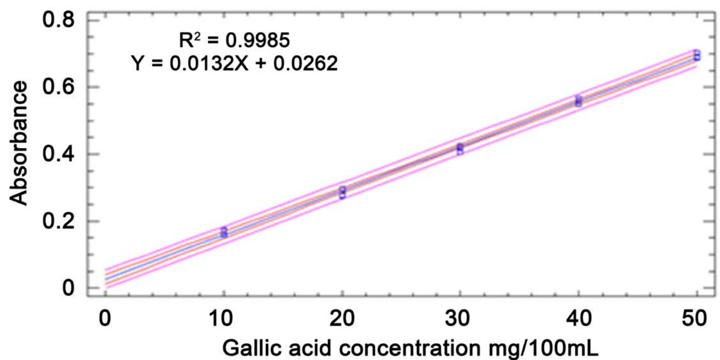


Figure 1. Calibration curve of gallic acid for the determination of total phenols.

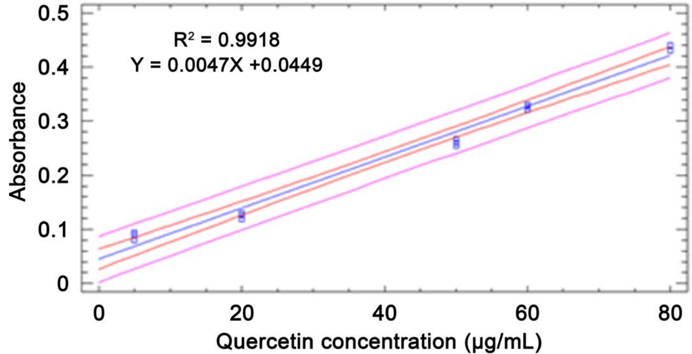


Figure 2. Quercetin calibration curve for the determination of total flavonoids.

230˚C was used in the ion source and the data compounds were collect with the full scan mode (40 - 600 amu) in the quadrupole mass analyzer.

2.8. Determination of Antioxidant Activity

The determination of the antioxidant activity was carried out by three methods: ferro-reducing activity, sequestration capacity of the DPPH radical and percentage inhibition of the ABTS radical.

Antioxidant activity by the FRAP method (*ferro-reducing capacity*): The capacity to reduce hydroalcoholic extracts was measured according to the procedure described by Benzie y Strain [21]. The determinations were spectrophotometric, a UV spectrophotometer was used (Rayleigh UV-1601, Shanghai, China) at an absorbance of 593 nm. The results were expressed as μmol equivalents of ascorbic acid (EAA) and as μmol equivalents of $FeSO_4$, from the calculation interpolating the optical density (OD) of the samples in the calibration curves of both reference substances at concentrations of 50, 100, 200, 400 and 500 μM. The readings were made in triplicate at four minutes.

Sequestration capacity of the 2,2*-diphenyl-*1*-pyrylhydrazyl radical* (*DPPH*): For the quantitative determination the DPPH free radical method was used [22] [23]. A UV-visible spectrophotometer was used and the determinations were measured at 517 nm after 30 min. The results were expressed as percentage inhibition of the DPPH radical.

ABTS test●+ (2,2'*-azino-bis*(3*-ethylbenzothiazoline*)-6*-sulfonic acid*): The test was performed according to the methodology of Re *et al.*, Agudo y Arnao *et al.* [24] [25] [26]. The results were expressed as percentage inhibition of the radical ABTS●+. The mean inhibitory concentration (IC50) was determined with the help of the statistical program Graphprism 5.0.

2.9. Estatistical Analisys

The results of the quality control of the drugs and extracts and the determination of the total phenols and total flavonoids, were processed to calculate the medium values and the standard deviations. The program Statgraphics®Plus, version 5.0 was used with a normality test (Kolmogorov-Smirnov), after which an analysis of variants and for the comparison of the Duncan's stockings.

The statistical program SPSS for Windows version 8.0 was used for the processing and statistical analysis of the results of the antioxidant study. The experimental values were expressed as the mean ± standard deviation (SD). The data was analyzed by one-way ANOVA, followed by a test of multiple comparisons of Tukey means with a $p \leq 0.05$.

3. Results

3.1. Physical Chemical Parapemeters of the Vegetable Material

For the results of the physical chemical parameters the results are shown in [**Table 1**].

All parameter where within the ranges informed by Pharmacopeas.

3.2. Phytochemical Screening of Plant Material

A qualitative analisys to a different variety of mashua was done, in order to determine the secondary metabolites present in each of them and the results are presented in [Table 2].

3.3. Organoleptic Parameters, Physical Chemical, and Phytochemical Screening of the Extracts

With the purpose of determining the quality of the extracts, we did a physical chemical parameter analysis, considering the organoleptic properties (odor and color), pH, total solids, refraction index and relative density; in Table 3 we present the results of the study.

Table 1. Physical-chemical parameters of the powdered drugs of the three varieties of Mashua.

Parameters	Variety		
	Yellow	Pink	Black
Total humidity (%)	92.86/0.04	90.26/0.07	91.77/0.56
Residual humidity (%)	7.09/0.08	6.59/0.10	8.23/0.05
Total ashes (%)	3.9/0.11	3.48/0.20	5.09/0.10
HCl insoluble ashes	0.37/0.10	0.31/0.003	0.15/0.02

Legend: X/S average value/standard deviation, n = 3.

Table 2. Phytochemical screening of the tubers of the three varieties of *T. tuberusum.*

Metabolite	Variety		
	Yellow	Pink	Black
Ethereal extract			
Oils	++	+	++
Alkaloids	+++	–	+++
Lactones	++	–	–
Triterpenes/Steroids	+++	–	++
Alcoholic extract			
Reducing compounds	+++	+	+++
Alkaloids	+++	–	–
Lactones	+++	–	–
Triterpenes/Steroids	+++	–	++
Phenolic compounds	+++	+++	–
Anthocyanins	+	+	+++
Flavonoids	–	–	+++
Aqueous extract			
Alkaloids	+++	–	+++
Phenolic compounds	+++	–	+++
Flavonoids	+	–	–
Reducing compounds	+++	+++	–

Legend: + (positive); – (negative).

3.4. Phytochemical Screening of the Hydroalcoholic Extracts of *T. tuberosum*

In the same way, to the extracts we did a phytochemical screening and the results are presented in [Table 4].

3.5. Total Phenol Content and Total Flavonoid Content of the Hydro Alcoholic Extracts of *T. tuberosum*

Another aspect evaluated to the extracts was the content of total phenols and total flavonoids and the results are presented in [Table 5].

Table 3. Organoleptic characteristics and physicochemical parameters of hydroalcoholic extracts of different varieties of *T. tuberosum.*

Parameters	Results		
	Yellow mashua	**Pink mashua**	**Black mashua**
Organoleptic	Slightly viscous liquid (less than the pink variety), translucent, yellow-orange color, characteristic smell	Viscous liquid Little translucent Yellow color Characteristic smell	Slightly viscous liquid (less than the yellow variety) Little translucent Wine red color Characteristic smell
pH	$3.92/0.02^{b}$	$4.22/0.01^{a}$	$4.06/0.03^{c}$
Total solids (%)	$1.18/0.05^{e}$	$2.02/0.01^{d}$	$2.23/0.06^{f}$
Refractive index	$1.3296/0.0002^{h}$	$1.3308/0.0001^{g}$	$1.3318/0.0002^{i}$
Relative density (g/mL)	$1.0171/0.0006^{k}$	$1.0197/0.0002^{j}$	$1.0221/0.0003^{l}$

Legend: X/S: mean value of the determinations/standard deviation, n = 3. Equal letters in a row show that there are no significant differences (p > 0.05) and different letters than if there are significant differences (p < 0.05) for 95% confidence, according to Duncan.

Table 4. Phytochemical screening of hydroalcoholic extracts of *T. tuberosum.*

Metabolites	Extracts		
	Pink Variety	**Variety amarilla**	**Variety negra**
Alcaloids	–	–	–
Tannins/Phenols	+	+	++
Saponins	+	+	+
Flavonoids	+ Light yellow	+ Intense yellow	++ Red
Reducing substances	++	++	
Mucílage	++	+	+
Bitter and astringent principles	–	–	–

Legend: + possitive test; ++ very possitive test; – negative test; /doubtful test.

Table 5. Content of total phenols and total flavonoids in hydroalcoholic extracts of *T. tuberosum.*

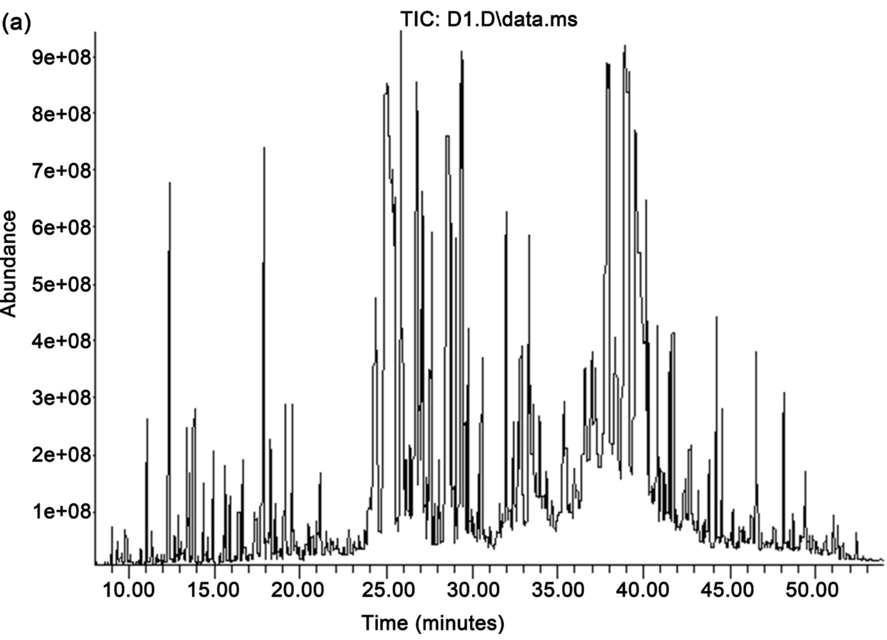
Extracts	Total phenols mg/mL $\bar{X} \pm DS$	Total flavonoids mg/mL $\bar{X} \pm DS$
Yellow variety	$0.48/0.01^{b}$	$0.19/0.005^{b}$
Pink variety	$0.31/0.01^{a}$	$0.28/0.01^{a}$
Black variety	$0.66/0.01^{c}$	$0.10/0.003^{c}$

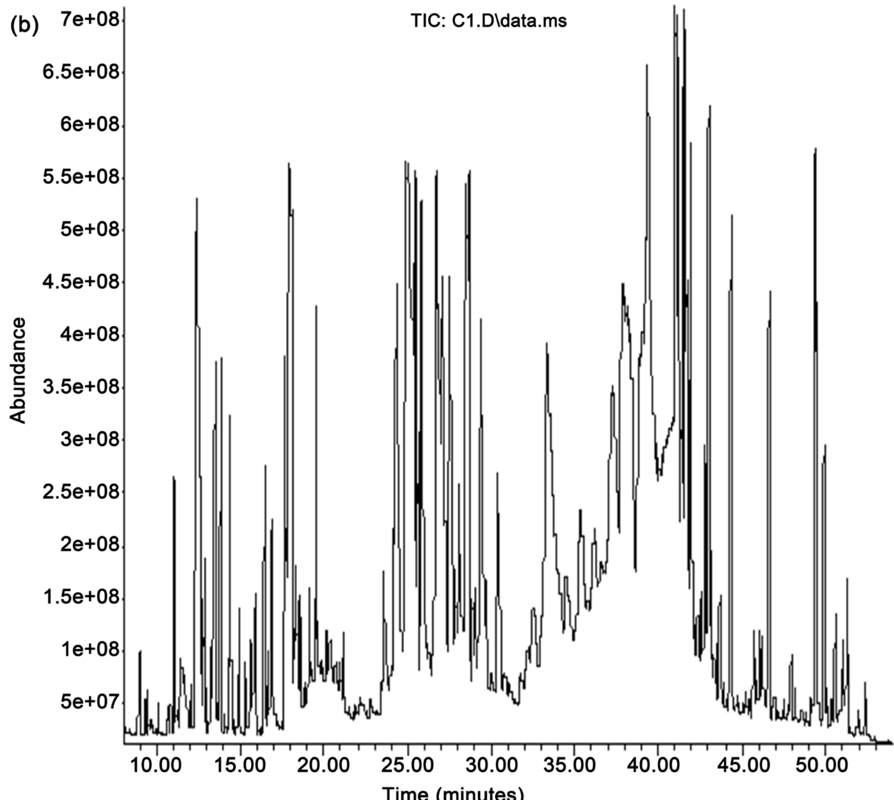
Legend: $\bar{X} \pm DS$: mean value of the determinations/standard deviation for n = 3. Equal letters show that there are no significant differences ($p > 0.05$) and different letters than if there are significant differences ($p < 0.05$) for 95% confidence, according to Duncan.

3.6. Analysis of the Extracts by Gaseous Chromatography Coupled System-Mass Spectrometry

The chromatograms from the hydro alcoholic extracts of the different varieties are presented in [Figure 3].

The components to which structures were assigned by comparison of their mass spectra with those of the equipment library are indicated in [Table 6].





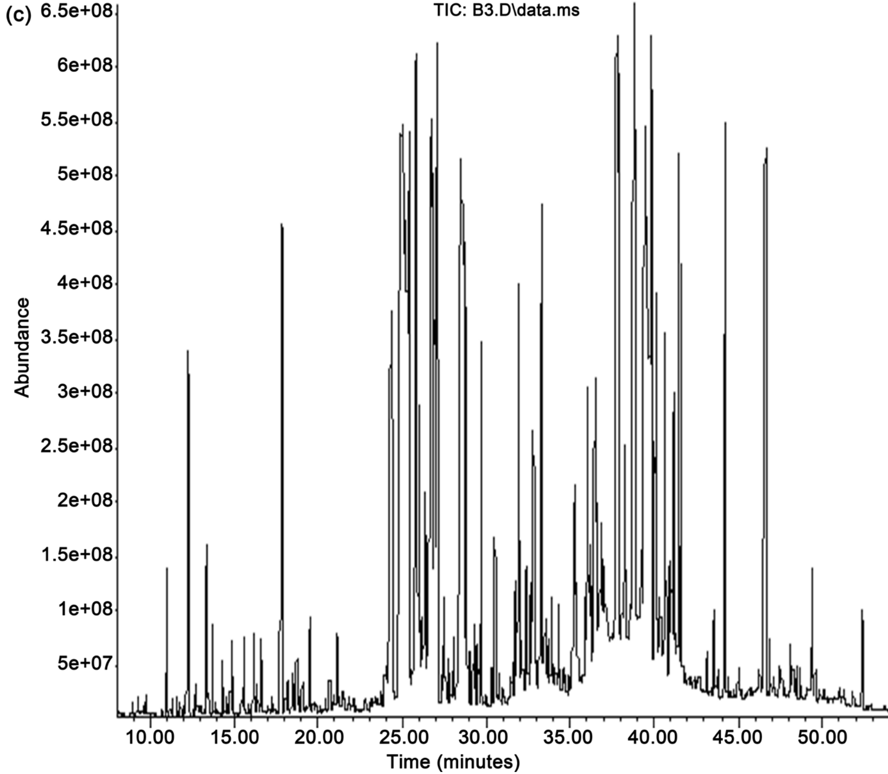


Figure 3. Chromatographic profile of ethanolic extracts of yellow (a), pink (b), and black (c) *Tropaeolum tuberosum.* Ruíz & Pavón, Kuntze, (mashua).

3.7. Antioxidant Activity of the Extracts

The antioxidant activity of plant extracts depends greatly on its composition and the test system, so it is necessary to perform more than one type of test to measure it and thus have a measure of the mechanism of antioxidant action [27]. That is why in this work several methods were used for this analysis, among which are FRAP, DPPH and ABTS. The results achieved are presented in [**Table 7**].

4. Discussion

4.1. Physicochemical Parameters and Phytochemical Screening of Raw Drugs

Regarding the physicochemical parameters [**Table 1**], the black mashua had the highest residual moisture value and total ash, and the lowest value of insoluble ashes in hydrochloric acid, which is indicative that the minerals present are of the alkaline and alkaline earth type. The pink variety had the lowest percentage of moisture and total ashes. According to NRC [28] and Monteros [29], among the minerals, the most concentrated are phosphorus and magnesium, followed by zinc and iron with low contribution in Na and its high content in K.

The phytochemistry of the *T. tuberosum* species has been studied for several decades. However, in compiled literature, comparative studies between tubers of different colors are missing.

Table 6. Compounds identified by CG-EM in the hydroalcoholic extracts of the three varieties of Mashua.

Peak	Retention time (min)	Compounds yellow variety	Abundance/S (%)
1	11.805	Serine	0.69/0.00
2	13.747	propanoic acid	4.11/0.00
3	14.545	Nonanoic acid	1.37/0.10
4	15.14	Treonine	0.34/0.00
5	16.136	1-Aspartic acid	6.85/0.01
6	21.12	Arabinonic acid	8.90/0.01
7	21.411	D-(-)-Ribose	2.06/0.00
8	23.369	Arabinonic acid	2.74/0.01
9	28.473	glucitol	5.14/0.01
10	27.788	D-(+)-talofuranose	4.45/0.00
11	30.323	Myo-Inositol	5.48/0.00
12	31.483	Heptadecanoic acid	4.80/0.04
13	32.625	Linoleic acid	13.36/0.01
14	32.751	Linolenic acid	11.30/0.00
15	33.511	*A*-D-Galactofuranose-(-)-fructuose	8.90/0.00
16	34.904	Cholesta-7.9(11)-dien-3-ol	4.795/0.00
17	43.135	D-Glucopyranose	8.56/0.00
18	51.271	Palatinose	4.80/0.00
Peak	**Retention time (min)**	**Compounds pink variety**	**Abundance/S (%)**
1	18.782	Arachidonic acid	6.49/0.02
2	21.504	α-linolenic acid	6.25/0.05
3	21.883	cis-5,8,11-eicosatrienoic acid	4.06/0.03
4	21.755	Estearic butil ester acid	3.25/0.01
5	22.011	N-mirystoil l-glicine	2.84/0.01
6	29.699	Palmític acid	34.90/0.02
7	31.448	3β-$5\alpha11\beta$-androstane3,11-diylbisoxy	10.55/0.03
8	35.802	β-dl-lyxopyranose	31.66/0.00
Peak	**Retention time (min)**	**Compounds black variety**	**Abundance/S (%)**
1	11.788	3-buten-1-ol	0.94/0.00
2	11.864	Serine	1.41/0.00
3	14.580	Nonanoic acid	3.76/0.00
4	14.821	2(3H)-Furanona	3.14/0.00
5	15.722	Nootkatone	1.88/0.00
6	16.518	Arachidonic acid	0.65/0.46
7	19.243	Alpha linoleic acid	1.41/0.00
8	23.381	Xylonic acid	15.05/0.00
9	27.391	Glucitol	15.05/0.01
10	32.665	9,12-Octadecanoic acid	44.21/0.01
11	48.552	β-Sitosterol	10.82/0.00

*Data are expressed as mean values/standard deviation (n = 3).

In this work some differences were found between the three varieties studied, mainly in the intensity of the colorations [**Table 2**]; the pink mashua was the one that gave negative results for most of the metabolites tested. The presence of fatty compounds in the tubers has been reported by Simopoulos [30], which points out the presence of linoleic and *α*-linoleic acids as the most abundant, in this work the fatty compounds were more abundant in the yellow and pink varieties. On the other hand, Albado *et al.* [31], has reported the presence of erucic acid in *T. tuberosum* in a percentage of 4.46% and 5.91% of dry tubers of two different samples using gas chromatography. The presence of triterpenoids was also detected, which were more abundant in the yellow variety and somewhat less in the black variety; these metabolites were reported by Chasquibol-Silva *et al.* [32], using the test of Lieberman-Burchard. Almagro [33] points out that the Lieberman-Buchard test does not allow to know the exact chemical structure of the molecules, nor the nature and conformation of the substituents. In addition, there are no other reports on the presence of phytosterols in *T. tuberosum*. Therefore, he considers that it would be interesting to study the chemical composition of phytosterols in the species to find out if they are responsible for some of the traditional uses such as anti-inflammatory, anti-diabetic and anti-cancer. The phenolic compounds were the metabolites that had greater intensity in the alcoholic extract for the yellow and pink varieties and in the aqueous extract for the yellow and black varieties. On the other hand, in the black mashua had greater intensity in the anthocyanidin test, these results are consistent with that reported by Chirinos [6] [11] [12], Campos [9], Tsao [34] y Tena & Apaza [35]. The alkaloids gave positive results for the black and yellow varieties in the three types of extracts tested, which may be due to the presence of nitrogen compounds such as the alkamines reported by Zhao [36] and Apaza *et al.* [37].

4.2. Organoleptic Parameters, Chemical Physicist and Phytochemical Screening of Extracts

Once the extracts of the three varieties were obtained, their organoleptic and physicochemical characteristics were established [**Table 3**]. The main deference found in the organoleptic analysis was the coloring of the extracts. In relation to the physical parameters, all presented acidic characteristics with a lower pH for the yellow variety, which also presented the lowest amount of total solids. There were significant differences in all the parameters studied for the three varieties. For the phytochemical screening some differences were obtained compared with the drug extract, mainly in the presence of alkaloids, which may be due to the concentration of these product of solubility [**Table 4**].

Total phenol content and total flavonoids in hydroalcoholic extracts of T. tuberosum

The statistical analysis of the results allowed to verify significant differences in the content of total phenols and total flavonoids in the evaluated extracts [**Table 5**], the highest concentration of total phenols was presented by the extract from the black variety, while the pink variety differed by presenting greater total fla-

vonoid content.

4.3. Analysis of the Extracts by the Coupled Gas Chromatography-Mass Spectrometry System

The analytical gas chromatograms of the different extracts were very complex, presenting numerous high intensity chromatographic peaks [Figure 3]. However, 18 compounds for the yellow variety, 8 for the pink variety and 12 for the black variety were identified by comparison of their mass spectra with those of the equipment library [Table 6]. The presence of acidic compounds, sugars and sterols stand out in all extracts. The yellow variety was the one with the highest number of acidic components (4), which reaffirms the lowest pH value found for the extract, also presented 7 compounds of a glycosidic nature, two amino acids and a steroid (derived from androstane), which confirm the results of phytochemical screening. The fatty acids found correspond to those reported in the literature [30]. In the pink variety, five fatty acids, one sugar, one carboxylic acid and one sterol (dehydro-cholesterol) were assigned, while in the black variety four acidic compounds, one amino acid, three sugars, two fatty acids and one sterol (dehydro-cholesterol).

In all the varieties studied, the presence of linoleic and linolenic fatty acids was found, but quantitative differences were found in their concentrations depending on the variety, Ramollo [38], also reported, the one with the highest content in fatty compounds being the yellow variety.

4.4. Antioxidant Activity of the Extracts

The results of the evaluation of the antioxidant activity by the three methods tested [Table 7], are analyzed below.

For the ferro-reducing activity of the three extracts evaluated, concentration dependent antioxidant activity was evidenced. At the lowest concentration tested, there were no significant differences between the extracts, however, from 80 μg/mL the black mashua extract showed the highest equivalent μM values of ascorbic acid and $FeSO_4$. The greatest capacity to reduce Fe^{3+} was manifested at the highest concentration evaluated.

The results allowed us to suggest that the three extracts have antioxidant activity, which translates into the equivalent μM values expressed as a function of the reference substances tested.

In the DPPH radical inhibition test against the concentration for each extract and reference substances tested, there is a tendency to increase the inhibition capacity of said radical as the concentration increases, the antioxidant activity being higher for the reference substances Vitamin C and Trolox, however, all three extracts were also able to inhibit this radical.

As can be seen in Table 7, there were significant differences in the percentages of DPPH radical inhibition between the extracts and in comparison with the reference substances at the same concentration. The highest values were evidenced

Table 7. Antioxidant activity of extracts of *Tropaeolum tuberosum* Ruiz & Pav. Kuntze.

Ferro-reducing activity of the extracts of *Tropaeolum tuberosum* Ruiz & Pav. Kuntze						
Conc. μg/mL	Ferro-reducing activity ± SD					
	μM equivalents of ascorbic acid			μM equivalents of $FeSO_4$		
	Pink mashua	Yellow mashua	Black mashua	Pink mashua	Yellow mashua	Black mashua
40	66.38 ± 3.85^{a}	74.16 ± 6.66^{a}	74.72 ± 9.18^{a}	58.90 ± 3.29^{b}	65.57 ± 5.71^{b}	66.04 ± 7.86^{b}
50	69.72 ± 3.47^{c}	88.61 ± 6.73^{cd}	99.72 ± 17.02^{d}	62.71 ± 4.29^{e}	77.95 ± 5.77^{ef}	87.47 ± 14.59^{f}
80	81.94 ± 12.94^{g}	140.28 ± 66.77^{g}	263.61 ± 34.21^{h}	72.23 ± 11.09^{i}	92.23 ± 12.14^{i}	227.95 ± 29.32^{j}
100	91.94 ± 12.72^{k}	190.83 ± 29.05^{l}	374.16 ± 16.91^{m}	80.80 ± 10.91^{n}	165.57 ± 24.90^{o}	326.04 ± 16.49^{p}
150	115.27 ± 15.12^{q}	270.83 ± 12.58^{r}	474.71 ± 15.03^{s}	100.80 ± 12.96^{t}	220.80 ± 15.80^{u}	408.90 ± 12.88^{v}

Conc. μg/mL	Radical Kidnapping Percentage DPPH (%) ± SD				
	Pink mashua	Yellow mashua	Black mashua	Vitamin C	Trolox
50	19.82 ± 2.01^{a}	31.99 ± 3.48^{b}	47.37 ± 3.96^{c}	85.31 ± 0.76^{d}	83.75 ± 0.86^{e}
100	30.74 ± 0.79^{f}	48.80 ± 2.63^{g}	53.12 ± 1.04^{h}	87.76 ± 1.09^{i}	86.42 ± 0.88^{i}
150	38.64 ± 5.54^{j}	55.31 ± 5.50^{k}	75.22 ± 2.18^{l}	88.62 ± 1.07^{m}	87.65 ± 0.78^{m}
200	60.04 ± 2.71^{n}	66.03 ± 6.00^{o}	77.58 ± 1.41^{p}	89.38 ± 0.61^{q}	88.70 ± 0.94^{q}
250	63.10 ± 2.48^{r}	75.45 ± 1.54^{s}	83.81 ± 0.86^{t}	90.40 ± 0.93^{u}	90.01 ± 0.54^{u}
Conc. μg/mL	Radical Kidnapping Percentage ABTS•+ (%) ± SD				
	Pink mashua	Yellow mashua	Black mashua	Vitamin C	Trolox
500	21.10 ± 1.54^{a}	33.89 ± 1.76^{b}	41.87 ± 1.79^{c}	86.38 ± 1.42^{d}	84.49 ± 0.65^{d}
600	23.92 ± 1.63^{e}	40.20 ± 2.40^{f}	56.15 ± 1.80^{g}	89.75 ± 1.24^{h}	89.10 ± 0.78^{h}
700	57.82 ± 1.45^{i}	63.94 ± 2.87^{j}	70.32 ± 2.20^{k}	91.42 ± 1.23^{l}	90.87 ± 1.20^{l}
800	62.64 ± 1.53^{m}	70.92 ± 1.81^{n}	84.60 ± 1.67^{o}	93.43 ± 1.11^{p}	91.79 ± 1.41^{p}
900	70.17 ± 1.40^{q}	76.48 ± 1.35^{r}	87.86 ± 1.76^{s}	94.47 ± 1.06^{t}	94.54 ± 1.02^{t}
	Inhibitory concentration media (IC_{50})				
	669.40 ± 0.85	667.05 ± 0.78 ^{a}	657.55 ± 2.33 ^{ab}	645.20 ± 11.03^{b}	652.25 ± 13.79^{ab}

The mean (n = 3) ± standard deviation (SD) is indicated. Different letters in a row indicate significant differences and equal letters that there are no significant differences, at the same concentration ($p \le 0.05$), according to the Tukey multiple comparison test.

for vitamin C and trolox, followed by black mashua extract. However, the extracts of the pink and yellow variety also showed good antioxidant activity with percentages greater than 60% from 200 μg/mL.

An important aspect to consider is the determination of the IC_{50} (concentration value at which 50% inhibition of the maximum DPPH sequestration effect is reached), in this regard, there were no significant differences between yellow and black mashua extracts with respect to the two reference substances, which indicates a similar antioxidant behavior, an aspect that denotes the high antioxidant power of these extracts.

The percentages of inhibition of the ABTS radical, of the extracts, showed a high sequestration capacity (greater than 55%) from 700 μg/mL, being higher for

the black variety extract. The statistical analysis of the results allowed to detect significant differences between the extracts at the same concentration with respect to the reference substances, which reached the highest percentages.

The analysis of the IC_{50} (concentration value at which 50% inhibition of the maximum abduction effect of ABTS is reached) allowed to verify a similar behavior between the three extracts with the trolox pattern and that of the black variety also with vitamin C, which translates into a good sequestration ability and therefore a high antioxidant activity by the mechanism of action evaluated.

Considering the results of the three in vitro test methods, it was found that as the concentration of the extracts increased, the reducing power (FRAP test) and the anti-radical activity (DPPH and ABTS tests) of the extracts increased, manifesting a high antioxidant activity, although lower than the reference substances used. Of the three extracts evaluated, the one from the variety of black mashua was the one that showed the greatest antioxidant capacity by the three methods tested.

For the antioxidant activity of mashua extracts, there is information in the literature, both for raw and purified extracts, with differences between them. The antioxidant activity recorded in purple tubers may be mainly due to anthocyanins [39] [40], which are the best natural source of pigments.

The antioxidant activity of the yellow tubers of *T. tuberosum* may be due to phenolic derivatives (flavonoids, anthocyanins) and hydroxycinnamic acids generated by the hydrolysis of glucosinolates [41].

Oxidative stress is considered an important contributor to the pathogenesis of several chronic diseases, and for this reason antioxidant behavior is one of the most commonly determined biological activities in plant extracts. A wide variety of antioxidant assays are used to determine the activity of plant extracts, two of which are based on the elimination of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) (DPPH assay) and the activity potential of iron reduction (FRAP trial) [42].

Many use the DPPH and FRAP assays in their plant activity detection programs, presumably, assuming that a combination of the data would provide a better description of the antioxidant activity than that obtained from a single test [42].

The three methods used (DPPH, FRAP and ABTS) for the evaluation of the antioxidant activity of hydroalcoholic extracts of *Tropaeolum tuberosum* were spectrophotometric methods.

The DPPH test is widely reported [43] [44] [45] for the determination of antioxidant activity, it is not a very tedious method in terms of chemical preparation and implementation of the test and, therefore, can be used for its operational simplicity. The radical used (DPPH) is one of the few stable and commercially available organic nitrogen radicals [45] [46].

In the present study, the antioxidant activity of each sample was measured several times to test the reproducibility of the assay. The only disadvantage of

this test is that it is not very profitable and is not suitable for measuring the antioxidant capacity of plasma, because proteins precipitate in the alcoholic reaction medium [45] [47]. As a result, this inconvenience was not appreciated because the extracts were hydroalcoholic and soluble in the alcoholic medium used.

The FRAP test is more tedious and time consuming in terms of preparing the chemicals in the work solution. It is a simple and economical method and does not require the use of any exclusive chemical. The results obtained are reproducible for all concentrations. Therefore, FRAP is a suitable method for the determination of antioxidant activity.

The ABTS test uses ABTS radicals performed by oxidation thereof with potassium persulfate. Therefore, this analysis takes a long time in terms of waiting for ABTS radicals to be generated, since it takes about 12 - 16 hours for the reaction to take place, unlike the DPPH test where you do not have to wait for the radicals to be generated. However, once the radicals are generated, it is a very simple test in terms of their realization. The ABTS radical is soluble in water and organic solvents, which allows the determination of the antioxidant capacity of hydrophilic and lipophilic compounds/samples [45]. A major drawback of this test is that the radicals formed are not very stable and the results are sometimes not reproducible. However, this assay is also widely reported for the measurement of antioxidant activity.

The synergism between the antioxidants in a mixture causes that the antioxidant activity depends not only on its concentration but on the interaction between them. The antioxidant capacity of an extract is not only given by the sum of the antioxidant capacities of each of its components, it also depends on the microenvironment in which they are found. The compounds interact with each other and may produce synergistic or inhibitory effects [48].

5. Conclusions

Significant differences were found in the physical chemical parameters as well as in the intensity of the coloration of the qualitative tests between the three varieties of mashua studied.

The highest concentration of total phenols was presented by the extract from the black variety, while the pink variety differed by presenting greater total flavonoid content.

Differences were also detected in the chemical components identified by GC-EM for the three varieties.

It was found that as the concentration of the extracts increased, the reducing power (FRAP test) and the anti-radical activity (DPPH and ABTS tests) of the extracts increased, manifesting a high antioxidant activity, although lower than the reference substances used. Of the three extracts evaluated, the one from the variety of black mashua was the one that showed the greatest antioxidant capacity by the three methods tested.

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Authors' Contribution

MEJ, worked on the experimental design of the work and on its writing. YGG and MMM, performed the results processing and collaborated in the writing of the work. IChG, performed the chromatographic analysis and participated in the review of the work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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