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Pharmacognostic, Chemical and Anti-inflammatory Activity Study of Two Varieties of *Tropaeolum tuberosum* (Ruiz & Pav.) Kuntze (Tropaeolaceae)

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Abstract: Ecuador has among its foods a series of potatoes and tubers that are not only nutritious, but also contain properties such as adjuvants which aid in the treatment of diseases caused by an inflammatory state. However, there is a lack of scientific evidence to support these hypotheses. The objective of this study was to carry out a comparative study of two varieties of the mashua tuber (*Tropaeolum tuberosum spp tuberosum* (Ruíz & Pavón, Kuntze)), by analyzing the pharmacognostic, chemical and anti-inflammatory characteristics. Since there are no previous reports regarding the macro- and micromorphological characteristics of the tubers, the following analyses were conducted; the establishment of physical-chemical parameters, the qualitative determination of the secondary metabolites, the quantification of phenols as well as flavonoids and the characterization of the extracts of ethyl acetate, ethyl alcohol and the coupling of the system through gas chromatography-mass spectrometry. Significant differences were found in the macro- and micromorphology, as well as in the secondary metabolite between the two varieties studied. The black mashua showed a higher concentration quantification of phenols and flavonoids. Some phytosterols and triterpenoids were reported for the first time in these species and the anti-inflammatory activity of the tubers was demonstrated. These findings can lead to further research for a future use in a clinical trial.

Keywords: Anti-Inflammatory, Chemical Composition, Macro and Micromorphology

1. Introduction

The misuse of products intended to combat inflammatory processes have become the cause of the emergence of other health problems that complicatethe health landscape of countries worldwide.

Inflammation is a result of a series of chronic degenerative diseases; therefore, the world scientific community has turned to nature for substances that contribute to the improvement of the symptoms of inflammatory processes, with less side effects.

Among the food products used since ancient times in Ecuador, there are severalplants rich in antioxidant substances that have been granted properties to combat degenerative and often catastrophic diseases [1]. Many of these plants do not have studies that endorse their properties, thusit is necessary to provide scientific support to everything that is said to be ancestral.

Tropaeolum tuberosum (Ruiz & Pav.) Kuntze, (mashua), tuber of the Tropaeolaceae family that grows between 2400 and 4300 masl, was discovered in the Andes, covering the

countries of Venezuela, Colombia, Ecuador, Peru, Bolivia, Chile and Argentina. It occupies the fourth position in the ranking of nutritional tubers after potatoes, oca and olluco [2, 3]. On the other hand, several studies have reported medicinal uses to relieve kidney and liver ailments, skin eczema, prostate diseases and diabetes, these therapeutic properties would be related to the presence of phenolic antioxidants due to the high content of anthocyanins [4-7].

The diversity of mashua tubers is indicated by the shape, color, characteristics of the yolks and pulp color. The skin varies in shades ranging from white, dark purple, yellow, orange, red and pink with either a single tone or spots. In general, they have a high content of proteins, carbohydrates, fiber, ascorbic acid and calories [8].

The objective was to carry out a comparative study between two varieties of mashua, covering the pharmacognostic, chemical and pharmacological aspects.

2. Materials and Methods

2.1. Collection, Drying and Milling

The tubers of the different varieties were collected in April 2019, in the province of Pichincha, Quito-Ecuador at 3360 masl, with the following coordinates: 0° 13'47.5 " S 78° 31'29.8 " O. The selection of tubers was carried out according to size, color and weight, washed with a 1% sodium hypochlorite solution, then rinsed with drinking water, drained, chopped and dried in Tecno stove Lab. SA at 50 ± 3°C until constant weight. Subsequently, milling was carried out in a Pulvex knife mill with a particle size of 2 mm.

2.2. Pharmacognostic Study

2.2.1. Macromorphological Characterization

The macromorphological characterization of the tuber was done before grinding it, with the help of a magnifying glass; the organoleptic characteristics, shape and dimensions of the different tubers were evaluated.

2.2.2. Anatomical Morpho Characterization

For histological analysis, cross-sections of the tubers were made in the fresh state, by the manual method, which were hydrated and rinsed with 1% sodium hypochlorite. They were colored with 1% safranine in water, fixed with glycerinated gelatin. Histochemical reaction was performed with the Lugol reagent for starch determination [9, 10].

To visualize the different internal anatomical characters of the plant, a NOVEL microscope (10X lens) with a model-coupled camera was used HDCE-50B.

2.2.3. Physicochemical Parameters

The physicochemical parameters were determined in triplicate (residual humidity, total ashes, water soluble ashes and insoluble ashes in hydrochloric acid), at the tubers powder of the two varieties as the procedures described by the WHO [11].

2.2.4. Phytochemical Screening

Phytochemical screening was performed on dry tubers, according to the procedure described by Miranda and Cuéllar [10]. An extraction system with a battery of solvents, of increasing polarity, was used on the same plant material, so that each metabolite was suitably extracted according to its selectivity by the solvent used. The samples were extracted successively with ethyl ether, ethanol and water, to obtain the corresponding extracts, which corresponded to the different tests.

2.3. Obtaining the Extracts

From the plant material, successive extractions were carried out by maceration, with solvents of increasing polarity (hexane, dichloromethane, ethyl acetate and ethanol), at a rate of 20g of drug per 100 mL of each solvent, for a period of seven days to each extraction, after the established time the volume was completed. Only ethyl acetate and ethanol extracts were considered for this study resemblingthe procedure described by Miranda and Cuéllar [10].

2.4. Total Phenols and Flavonoid Quantification

Were determined to the ethanolic extract according to the Folin-Ciocalteu method and by the colorimetric method of aluminum trichloride respectively [12–16] (table 1).

Table 1. *Working conditions for the quantification of phenols and flavonoids.*

	Quantification	
	Phenols	**Flavonoids**
Method	Folin-Ciocalteu	Colorimetric of aluminum trichloride
Dilution extract: water v: v	1:2	1:5
reference substance	gallic acid	quercetin stock solution
concentration	10, 20, 30, 40 and 50 mg/100 mL	5, 20, 50, 60 and 80 µg / mL (in 96% ethanol)
Absorbance calibration curve	against the standard concentration of the concentration of phenols/flavonoid in the extract expressed in mg/mL	

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

Extract ethyl acetate: gas chromatography mass spectrometry equipment Agilent Technologies (7890A GC system and 5975C inert XL MSD with triple axis detector). A capillary column HP-5MS (30 m × 0.25 mm) with phenyl methyl polysiloxane was used as stationary phase (0.25 micron film thickness) and helium as the carrier gas (0.9 mL/min). The injection of 0.5 µL of sample was done at a temperature of 250°C with splitless mode, the detector temperature was 280°C and the oven temperature was

maintained at 50°C for 0.5 minutes, then it was increased to 250°C at 4°C/min. The electron ionization to 70 eV and 230° C was used as ion source and the data compounds were collected with the full scan mode (40-1000 uma) in the quadrupole mass analyzer. Subsequently, compounds were identified by comparison of their mass spectra and mass reference of Wiley 9th with NIST 2011 MS Library.

Extract ethanolic: Dried samples were mixed with N-Trimethylsilyl-N-methyl trifluoroacetamide (MSTFA) and heated in a water bath to 80°C for 2 h to permit the silylation of metabolites [17]. Next, Gas Chromatography-Mass Spectometry (GC-MS) analysis was performed in a GC-MS equipment of the brand 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 the ninth version of the software Wiley, and NIST 2011 MS Library. An electron ionization of 70 eV at 230°C was used in the ion source and the data compounds were collected with the full scan mode (40-600 amu) in the quadrupole mass analyzer.

2.6. Anti-inflammatory Activity

The animal model used in this test were female Wistar albino rats, from CENPALAB (National Center to produce Laboratory Animals) with their corresponding quality certificates that guaranteed their health being able to perform this type of assay. The weight of the rats ranged from 160 to 200g. The room temperature was 20 ± 3°C, Relative Humidity: 30 - 70% ± 5%, light / dark cycle: 12/12 h. Water and food were supplied "ad libitum". The food was withdrawn 24 hours before the start of the trial, and they had access to water.

Four treatment groups were made with six animals each as shown in table 2.

Table 2. *Test groups for anti-inflammatory activity.*

Group		Carrageenan (3% aqueous solution)	Quantity	Test agent
Control	1 (-)	0,3 ml	10 mL/kh	NaCl (0,9%)
	2 (+)	0,3 ml	10 mg/kg	Indomethacin
Experimental	3	0,3 ml	500 mg/kg	T. tuberosum yellow
	4	0,3 ml	500 mg/kg	T. tuberosum black

All animals received an exact dosage according to the weight and route of administration used [18].

Initially the normal volumes of the right hind leg of the rats were measured with the use of a digital plethysmometer (Panlab, Spain). Subsequently, the test compounds were administered orally using an intragastric cannula. After 30 minutes, 3% aqueous carrageenan solution was administered in the right plantar aponeurosis of all animals. The volumes of the inflamed leg were measured at 1, 2, 3 and 5 hours after carrageenan administration. The difference between the initial value and subsequent readings for each study time showed the volume of edema. The percentages of inflammation inhibition were calculated by the following expression [19]:

$$\% \text{ inhibition} = Vc - Vt / Vc$$

Where:

Vc=mean value of edema volume of animals in the negative control group

Vt=mean value of the edema volume of the animals in the group treated with the test substance.

At the end of the test, the animals were sacrificed using a saturated ether atmosphere, always considering the refinement techniques currently proposed for testing experimental animals.

2.7. Ethical Considerations

All the procedures and the manipulation of the animals, were carried out following the ethical principles that govern animal experimentation, guaranteeing their welfare and protection, complying with the instructions recommended in the International Guidelines [20].

2.8. Statistical Analysis

Pharmacognostic and Chemical Study: Experiments were carried out in triplicate for each sample and data were expressed as means ± standard deviation. Differences were evaluated by the one-way analysis of variance (ANOVA), and comparison among means was determined according to Tukey test with 5% significance using InfoStat statistical software, version 2008 [21].

Pharmacology activity: Data was expressed as arithmetic mean / standard deviation. One-way ANOVA analysis was used was to determine if there was a statistically significant difference for the variable evaluated and then Kruskal-Wallis was applied, followed by the Friedman test. The level of significance set was $P<0.05$. The data obtained in each trial were processed using the SPSS statistical package for Windows version 8.0.

3. Results

3.1. Macro Morphological Analysis

Figure 1 shows the photograph of the tubers being studied and in Table 3, the results obtained for their macromorphological parameters are reflected.

(A) mashua yellow variety (B) mashua black variety.

Figure 1. *Macromorphological characteristics of the two mashua species.*

Table 3. *Macromorphological parameters of the two varieties of T. tuberosum tubers.*

Parameter	Variety	
	Yellow	Black
Length (cm)	11.30/0.20	10.92/073
Width (cm)	3.93/0.04	4.01/0.14
Weight (g)	45.95/7.60	43.55/3.95

Legend: X / S average value / standard deviation.

3.2. Morfo Anatomic Analysis

Figure 2 shows the histological sections of the fresh drugs of the two varieties (A, B, C and D) and the analysis of the drug powder (E, F).

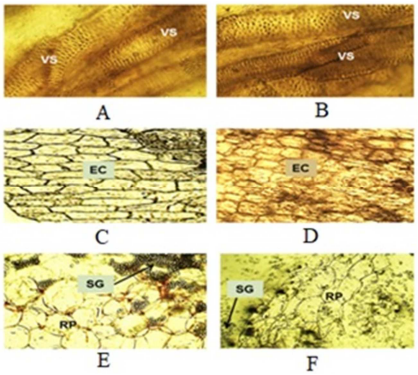


Figure 2. *Microscopic characteristics of T. tuberosum tubers.*

Legend: VS: vascular sistem, EC: epidermal cells, RP: reserve parenchyma, SG; starch grains.
A (yelow variety), B (black variety): scaleriform and pitted xylem vessels C (yelow variety), D (black variety): epidermal cells E (yelow variety), F (black variety): reserve parenchyma and starch grains.

3.3. Physicochemical Parameters of Plant Material

For the physical chemical parameters the results are shown in Table 4. All parameters were found within the ranges reported by the Pharmacopoeias.

Table 4. *Physico-chemical parameters of the two powdered drugs of Mashua varieties.*

Parameters	Yellow	Black
Total humidity (%)	92.86/0.04	91.77/0.56
Residual humidity (%)	7.09/0.08	8.23/0.05
Total ashes (%)	3.9/0.11	5.09/0.10
HCl insoluble ashes (%)	0.37/0.10	0.15/0.02

Legend: X / S average value / standard deviation.

3.4. Phytochemical Screening of Plant Material

For the different varieties of mashua, a qualitative analysis was performed, to determine in a preliminary way the secondary metabolites present in each of them and the results are shown in the Table 5.

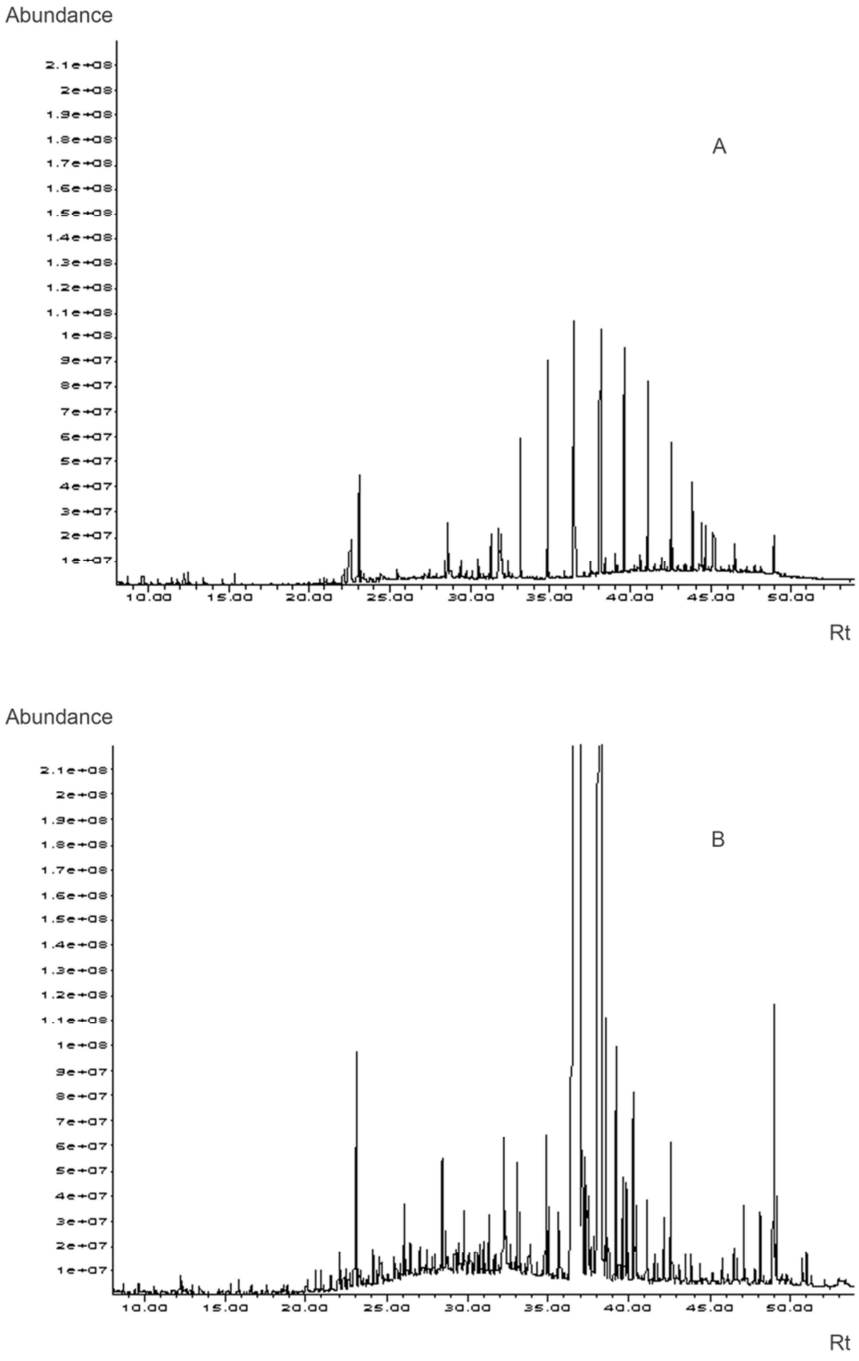
Table 5. *Phytochemical screening of the two varieties of T. tuberusum.*

Metabolite	Variety	
Ethereal extract		
	Yellow	Black
Oil	++	++
Alkaloids	+++	+++
Lactones	++	-
Triterpenes / Steroids	+++	++
Alcoholic extract		
Reducing compounds	+++	+++
Alcaloids	+++	-
Lactones	+++	-
Triterpenes / Steroids	+++	++
Tanins	+++	-
Anthocyanins	+	+++
Flavonoids	-	+++
Aqueous extract		
Alcaloids	+++	+++
Tanins	+++	+++
Flavonoids	+	-
Reducing compounds	+++	-

Legend: + slightly positive essay; ++ positive test; +++ very positive test; - negative test.

3.5. Content of Total Phenols and Total Flavonoids in Alcoholic Extracts of T. tuberosum

Another aspect evaluated was the content of total phenols and total flavonoids and the results are presented in Table 6.



(A) Yellow variety (B) Black variety.

Figure 3. *Analytical gas chromatogram of the ethyl acetate extract of the two varieties of mashua.*

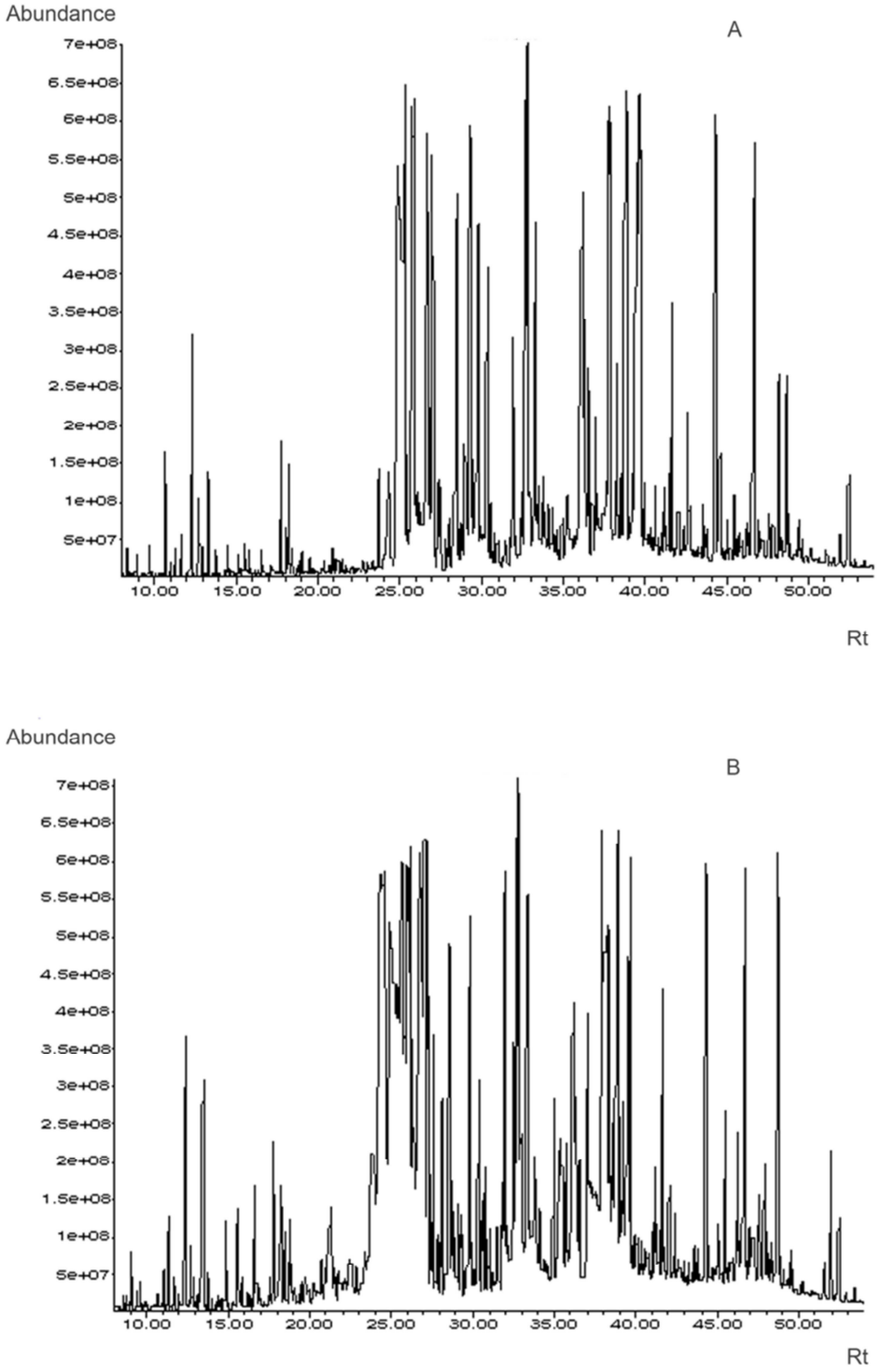
Table 6. *Total phenols and total flavonoids content in alcoholic extracts of T. tuberosum*

Extracts	**Phenols totals mg/mL** $\bar{X} \pm DS$	**Flavonoids totals mg/mL** $\bar{X} \pm DS$
Yellow variety	0.48 /0.01[b]	0.19 /0.005[b]
Black variety	0.66 /0.01[c]	0.10 /0.003[c]

Legen: $\bar{X} \pm DS$: media value of the determinations / standard deviation. 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 pun 95% confidence, according to Duncan.

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

Hexane and dichloromethane extracts were used as degreasers of plant material and only ethyl acetate and ethanol extracts were considered for this study. Chromatograms of ethyl acetate extracts and ethanolic extracts of the two varieties are presented in Figures 3 and 4.



(A) Yellow variety (B) Black variety.

Figure 4. *Analytical gas chromatogram of the ethanolic extract of the two varieties of mashua.*

The components to which structures were assigned by comparison of their mass spectra with those of the equipment library are indicated in the Tables 7 and 8.

Table 7. *Identified compounds by CG-EM in Ethyl acetate extract of the two mashua varieties.*

Peak	Yellow variety	FG	Tr	% Abundance
	anisaldehyde	$C_8H_8O_2$	12.237	0.630
	Hexadecane	$C_{16}H_{34}$	21.032	0.227
	Heptadecane	$C_{17}H_{36}$	23.288	0.586
	Myristic acid	$C_{14}H_{28}O_2$	24.506	0.483
	Octadecane	$C_{18}H_{38}$	25.439	0.557
	n-nonadecane	$C_{19}H_{40}$	27.490	0.737
	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	27.945	0.300
	Hexadecanoic acid	$C_{16}H_{32}O_2$	28.435	5.521
	β-Pregnane	$C_{21}H_{36}$	30.801	0.367
	Heneicosane	$C_{21}H_{44}$	31.320	2.581
	9.12-Octadecadienoic acid	$C_{18}H_{32}O_2$	31.827	4.956
	9-Octadecenoic acid	$C_{18}H_{34}O_2$	31.932	5.058
	Octadecanoic acid	$C_{18}H_{36}O_2$	32.352	2.859
	14-β-H-pregna	$C_{21}H_{36}$	32.439	0.410
	Docosane	$C_{22}H_{46}$	33.127	8.218
	Tricosane	$C_{23}H_{48}$	35.872	0.447
	n-Tetracosane	$C_{24}H_{50}$	36.531	28.586
	n-hexacosane	$C_{26}H_{54}$	39.637	16.869
	n-heptacosane	$C_{27}H_{56}$	41.100	13.365
	Squalene	$C_{30}H_{50}$	42.616	1.613
	triacontane	$C_{30}H_{62}$	45.198	2.639
	Stigmast-5-en-3-ol	$C_{29}H_{50}O$	48.946	2.991

Peak	Black variety	FG	Tr	% Abundance
	Benzaldehyde, 4-methoxy	$C_8H_8O_2$	12.243	1.615
	Benzenemethanol, 4-methoxy	$C_8H_{10}O_2$	12.989	0.412
	2,5-cyclohexadien-1-one	C_6H_6O	17.564	0.860
	Dodecanoic acid	$C_{12}H_{24}O_2$	20.071	7.565
	n-Hexadecane	$C_{16}H_{34}$	21.038	0.310
	Heptadecane	$C_{17}H_{36}$	23.294	0.996
	Tetradecanoic acid	$C_{14}H_{28}O_2$	24.564	1.512
	n-Octadecane	$C_{18}H_{38}$	25.450	1.651
	n-eicosane	$C_{20}H_{42}$	25.584	1.651
	Caffeine	$C_8H_{10}N_4O_2$	26.092	5.467
	n-nonadecane	$C_{19}H_{40}$	27.496	2.270
	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	27.951	1.685
	Hexadecanoic acid	$C_{16}H_{32}O_2$	28.633	4.952
	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	29.286	1.702
	9,12-Octadecadienoic, acid methyl ester	$C_{18}H_{30}O_2$	31.110	1.203
	n-Heneicosane	$C_{21}H_{44}$	31.331	3.851
	14β-Pregnane	$C_{21}H_{36}$	31.967	0.825
	Linoleic acid ethyl ester	$C_{20}H_{34}O_2$	32.334	5.329
	Octadecanoic acid,	$C_{18}H_{36}O_2$	32.963	1.821
	Docosane	$C_{22}H_{46}$	33.127	5.845
	1-Octadecene	$C_{18}H_{36}$	33.226	3.060
	3,5-Dimethoxystilbene	$C_{16}H_{16}O_2$	33.523	0.722
	Hexacosane	$C_{26}H_{54}$	39.637	5.021
	Heptacosane	$C_{27}H_{56}$	41,094	3.678
	n-Octacosane	$C_{28}H_{58}$	42.511	2.233
	Squalene	$C_{30}H_{50}$	42.645	8.218
	n-Nonacosane	$C_{29}H_{60}$	43.875	1.548
	Triacontane	$C_{30}H_{62}$	45.198	1.306
	α -Tocopherol	$C_{29}H_{50}O_2$	46.684	1.066
	Campesterol	$C_{28}H_{48}O$	47.821	1.340
	Stigmasterol	$C_{29}H_{48}O$	48.153	3.541
	γ-Sitosterol	$C_{29}H_{50}O$	48.992	16.745

Table 8. *Identified compounds by CG-EM in ethanolic extract of the two Mashua varieties.*

Peak	Yellow variety	FG	Tr	% Abundance
	Octanoic acid	$C_8H_{16}O_2$	12.033	0.348
	Butanedioic acid	$C_4H_6O_4$	13.618	0.348
	L-Threonic acid	$C_4H_8O_5$	19.482	1.742
	Arabinonic acid	$C_5H_{10}O_6$	21.120	1.742
	Heptadecanoic acid	$C_{17}H_{34}O_2$	31.501	7.665

Peak	Yellow variety	FG	Tr	% Abundance
	11-trans-Octadecenoic	$C_{18}H_{34}O_2$	32.923	13.240
	9-Octadecenamide	$C_{18}H_{35}NO$	35.242	24.042
	Squalene	$C_{30}H_{50}$	42.021	8.014
	Nonacosane	$C_{29}H_{60}$	45.886	4.879
	Eicosane	$C_{20}H_{42}$	45.885	4.529
	(+)- α-Tocopherol	$C_{29}H_{50}O_2$	46.200	6.271
	Stigmasterol	$C_{29}H_{48}O$	47.827	5.576
	β-Sitosterol	$C_{29}H_{50}O$	48.602	21.602
	Benzoic acid.	$C_7H_6O_2$	11.590	2.629
	Butanedioic ac. methyl ester	$C_5H_{10}O_2$	13.659	0.880
	2, butenodioic acid	$C_4H_4O_4$	14.335	1.310
	Nonanoic acid.	$C_9H_{18}O_2$	14.568	1.744
	L-Treitol	$C_4H_{10}O_4$	18.083	4.365
	Nookatone	$C_{15}H_{22}O$	19.301	3.498
	Xilonic acid.	$C_5H_{10}O_6$	21.166	13.363
	Squalene	$C_{30}H_{50}$	42.033	24.456
	Nonacosane	$C_{29}H_{60}$	45.903	13.683
	Stigmasterol	$C_{29}H_{48}O$	47.850	33.774

Black variety	FG	Tr	% Abundance
Benzoic acid.	$C_7H_6O_2$	11.590	2.629
Butanedioic ac. methyl ester	$C_5H_{10}O_2$	13.659	0.880
2, butenodioic acid	$C_4H_4O_4$	14.335	1.310
Nonanoic acid.	$C_9H_{18}O_2$	14.568	1.744
L-Treitol	$C_4H_{10}O_4$	18.083	4.365
Nookatone	$C_{15}H_{22}O$	19.301	3.498
Xilonic acid.	$C_5H_{10}O_6$	21.166	13.363
Squalene	$C_{30}H_{50}$	42.033	24.456
Nonacosane	$C_{29}H_{60}$	45.903	13.683
Stigmasterol	$C_{29}H_{48}O$	47.850	33.774

3.7. Anti-inflammatory Activity

The anti-inflammatory activity of the alcoholic extracts of the (*Tropaeolum tuberosum* Ruiz & Pav.) Kuntze tubers (yellow and black variety) was evaluated at the first sign that acute inflammation appear (mainly edema during the first five hours) by testing of plantar edema due to carrageenan. This is currently used mostly for the evaluation of anti-inflammatory drugs. The results are presented in the Table 9.

Table 9. *Average volume of plantar edema over time.*

Groups	Edema volume (mL)			
	1h	2h	3h	5h
NaCl 0.9%	0.98/0.05a	0.99/0.07a	1.04/0.05a	1.10/0.03a
Indometacine	0.76/0.04b	0.74/0.05b	0.60/0.06b	0.49/0.02b
Yellow mashua extract	0.89/0.03c	0.85/0.07c	0.70/0.08c	0.52/0.05b
Black mashua extract	0.87/0.04c	0.85/0.07c	0.72/0.08c	0.52/0.04b

Legend: The values represent the average of the edema / standard deviation volumes (n=6); different letters in a column indicate significant differences $p<0.05$ (Kruskall-Wallis and Friedman).

The percentages of edema inhibition of the groups under study are illustrated in the figure 5.

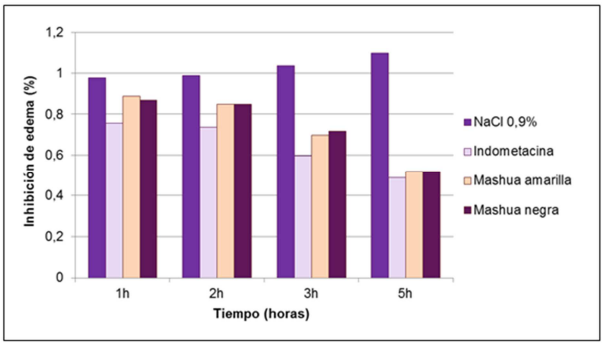


Figure 5. *Inhibition percentage of plantar edema over time.*

4. Discussion

4.1. Macromorphological Characteristics

Differences in all macromorphological characteristics are observed mainly in color, length, width and average weight. The yellow variety is longer and heavier than the black one.

4.2. Morpho Anatomical Characteristics

A set of conductive bundles of the xylem tissue, represented by vessels with thickening of the scaler type and dotted in the three samples evaluated was observed in the cross-section of the tubers in the fresh state (Figure 2 A, B) [22].

Epidermal tissue cells (C, and D) of elongated shape and variable size in the two varieties of mashua was visualized. Reserve parenchyma (amyliferous) rounded cells and blackish colored starch grains were found with the lugol reagent (Figure 2 E y F).

Observations were made trying to finda significant difference that was consistent with the morphological differences (color and size), but nothing relevant was found. The most relevant observation of the study was to find many starches from the rice group, in the two samples evaluated, more abundant in yellow mashua [23].

4.3. Physical-chemical Parameters and Phytochemical Screening of Raw Drugs

The norms and pharmacopoeias establish a residual moisture content of no more than 14%, depending on the plant material [11, 24, 25]. In the study carried out, the residual moisture value was below the maximum allowable limit for vegetable drugs.

Some Pharmacopoeias pose a total ash index up to 5% [11, 24] and another, such as the Chinese Pharmacopoeia, refers to up to 15% [26]. In the trial carried out, the percentage was below the maximum allowed limit for the yellow variety, the black variety being within the allowed limit. On the other hand, acid-insoluble ashes were also small, in both cases less than 2%, although in the black variety the lowest value was obtained which is indicative that the minerals present are of the type of alkaline and earth alkaline.

In the phytochemical screening, some differences were found between the two varieties, mainly in the intensity of the colorations, in the black mashua, greater intensity was found in the anthocyanidin test, which is consistent with the findings reported by Chirinos *et al*, [27].

4.4. Total Phenols Content and Total Flavonoids in Alcoholic 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, the highest concentration of total phenols was presented by the extract from the black variety, while in the yellow variety it presented a higher content of total flavonoids. Similar results were reported by Tena and Apaza, which indicated that the black variety had a higher concentration of phenolic compounds [28].

Campos et al., compared different Andean tubers among different varieties of *T. tuberosum,* observing significant differences in the content of these metabolites, where *T. tuberosum* presented a very high phenolic content. They also reported that the purple genotypes of *T. tuberosum* had a higher concentration of polyphenols [29].

Phenolic compounds in aqueous extracts of the black variety were identified by Chirinos et al., which determined the presence of gallic acid, gallocatechin, epigallocatechin, procyanidin B2 as well as derivatives of hydroxybenzoic acid, hydroxycinnamic acid, myricetin, rutin and epigallocatechin [5, 30-32].

The mixture of polyphenols was capable of scavenging peroxyl radicals, which is in concordance with the antioxidant activity of polyphenols.

Jiménez et al, conducted a study in three varieties of Mashua and in three methods of determining antioxidant capacity (FRAP, DPPH and ABTS) [7]. In the study, they were able to verify that as the concentration of the extracts increased, the reducing power increased (FRAP test) and the anti-radical activity (DPPH and ABTS tests) of the extracts, manifesting a high antioxidant activity, although lower than the reference substances used (vitamin C and Trolox). Of the three extracts evaluated, the variety of black mashua showed the greatest antioxidant capacity of the three methods tested.

4.5. Analysis of the Extracts by the Coupled gas Chromatography-mass Spectrometry System

According to Tena and Apaza, [33], the phytochemistry of the *T. tuberosum* species has been studied for several decades, however, no comparative chemical or pharmacological studies between tubers of different colors have been reported.

4.5.1. Ethyl Acetate Extract

The analytical gaseous chromatograms of the ethyl acetate extract (yellow and black variety tubers) presented in Figure 3 shows that the black variety has a greater chromatographic complexity than the yellow one.

a. Yellow variety.

In the yellow variety, a total of 22 compounds were identified by comparing their mass spectra with those of the equipment library (Table 5), of which 11 corresponded to hydrocarbons, six to fatty acids, four to triterpenes and / or steroids and 1 aromatic compound.

In the yellow variety, a total of 22 compounds were identified by comparing their mass spectra with those of the equipment library (Table 5) of which 11 corresponded to hydrocarbons, six to fatty acids, four to triterpenes and / or steroids and 1 aromatic compound.

Other components abundant in the extract were fatty acids with 18,872% of them 9-octadecenioc acid (oleic acid), which is a monounsaturated fatty acid of the omega 9 series typically found in vegetable oils, which exerts a beneficial action on blood vessels reducing the risk of cardiovascular disease. 9,12-octadecenoic acid was the other fatty acid of relative abundance with 4,956%; which is an essential fatty acid of the omega 6 series and attributed important beneficial activities for health [34 – 37].

The presence of fatty acids in the tubers was detected qualitatively in the phytochemical screening in the ethereal extract.

Ramallo determined the percentage and type of fatty acids in six different samples of *T. tuberosum* using gas phase chromatography [38]. The results showed a high content of polyunsaturated fatty acids (70.8%) in the six samples of T. tuberosum; highlighting the presence of linoleic acid (48.7%) and α-linoleic acid (22.1%). In the yellow variety, which is one of the most consumed in Ecuador, although there is

presence of omega 6 and 9 fatty acids, the percentages do not reach those figures.

The triterpenoid and steroidal compounds were also detected in this extract, with Stigmast-5-en-3-ol being the highestwith 2,991%. These metabolites were also detected by phytochemical screening in ethereal and alcoholic extracts.

Chasquibol-Silva et al., reported the presence of steroid compounds in dry tubers of *T. tuberosum* extracted with ethanol, using the Lieberman-Burchard reagent, which is appropriate to determine the existence of triterpenes and sterols in the crude extract [39]. However, as a qualitative method, it is not possible 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*.

Almagro, points out that there is a lack of information on these metabolites, to know if they are responsible for some traditional uses (anti-inflammatory, anti-diabetic and anti-cancer) [40].

Stigmasterol, identified in this extract is part of a group of plant sterols, or phytosterols, which include β-sitosterol, campesterol and ergosterol, considered provitamin D2. Research has indicated that stigmasterol may be useful in the prevention of certain cancers, including ovarian, prostate, breast, and colon cancer. It also has properties as a potent antioxidant, hypoglycemic agent and in the inhibition of thyroid gland function [41].

b. Black variety.

In the extract of the black variety a greater variability of compounds was observed; a total of 32 components were identified, 13 hydrocarbons (33.42%), 8 fatty acids (25,769%), 2 aromatic compounds (2,027%), 5 triterpenes / steroids (30,669%), an aliphatic ketone (0.860); a polyphenol derivative (0.722); caffeine (5.467%); and α-tocopherol (1,066%) (Table 5).

The hydrocarbons docosane, hexacosane and heptacosane presented greater abundance, correspond to compounds that can be part of the epicuticular waxesand are abundant in many plant species.

Fatty acids had a higher percentage in this variety, but saturated fatty acids were more abundant, with only 6,531% corresponding to unsaturated fatty acids, mainly methyl and ethyl esters of linoleic acid.

Triterpenes / steroids had a high percentage compared to the yellow variety. Of these, γ-sitosterol was the major component of the extract with 16,745%, followed by scualene with 8,218%.

For scualene several studies have shown that it has anti-inflammatory properties, a beneficial effect for atherosclerosis, is an antioxidant, is present in skin lipids and plays a role in aging and skin pathologies. It is also used as an adjuvant in vaccines and has been tested in other fields such as cancer and dyslipidemia. On the other hand, γ-sitosterol is a potent inhibitor of the complement component C1 complex and has demonstrated potential as a treatment for diabetes in rats [42-46].

The presence of caffeine in tuber extract with a percentage of 5,467% is striking. In the phytochemical screening the presence of alkaloids for the tubers was obtained, which could be due to the presence of this xanthine. Some authors have suggested that caffeine appears in some plants and that it has among others the function of preventing other species that it competes with from growing around it or that can serve as an attractant of bees for pollination [47]. The truth is that farmers traditionally argue that it is good to sow the black variety in potato crops to prevent fungal contamination.

Another of the components detected, although in a low proportion was 1,066% α-tocopherol, which could also justify the antioxidant activity of the plant. This compound is frequently found in many medicinal plants [48].

4.5.2. Ethanolic Extract

The analytical gaseous chromatograms of the ethanol extracts (figure 4) were extremely complex for both species and only 13 structures of the yellow variety and 10 of the black variety could be assigned structures (Table 6)

a. Yellow variety.

In the yellow variety, the majority component was 9-octadecenamide, an oleic acid amide known as oleamide (24,042%).

Apaza et al., reported that from the heptane extracts of the dry tubers of the purple and black varieties of *T. tuberosum*, the macamide N-oleoyldopamine and the alkamide N- (2-hydroxyethyl) -7Z, 10Z, 13Z, were isolated 16Z-docosatetraenamide) [33]. These authors reported that alkamides and macamides isolated in *T. tuberosum* inhibited the production of TNF-α. In addition, Woelkart, noted the in vitro inhibitory activity of alkalides in cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [49]. The inhibition of cyclooxygenases and TNF-α are effective strategies to suppress pain and inflammation. These results could explain the traditional use of *T. tuberosum* against pain and inflammation.

The other abundant metabolite in this extract was β-sitosterol (21,602%), this compound has been assigned the following properties: anticancer, hypoglycemic and for the treatment of benign prostatic hyperplasia [50-52].

Other major components were 11-E-Octadecenoic acid, squalene (8.014%), heptadecanoic acid (7.665%), α-tocopherol (6.271%) and stigmasterol (5.576%). The rest of the components were organic acids, acids from the oxidation of sugars and hydrocarbons.

b. Black variety.

Unlike the yellow variety, the presence of alkamides was not identified in the black variety. The major components in this extract were the acyclic triterpene scualene (24,456%) and the phytosterol stigmasterol (33,774%), of which properties have already been discussed since they were found in the ethyl acetate extract. It is followed in abundance by nonacosane hydrocarbon and xylonic acid produced by the oxidation of xylose sugar. The rest of the components of this fraction are minority and are composed of organic acids, alcohols and Nookatone ketone (3,498%), with insecticidal properties not toxic to man [53].

4.6. Anti-inflammatory Activity

The anti-inflammatory activity evaluation of 30% hydroalcoholic extracts of the *Tropaeolum tuberosum* (Ruiz & Pav.) Kuntze tubers (yellow and black variety) were performed on the first signs that appear during acute inflammation (mainly edema during the first five hours), by means of the carrageenan plantar edema test, which is mostly used today for the evaluation of anti-inflammatory drugs.

This method makes it possible to quantify in a reproducible and simple way two of the most characteristic parameters of inflammation such as edema and plasma extravasation by inducing an acute inflammation located in the animal's leg after the administration of carrageenan in the plantar aponeurosis of the rat [54].

In table 7 it was observed that, with the exception of the negative control group whose volume of edema was increasing over time, all treated groups managed to reduce the edema produced by carrageenan, mainly at the third and fifth hour that the lowest value is obtained, which demonstrates the anti-inflammatory effect of indomethacin (used as a positive control) and the extracts tested.

An increase in the volume of edema was seen in the negative control group, a logical result of waiting if one considers that no anti-inflammatory agent was administered. On the contrary, in the groups treated with indomethacin and the extracts an anti-inflammatory tendency was observed, which translates into a decrease in the volume of edema at the time of study.

When applying the Kruskall-Wallis test, it was found that there were significant differences ($p<0.05$) between all the treated groups with respect to the negative control group. The groups that received the extracts (yellow and black variety) had a similar behavior among them. At the fifth hour, both extracts of mashua had a behavior comparable to indomethacin.

When applying the Friedman test (for the same treatment) it was observed that the volume of edema was significant, mainly when the first hour was compared with the last one. A graphic representation of edema volumes (Figure 5) showed how extracts together with indomethacin reached the lowest edema volumes from the third and fifth hour, resembling the fifth hour of testing.

Among the positive controls that are frequently used in this experimental model is indomethacin that inhibits cyclooxygenases (COX) and therefore inhibits the formation and release of prostaglandins, which in the second phase (between 3 and 4 hours) acquires its maximum manifestation, mainly the PGE2 [55].

Tropaeolum tuberosum, commonly known as "Mashua", it is one of the plants most used by the Andean (Peruvian-Bolivian) as food and medicine. It is used as a remedy against a wide range of diseases, especially those related to inflammation [34].

5. Conclusion

The varieties under study showed significant differences in the macro and micromorphological characteristics; the yellow variety is generally longer, heavier, and has a greater number of starch granules than the black variety.

In the phytochemical screening, the yellow variety exhibited a greater abundance of lactones, reducing compounds and alkaloids than the black variety, the latter, however, presented greater intensity of color in the reaction of flavonoids in the alcoholic extract.

The quantitative analysis of phenols and flavonoids showed that the black variety has a significantly higher total phenol content than the yellow variety, while in the latter the concentration of total flavonoids is higher.

The chemical composition of the ethyl acetate extracts differs qualitatively, the highest number of components was detected in the black variety. In the ethanolic extract, on the contrary, the greatest number of compounds were identified for the yellow variety. It is important to note that the triterpenes and sterols identified in this work, squalene, campesterol, stigmasterol and γ-sitosterol, are the first reports for the species and its varieties, as well as the presence of oleamide, α -tocopherol and caffeine.

The two varieties had a similar anti-inflammatory effect, which may be due to the presence of stigmasterol as the majority component of the extracts of both varieties. This result corroborates one of the traditional uses attributed to the spice.

Authors' Contribution

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

References

[1] Rosset, Peter. “Food Sovereignty: Global Rallying Cry of Farmer Movements.” Institute for Food and Development Policy Backgrounder. 2003; 9 (4): 1-4.

[2] CIP. Centro Internacional de la Papa. Oca, ulluco y mashua. [International Potato Center. Oca, ulluco and mashua]. Lima, Perú. 2015. Aviable in: https://cipotato.org/es/raices-y-tuberculos/oca-ulluco-y-mashua/.

[3] Aruquipa, R., Trigo, R., Bosque, H., Mercado, G., y Condori, J. El Isaño (*Tropaeolum tuberosum*) un cultivo de consumo y medicina tradicional en Huatacana para el beneficio de la población boliviana. [The Isaño (*Tropaeolum tuberosum*) a consumer crop and traditional medicine in Huatacana for the benefit of the Bolivian population]. Revista de Investigación e Innovación Agropecuaria y de Recursos Naturales. 2016; 3 (2): 146-151.

[4] Rivera, F. Raíces y tubérculos con alto contenido energético y medicinal. [Roots and tubers with high energy and medicinal content]. Desafío. 2005. Aviable in: http://repositorio.educacionsuperior.gob.ec/bitstream/28000/675/1/T-SENESCYT-0127.pdf.

[5] Chirinos, R.; Campos, D. Costa, N.; Arbizu, C.; Pedreschi, R.; Larondelle, Y. Phenolic profiles of andean mashua (Tropaeolum tuberosum Ruíz & Pavón) tubers: Identification by HPLC-DAD and evaluation of their antioxidant activity. Food Chemistry. 2008a; 106: 1285-1298.

Villacres, E. Quelal, M. & Álvarez, J. Nutrición, Procesamiento y Gastronomía de Raíces y Tubérculos Andinos en Ecuador: Una Revisión Bibliografía de Papa, Melloco, Oca, Mashua, Zanahoria Blanca y Jícama. [Nutrition, Processing and Gastronomy of Andean Roots and Tubers in Ecuador: A Bibliography Review of Potato, Melloco, Oca, Mashua, White Carrot and Jicama]. 2013. Aviable in: http://repositorio.iniap.gob.ec/bitstream/41000/2816/1/iniapsc302.pdf.

[6] Jiménez, H. M, Gutiérrez, GY, Chóez, GI, Miranda, MM. Chemical Study and Determination of the Antioxidant Activity of Three Varieties *Tropaeolum tuberosum* (Mashua). American Journal of Plant Sciences. 2019; 10: 2279-2297. https://doi.org/10.4236/ajps.2019.1012159.

[7] Morillo, C. A. C. Morillo, C. Y. & Tovar L. Y. Molecular characterization of cubios (*Tropaeolum tuberosum* Ruíz and Pavón) in the department of Boyacá. Revista de Ciencias Agrícolas. 2016; *33* (2): 32-42. https://doi.org/10.22267/rcia.163302.50.

[8] Gattuso MA, Gattuso SJ. Manual de procedimientos para el análisis de drogas en polvo. [Procedures manual for the analysis of powdered drugs]. Editorial de la Universidad Nacional de Rosario Urquiza. Argentina. 1999. ISBN 950-673-199-3.

[9] Miranda, MM, Cuéllar, AC. Manual de prácticas de laboratorio. Farmacognosia y productos naturales. [Laboratory practice manual. Pharmacognosy and natural products]. 2000; 1-7, 18-32. Ciudad Habana.

[10] WHO. World Health Organization. Quality control methods for medicinal plant materials. WHO/PHARM/92.559. Updated edition of Quality control methods for medicinal plant materials, 1998. ISBN 978 92 4 150073 9. Geneva. 2011.

[11] Pourmorad F, Hosseinimerhr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology. 2006; 5 (11): 1142-1145.

[12] Memnune S, Hilal Y, Neva G, Bulent C, Zeynep E, Sezai E. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak. J. Pharm. Sci. 2009; 22 (1): 102-106.

[13] Kale, A, Gaikwad, S, Mundhe, K, Deshpande, N, Salvekar, J. Quantification of phenolics and flavonoids by spectrophotometer from *Juglans regia*, Int. J. Pharma. Bio Scs. 2010; 1: 1-4.

[14] Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P. (2012). Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT-Food Science and Technology. 2010; 46: 548-555.

[15] Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis. 2002; 10 (3): 178-182.

[16] Saitta M, Curto S, Lo Salvo F, Bella GDi, & Dugo G. Gas chromatographic-tandem mass spectrometric identification of phenolic compounds in Sicilian olive oils. Analytica Chimica Acta. 2002. https://doi.org/10.1016/S0003-2670(02)00572-X.

[17] Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, et al. A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes. J. Appl. Toxicol. 2001; 21: 15-23.

[18] Balamurugan K., Sakthidevi G, Mohan VR. Antiinflammatory activity of leaf of *Melastoma malabathricum* L. (Melastomataceae). International Journal of Research in Ayurveda and Pharmacy. 2012; 3 (6): 801-802.

[19] The World Medical Association. Declaración de la AMM sobre el Uso de Animales en la Investigación Biomédica. [WMA Statement on the Use of Animals in Biomedical Research] [Internet]. 2016 [citado 2 de marzo de 2017]. Aviable in: https://www.wma.net/e;s/policies-post/declaracionde-la-amm-sobre-el-uso-de-animales-en-la-investigacionbiomedica/.

[20] Esau K. Anatomía vegetal. [Plant anatomy]. Edición Revolucionaria. Cuba; 1972; 355-410.

[21] Khandelwal, KR. Practical Pharmacognosy. Techniques and experiments. Twelveth edition. 2004; 138.

[22] Lou Zhi-cen. General control methods for vegetable drugs. Comparative study of methods included in thirteen pharmacopoeias a proposal on their international unification. WHO/PHARM/80.502: 8-39. 1980.

[23] Miranda MM, Cuéllar AC. Farmacognosia y productos naturales. [Pharmacognosy and natural products]. Editorial Félix Varela. Segunda edición, La Habana. 2012; 135-145, 261-280.

[24] Commission CP. Pharmacopoeia of the People's Republic of China. Chinese Medical Science and Technology Press. Peking. 2015; 337.

[25] Chirinos R, Campos D, Betalleluz I, Giusti MM, Schartz SJ, Tian Q, et al. High performance liquid chromatography with photodiode array detection (HPLC/DAD)/HPLC-Mass spectrometry 1070 (MS) profiling of anthocyanins from Andean Mashua tubers (*Tropaeolum tuberosum* Ruíz & Pavón) and their contribution to the overall antioxidant activity. J. Agr. Food Chem. 2006; 54: 7089-7097.

[26] Tena Pé V, Luis Nestor AT. Ethnobotanical Uses, Secondary Metabolites and Biological Activities of Mashua (*Tropaeolum tuberosum* Ruíz & Pavón). Journal of Ethnopharmacology. 2019; 247, Article ID: 112152. https://doi.org/10.1016/j.jep.2019.112152.

[27] Campos D, Noratto G, Chirinos R, Arbizu C, Roca W, Cisneros-Zevallos L. Antioxidant capacity and secondary metabolites in four species of Andean tuber crops: native potato (Solanum sp.), Mashua (*Tropaeolum tuberosum* Ruíz & Pavón), Oca (Oxalis tuberosa Molina) and Ulluco (Ullucus tuberosus Caldas). J. Sci. Food Agric. 2006; 86: 1481-1488.

[28] Chirinos R, Campos D, Warnier M, Pedreschi R, Rees JF, Larondelle Y. Antioxidant properties of Mashua (*Tropaeolum tuberosum*) phenolic extracts against oxidative damage using biological in vitro assays. Food Chem. 2008b; 111: 98-105.

[29] Chirinos R, Campos D, Arbizu C, Rees JF, Roez H, Larondelle Y. Effect of genotype, maturity stage and post-harvest storage on phenolic compounds, carotenoid content and antioxidant capacity of Andean Mashua tubers (*Tropaeolum tuberosum* Ruíz y Pavón). Journal of the Science of Food and Agriculture. 2007a; 87: 437-446.

[30] Chirinos R, Rogez H, Campos D, Pedreschi R, Larondelle Y. Optimization of extraction conditions of antioxidant phenolic compounds from Mashua (*Tropaeolum tuberosum* Ruíz & Pavón) tubers. Sep. Purif. Technol. 2007b; 55: 217-225.

[31] Apaza TL, Tena PV, Serban AM, Alonso NMJ, Rumbero A. Alkamides from *Tropaeolum tuberosum* inhibit inflammatory response induced by TNF-α and NF-κB. J. Ethnopharmacol. 2019; 235: 199-205.

[32] Moreno JJ, Mitjavila MT. The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (review). J. Nutr Biochem. 2003; 14 (4): 182-195.

[33] Harvey KA, Walker CL, et al. (2010a). "Long-chain saturated fatty acids induce pro-inflammatory responses and impact endothelial cell growth." Clin Nutr 29 (4): 492-500.

[34] Harvey KA, Walker CL, Xu Z, Whitley P, Pavlina TM, Hise M, et al. Oleic acid inhibits stearic acid-induced inhibition of cell growth and pro-inflammatory responses in human aortic endothelial cells. J Lipid Res. 2010; 51 (12): 3470-3480.

[35] Harvey KA, Walker CL, Xu Z, Whitley P, Pavlina TM, Hise M, et al. Trans fatty acids: induction of a pro-inflammatory phenotype in endothelial cells. Lipids. 2012; 47 (7): 647-657.

[36] Ramallo ZR. Análisis exploratorio de los ácidos grasos del isaño (*Tropaeolum tuberosum*). [Exploratory analysis of isaño fatty acids (*Tropaeolum tuberosum*)]. Rev. Investigación & Desarrollo. 2004; 4: 71-77.

[37] Chasquibol-Silva N, Delmás-Robles DI., Rivera-Castilla D, Lengua-Calle RL, Aguirre-Medrano R, Bazán-Gutiérrez D, Becerra-Vásquez E, Bautista-Castro M. Contribución a la normalización de productos tradicionales andinos: Maca, Kiwicha, Cañihua, Mashua. [Contribution to the normalization of traditional Andean products: Maca, Kiwicha, Cañihua, Mashua]. Rev. Per. Quim. Ing. Quim. 1999; 2 (1): 9-21.

[38] Almagro L, Miras-Moreno B, Sabater-Jara AB. Bioactivity of phytosterols and their production in plant in vitro cultures. J. Agric. Food. Chem. 2016; 38: 7049-7058.

[39] Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. Fitoterapia. 2009; 80 (2): 123-126.

[40] Khan MR, Mlungwana SM. γ-sitosterol, un esterol citotóxico de Markhamia zanzibarica y Kigelia africana.[γ-sitosterol, a cytotoxic sterol from Markhamia zanzibarica and Kigelia africana]. Fitoterapia. 1999; 70 (1): 96-97.

[41] Fernández C, Martín M, Gómez-Coronado D, Lasunción MA. Efecto de los fitosteroles sobre la biosíntesis de colesterol y la proliferación en células humanas. [Effect of phytosterols on cholesterol biosynthesis and proliferation in human cells]. Clínica e Investigación en Arteriosclerosis. 2003; 15 (5): 175-183.

[42] Rangachari B, Veeramuthu D, Savarimuthu I. Antidiabetic activity of γ-sitosterol isolated from *Lippia nodiflora* L. in streptozotocin induced diabetic rats. European Journal of Pharmacology. 2011; 667 (1–3): 410-418.

[43] Shenbagamoorthy S, Ramar T, Vellingiri S, Krishnasamy K, Palani G, Shanmugam A, Soundarapandian K. (2011). Sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. European Journal of Pharmacology. 2011; 667 (1–3): 410-418.

[44] Ferrís-Tortajada J, Berbel-Tornero O, García-Castell J, Ortega-García JA, López-Andreue JA. Dietetic Factors Associated with Prostate Cancer. Protective Effects of Mediterranean Diet. Actas Urológicas Españolas. 2012; 36 (4): 239-245.

[45] Chittka L, Peng F. Neuroscience. Caffeine boosts bees' memories. Science.2013; 339 (6124): 1157-1159.

[46] Gutiérrez GY, Miranda MM, Bello A, Hernández S, Montes de Oca PR. Chemical characterization using gas chromatography/mass spectrometry of two extracts from *Phyllanthus orbicularis* HBK. Revista Cubana de Farmacia. 2011; 45 (3): 405-413.

[47] Woelkart K, Bauer R. The role of alkamides as an active principle of Echinacea. Planta Med. 2007; 7: 615-623.

[48] Awad AB, Chen YC, Fink CS, Hennessey T. β-Sitosterol inhibits HT-29 human colon cancer cell growth and alters membrane lipids. Anticancer Res. 1996; 16 (5a): 2797-2804.

[49] Ivorra MD, D'Ocon MP, Paya M, Villar A. Antihyperglycemic and insulin-releasing effects of beta-sitosterol 3-beta-D-glucoside and its aglycone, beta-sitosterol. Archives Internationales de Pharmacodynamie et de Therapie. 1988; 296: 224-231.

[50] Klippel KF, Hiltl DM, Schipp B. A multicentric, placebo-controlled, double-blind clinical trial of β-sitosterol (phytosterol) for the treatment of benign prostatic hyperplasia. British Journal of Urology. 2003. https://doi.org/10.1046/j.1464-410X.1997.t01-1-00362.x.

[51] Dolan MC, Jordan RA, Schulze TL, Schulze CJ, Manning MC, Ruffolo D, et al. Capacidad de dos productos naturales, nootkatone y carvacrol, para suprimir *Ixodes scapularis* y *Amblyomma americanum* (Acari: Ixodidae) en un área endémica de la enfermedad de Lyme de Nueva Jersey. [Ability of two natural products, nootkatone and carvacrol, to suppress Ixodes scapularis and Amblyomma americanum (Acari: Ixodidae) in an area endemic for Lyme disease in New Jersey. J Econ Entomol. 2009; 102 (6): 2316–24.

[52] Fernández RGA, Cruzado LM, Bonilla RPE, Ramírez CFJM, Toche TA, Curay CVL. Identificación de metabolitos secundarios y efecto antiinflamatorio del extracto etanólico de hojas de *Chromolaena leptocephala* (DC) R. M. King & H. Rob. "chilca negra". [Identification of secondary metabolites and anti-inflammatory effect of the ethanolic extract of leaves of *Chromolaena leptocephala* (DC) R. M. King & H. Rob. "Black chilca". Revista Peruana de Medicina Integrativa. 2017; 2 (3): 779-84.

[53] Dirosa M, Giround J. Studies of the mediators of acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J. Pathol. 1971; 15-29.