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* (Ruiz & 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 complicate the 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 several plants 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, thus it 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 resembling the 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>
<thead>
<tr>
<th>Table 1. Working conditions for the quantification of phenols and flavonoids.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantification</strong></td>
</tr>
<tr>
<td>Method</td>
</tr>
<tr>
<td>Dilution extract: water v: v reference substance</td>
</tr>
<tr>
<td>concentration</td>
</tr>
<tr>
<td>Absorbance calibration curve</td>
</tr>
</tbody>
</table>

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...
performed at 250°C with splitless mode. The oven treatment with the test substance. of metabolites [17]. Next, Gas Chromatography-Mass Spectrometry (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 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 Spectrometry (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 their mass spectra and mass reference of Wiley 9th with NIST 2011 MS Library.

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>
<thead>
<tr>
<th>Group</th>
<th>Carrageenan (3% aqueous solution)</th>
<th>Quantity</th>
<th>Test agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0,3 ml</td>
<td>10 mL/h</td>
<td>NaCl (0,9%)</td>
</tr>
<tr>
<td>Experimental</td>
<td>0,3 ml</td>
<td>10 mg/kg</td>
<td>Indomethacin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 mg/kg</td>
<td>T. tuberosum yellow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 mg/kg</td>
<td>T. tuberosum black</td>
</tr>
</tbody>
</table>

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]:

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variety</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yellow</td>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>11.30/0.20</td>
<td>10.92/0.73</td>
<td></td>
</tr>
<tr>
<td>Width (cm)</td>
<td>3.93/0.04</td>
<td>4.01/0.14</td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>45.95/7.60</td>
<td>43.55/3.95</td>
<td></td>
</tr>
</tbody>
</table>

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.

A (yellow variety), B (black variety): scalariform and pitted xylem vessels C (yellow variety), D (black variety): epidermal cells E (yellow 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Variety</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total humidity (%)</td>
<td>92.86/0.04</td>
<td>91.77/0.56</td>
<td></td>
</tr>
<tr>
<td>Residual humidity (%)</td>
<td>7.09/0.08</td>
<td>8.23/0.05</td>
<td></td>
</tr>
<tr>
<td>Total ashes (%)</td>
<td>3.90/0.11</td>
<td>5.09/0.10</td>
<td></td>
</tr>
<tr>
<td>HCl insoluble ashes (%)</td>
<td>0.37/0.10</td>
<td>0.15/0.02</td>
<td></td>
</tr>
</tbody>
</table>

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. tuberosum*.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Variety</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethereal extract</td>
<td>Yellow</td>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>Oil</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Lactones</td>
<td>++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Triterpenes / Steroids</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Reducing compounds</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Alcaloids</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lactones</td>
<td>+++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Triterpenes / Steroids</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tanins</td>
<td>+++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>+</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Alcaloids</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tanins</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Reducing compounds</td>
<td>+++</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

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.

![Analytical gas chromatogram of the ethyl acetate extract of the two varieties of mashua.](image)

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

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Phenols totals mg/mL $\bar{x} \pm D \bar{S}$</th>
<th>Flavonoids totals mg/mL $\bar{x} \pm D \bar{S}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow variety</td>
<td>$0.48 \pm 0.01^a$</td>
<td>$0.19 \pm 0.005^b$</td>
</tr>
<tr>
<td>Black variety</td>
<td>$0.66 \pm 0.01^c$</td>
<td>$0.10 \pm 0.003^c$</td>
</tr>
</tbody>
</table>

Legen: $\bar{x} \pm D \bar{S}$: 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.

![Analytical gas chromatograms of the extracts of mashua varieties](image)

(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.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Yellow variety</th>
<th>FG</th>
<th>Tr</th>
<th>% Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anisaldehyde</td>
<td></td>
<td>C₆H₄O₂</td>
<td>12.237</td>
<td>0.630</td>
</tr>
<tr>
<td>Hexadecane</td>
<td></td>
<td>C₁₆H₃₄</td>
<td>21.032</td>
<td>0.227</td>
</tr>
<tr>
<td>Heptadecane</td>
<td></td>
<td>C₁₇H₃₆</td>
<td>23.288</td>
<td>0.586</td>
</tr>
<tr>
<td>Myristic acid</td>
<td></td>
<td>C₁₄H₂₄O₂</td>
<td>24.506</td>
<td>0.483</td>
</tr>
<tr>
<td>Octadecane</td>
<td></td>
<td>C₁₈H₃₈</td>
<td>25.439</td>
<td>0.557</td>
</tr>
<tr>
<td>n-Nonadecane</td>
<td></td>
<td>C₁₉H₃₈</td>
<td>27.490</td>
<td>0.737</td>
</tr>
<tr>
<td>Hexadecanoic acid methyl ester</td>
<td></td>
<td>C₁₇H₃₄O₂</td>
<td>27.945</td>
<td>0.300</td>
</tr>
<tr>
<td>Hexadecanoic acid</td>
<td></td>
<td>C₁₈H₃₈</td>
<td>28.435</td>
<td>5.521</td>
</tr>
<tr>
<td>β-Pregnane</td>
<td></td>
<td>C₂₃H₄₈</td>
<td>30.801</td>
<td>0.367</td>
</tr>
<tr>
<td>Heneicosane</td>
<td></td>
<td>C₂₁H₄₄</td>
<td>31.320</td>
<td>2.581</td>
</tr>
<tr>
<td>9,12-Octadecadienoic acid</td>
<td></td>
<td>C₁₉H₃₈</td>
<td>31.827</td>
<td>4.956</td>
</tr>
<tr>
<td>9-Octadecanoic acid</td>
<td></td>
<td>C₁₈H₃₈</td>
<td>31.932</td>
<td>5.058</td>
</tr>
<tr>
<td>Octadecanoic acid</td>
<td></td>
<td>C₁₈H₃₈</td>
<td>32.352</td>
<td>2.859</td>
</tr>
<tr>
<td>14-β-H-pregnina</td>
<td></td>
<td>C₁₈H₃₈</td>
<td>32.439</td>
<td>0.410</td>
</tr>
<tr>
<td>Docosane</td>
<td></td>
<td>C₂₂H₄₄</td>
<td>33.127</td>
<td>8.218</td>
</tr>
<tr>
<td>Tricosane</td>
<td></td>
<td>C₂₃H₄₈</td>
<td>35.872</td>
<td>0.447</td>
</tr>
<tr>
<td>n-Tetracosane</td>
<td></td>
<td>C₂₄H₅₀</td>
<td>36.531</td>
<td>28.586</td>
</tr>
<tr>
<td>n-Hexacosane</td>
<td></td>
<td>C₂₅H₅₄</td>
<td>39.637</td>
<td>16.869</td>
</tr>
<tr>
<td>n-Heptacosane</td>
<td></td>
<td>C₂₇H₅₆</td>
<td>41.100</td>
<td>13.365</td>
</tr>
<tr>
<td>Squalene</td>
<td></td>
<td>C₂₅H₅₀</td>
<td>42.616</td>
<td>1.613</td>
</tr>
<tr>
<td>Triacontane</td>
<td></td>
<td>C₂₅H₅₂</td>
<td>45.198</td>
<td>2.639</td>
</tr>
<tr>
<td>Tricosane</td>
<td></td>
<td>C₂₃H₅₄</td>
<td>48.946</td>
<td>2.991</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Peak</th>
<th>Yellow variety</th>
<th>FG</th>
<th>Tr</th>
<th>% Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butanoic acid</td>
<td></td>
<td>C₆H₁₀O₂</td>
<td>12.033</td>
<td>0.348</td>
</tr>
<tr>
<td>Butanedioic acid</td>
<td></td>
<td>C₆H₁₀O₄</td>
<td>13.618</td>
<td>0.348</td>
</tr>
<tr>
<td>L-Threonine acid</td>
<td></td>
<td>C₆H₁₀O₂</td>
<td>19.482</td>
<td>1.742</td>
</tr>
<tr>
<td>Arabionic acid</td>
<td></td>
<td>C₆H₁₀O₂</td>
<td>21.120</td>
<td>1.742</td>
</tr>
<tr>
<td>Heptadecanoic acid</td>
<td></td>
<td>C₁₇H₃₈O₂</td>
<td>31.501</td>
<td>7.665</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Edema volume (mL.)</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>5h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl 0.9%</td>
<td>0.98/0.05a</td>
<td>0.99/0.07a</td>
<td>1.04/0.05a</td>
<td>1.10/0.03a</td>
<td></td>
</tr>
<tr>
<td>Indometacine</td>
<td>0.76/0.04b</td>
<td>0.74/0.05b</td>
<td>0.60/0.06b</td>
<td>0.49/0.02b</td>
<td></td>
</tr>
<tr>
<td>Yellow mashua extract</td>
<td>0.89/0.03c</td>
<td>0.85/0.07c</td>
<td>0.72/0.08e</td>
<td>0.52/0.05b</td>
<td></td>
</tr>
<tr>
<td>Black mashua extract</td>
<td>0.87/0.04c</td>
<td>0.85/0.07c</td>
<td>0.72/0.08e</td>
<td>0.52/0.04b</td>
<td></td>
</tr>
</tbody>
</table>

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.

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 scalar 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 (amyloplast) rounded cells and blackish colored starch grains were found with the lugol reagent (Figure 2 E y F).

Observations were made trying to find 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 allowed 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 between the two varieties, mainly in the intensity of coloration, in the black mashua, greater intensity was found. Difference that was consistent with the morphological complexity than the yellow one. 

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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, galloycatechin, 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.

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.

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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-octadecenioc 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 etheral 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
The 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 highest with 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, hypnotiglycemic agent and in the inhibition of thyroid gland function [41].

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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 waxes in which 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 sculene with 8,218%.

For sculene 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 macamides 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, hypnotiglycemic 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 sculene (24,456%) and the phytosterol stigmastanol (33,774%), of which properties have already been discussed since they were found in the ethyl acetate extract. It is followed in abundance by nonacosenoic 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


