

Evaluation Polyphenol Content and Antioxidant Activity in Extracts of *Cucurbita spp*

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Abstract

It is increasingly evident that oxidative stress and the presence of free radicals are a major cause of many disease processes that may aggravate the disease course, or create new effects that could cause further alterations in patient's health or impair their quality of life. The purpose of this study was to determine the antioxidant activity of extracts from seeds of four varieties of *Cucurbita spp*: (Hybrid between *C. moschata* and *C. maxima* Duchesne ex Lam) Tetsukabuto, *C. mixta* Pangalo (calabaza rayada), *C. moschata* (Duchesne ex Lam.) Duchesne ex Poir. (coreanito) and *C. maxima* Duchesne (calabaza plomo), in relation to their content of total phenols and flavonoids. Extracts of decreasing polarity were obtained using the following solvents: acidified water, methanol, acetone and ethyl acetate, the total phenols and flavonoids were quantified by the Folin-Ciocalteu and complexation with $AlCl_3$ to 5% respectively. To determine the antioxidant activity of extracts bleaching technique free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used. The total phenolic content was to Tetsukabuto (165.48 ± 0.94), calabaza rayada (275.10 ± 6.86), coreanito (118.79 ± 3.72) calabaza plomo (212.87 ± 7.51) (mol GAE/g of sample). Flavonoids values ranged from (32.03 ± 1.39) and (96.63 ± 1.14) (quercetin mg/g sample). The antioxidant activity expressed as median inhibitory concentration (IC₅₀) showed the following results: Tetsukabuto (117.69 ± 5.81) calabaza rayada (77.75 ± 3.64); coreanito (110.67 ± 1.70), calabaza plomo (87.39 ± 8.64) (mg/ml). A one-way analysis of variance showed significant differences ($p < 0.05$) among the varieties studied. The results obtained show a clear relationship between the content of total phenolics and flavonoids with antioxidant activity, with *C. mixta* Pangalo variety showed higher antioxidant activity and can be considered a source of natural antioxidants to produce a beneficial effect on health.

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Keywords

Antioxidant Activity, Seeds, Pumpkin

1. Introduction

Pumpkin seeds (*Cucurbita spp*) from antiquity have multiple uses in different countries as food and for medicinal purposes [1]. The man from the beginning in a process of relationship with the environment always looked at nature, how to find cures to diseases that afflicted him; empirically in their search for the healing of their ailments, was discovering the effect the proper use of plants and over time researching and extracting their properties [2].

Phenolic compounds or polyphenols are a large group of chemicals with different structures and chemical properties and biological activity, encompassing over 8000 different compounds. The study of oxidative reactions has increased in recent years due to the oxidation of cellular components by the action of free radicals and other factors has been recognized as the cause cellular processes such as aging and numerous diseases such as cancer, Parkinson's disease, Alzheimer's and cardiovascular diseases [3] [4]. Therefore natural antioxidants present in grains, vegetables and fruits have received considerable attention for their potential applications in the prevention of numerous diseases [5].

The search for natural variants is further premise, therefore in this paper the content of total phenols, flavonoids and antioxidant activity in four varieties of seeds *Cucurbita spp* grown in the province of Chaco is determined: Tetsukabuto (hybrid *C. moschata* and *C. maxima* Duchesne ex Lam.), *C. mixta* Pangalo (calabaza rayada), *C. moschata* (Duchesne ex Lam.) Duchesne ex Poir. (coreanito) and *C. maxima* Duchesne (calabaza plomo) (Figure 1).

2. Materials and Methods

We proceeded to collecting seeds of different varieties of *Cucurbita spp* producer in the region. The samples were dried to constant weight and stored in plastic bags, hermetically sealed and identified with labels. Fresh samples of plants (leaf, flower and fruit) were deposited in the Department Pharmacobotany National University of the Chaco Austral for identification.

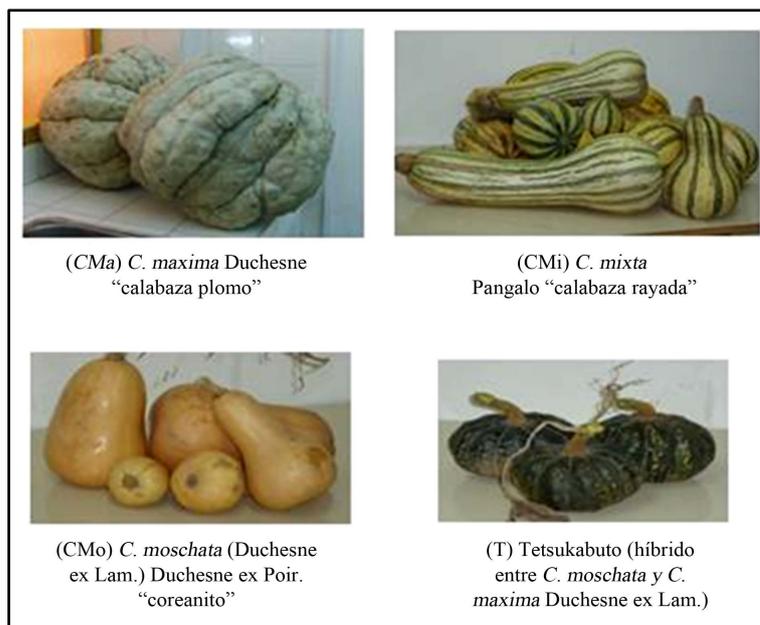


Figure 1. Images of four varieties of *Cucurbita spp*.

2.1. Obtention of Extracts with Solvent Decreasing Polarity

Ten grams of pumpkin seeds were homogenized in 50 ml of deionized water acidified (2% acetic acid) using a homogenizer (Ultra-Turrax T25 JANKE and Kunkel, IKA-Labortechnik). The suspension was left at room temperature for 10 min and centrifuged at 800 rpm for 10 min. The supernatant was collected in a flask and the pellet was treated with 50 ml of water acidified again. The suspension was centrifuged at 800 rpm for 10 min and the resultant supernatant was combined with the supernatant of the first centrifugation, the remaining pellet was homogenized in 50 ml of acidified methanol (2% acetic acid) and the aforementioned process is repeated. Two more solvents, acetone and ethyl acetate, were successively used for extraction of each fraction of the remaining residue. Four pumpkin seed extracts were finally collected: an aqueous extract (W), a methanol extract (M), one in acetone (Ac) and another ethyl acetate extract (ACET ETHYL). All fractions were evaporated under reduced pressure and redissolved in a volume of solvent that was originally used for extraction; the extraction process is performed for each of the four varieties of *Cucurbita spp* studied.

2.2. Quantification of Total Phenols

The total phenolic content was determined by the method of [6], which is based on the phenolic compounds reducing the Folin-Ciocalteu (tungsten and molybdate reagent) to form a blue complex that absorbs 765 nm, to 200 μ l of sample was added 2 ml of deionized water and 0.2 ml of Folin-Ciocalteu method, after 2 minutes 0.8 ml of 15.9% sodium carbonate was added, incubated at 50°C for 5 minutes and the absorbance at 765 nm on a UV-Visible Spectrophotometer Perkin Elmer Lambda 25 was measured. A calibration curve was performed with an alcoholic solution of gallic acid (GA). A stock concentration of 0.1 mg/ml solution was used, covering a range from 0.002 to 0.035 mg of acid per milliliter of solution. The results were expressed as μ mol of gallic acid per g of extract (AG μ mol/g extract).

2.3. Quantitation of Total Flavonoids

The total flavonoid content in the different extracts was determined by the method of [7] with modifications. To an aliquot of 0.2 ml of extract were added 1.8 ml of methanol and 1 ml of ethanol solution of 5% AlCl_3 . After one hour incubation at room temperature, the absorbance was measured at 425 nm on a UV-Visible Spectrophotometer Perkin Elmer Lambda 25. Quercetin solutions were used between 5 - 25 mg/ml, to construct a calibration curve. The total flavonoid content was calculated as mg equivalent of quercetin (QE)/g extract. This procedure was performed with each of the extracts of each variety in triplicate.

2.4. Antioxidant Activity

The method described by [8] was used. This method is based on reduction of an alcoholic solution of DPPH in the presence of a hydrogen donor antioxidant. The calculated amount of antioxidants in the sample required to reduce the initial concentration of DPPH radical 50%. The DPPH radical was dissolved in 95% ethanol, 2.5 ml of DPPH solution equal to 1.0 absorbance were taken, aliquots of the extract were added. Efficiency values of concentration were made after 10 minutes of reaction, decreasing absorbencies were recorded at 1 minute intervals. Worked at 515 nm as the peak wavelength in a UV-Visible Spectrophotometer Perkin Elmer Lambda 25, DPPH radical concentration in the reaction medium was calculated by nonlinear from a calibration curve obtained for different concentrations of extract concentration vs. regression DPPH radical.

The antiradical activity (AAR) was calculated by the equation:

$$\text{AAR} (\%) = 100 \left[1 - \frac{A_m^\infty}{A^0} \right]$$

where:

A^0 : absorbance at time 0 sample without added.

A_m^∞ : sample absorbance obtained in steady state by adjusting the kinetic profiles at infinite time.

However, the results obtained by this method are reported as IC50 that is the median inhibitory concentration, that is, the concentration of antioxidant compounds that can inhibit 50% of DPPH radical.

3. Data Analysis

The results were analyzed by one-way ANOVA ($p < 0.05$) and LSD test was used to compare means. STATGRAPHICS Plus software was used

4. Results

Content of total phenols Phenolic compounds are very important plant constituents because of their ability to sequester free radicals, which is related to the presence of the hydroxy group. In **Table 1**, the concentration of total phenolic compounds from each fraction for all four strains of *Cucurbita spp* seeds shown.

Total flavonoid content: the results (**Table 2**) showed lower values than those obtained for the analysis of phenols. This is within expected, being that flavonoids are a subgroup of the phenolic compounds.

Antioxidant activity: the results indicate that the antioxidant activities of all varieties of *Cucurbita spp* seeds were able to trap DPPH radical a concentration dependent manner. The antioxidant activity of these expressed as median inhibitory concentration (IC₅₀) is shown in **Figure 2**.

Table 1. Total phenols in extracts of pumpkin seeds, acid Galico umol/g acid extract.

Variety	Ex	W	M	Ac	Ac Et	Cont. Total
CMi		67.48 ± 1.22	95.96 ± 3.25	48.72 ± 3.46	80.91 ± 1.89	275.10 ± 6.86
CMo		14.15 ± 0.20	70.78 ± 0.20	22.57 ± 1.40	11.29 ± 0.21	118.79 ± 3.72
T		25.06 ± 0.78	68.80 ± 1.48	29.49 ± 2.67	42.12 ± 0.67	165.48 ± 0.94
CMa		53.88 ± 0.58	62.79 ± 3.34	30.75 ± 2.76	47.49 ± 0.13	212.87 ± 7.51

*Fractions: W: aqueous, M: methanol, Ac: acetone; Ac Et: ethyl; **Variety seed. **CMi**: *C. mixta* Pangalo; **CMo**: *C. moschata* (Duchesne ex Lam.) Duchesne ex Poir, **T**: Between hybrid *C. moschata* and *C. maxima* Duchesne ex Lam, **CMa**: *C. maxima* Duchesne.

Table 2. Total flavonoids in extracts of pumpkin seeds, Quercetin as mg/g extract.

Variety	Ex	W	M	Ac	Ac.Et	Cont. Total
CMi		1.35 ± 0.05	1.421 ± 0.15	39.12 ± 1.94	54.74 ± 0.46	96.63 ± 1.14
CMo		0.43 ± 0.07	0.41 ± 0.04	25.64 ± 0.12	53.55 ± 0.21	80.02 ± 0.18
T		1.72 ± 0.08	0.53 ± 0.01	20.66 ± 1.02	9.27 ± 0.18	32.03 ± 1.39
CMa		0.48 ± 0.06	0.29 ± 0.33	24.23 ± 1.85	52.77 ± 0.05	77.77 ± 1.82

*Fractions: W: aqueous, M: methanol, Ac: acetone; Ac Et: ethyl; **Variety seed. **CMi**: *C. mixta* Pangalo; **CMo**: *C. moschata* (Duchesne ex Lam.) Duchesne ex Poir, **T**: Between hybrid *C. moschata* and *C. maxima* Duchesne ex Lam, **CMa**: *C. maxima* Duchesne.

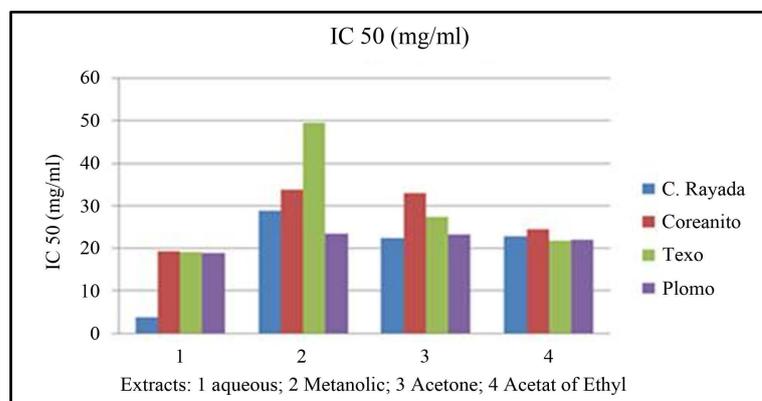


Figure 2. Median inhibitory concentration of pumpkin seed extracts. IC₅₀ (mg/ml).

5. Discussion

Phenolic compounds have a significant action on the stabilization of lipid oxidation, have been associated with antioxidant activity and are suggested to have inhibitory effect on mutagenesis and carcinogenesis [9]. The highest values of total phenols were observed in the methanolic fractions of the four varieties of *Cucurbita spp* with values ranging from 10.68 to 16.32 mg GA/g extract, higher than that reported by [10] who found values in the methanol fraction of 0.09 to 0.20 mg GA/g extract, pumpkin seeds, and are also higher than those reported by other authors [11] (1.58 mg AG/g flour) and methanol extracts of pumpkin seed oil [12] which reported a value of 0.98 mg AG/g oil. In turn, high concentration of phenolic compounds in the aqueous fraction can be attributed to the presence of proteins and other water soluble compounds containing phenolic rings are obtained, with *C. mixta* Pangalo (67.48 ± 1.22 mol GA/g extract) and *C. maxima* Duchesne (53.88 ± 0.58 mol GA/g extract) varieties showed higher content in this fraction. The Folin-Ciocalteu method is widely used to measure phenols; however there may be reactions with reducing substances such as phenolic compounds or ascorbic acid sugar [13] [14].

In fractions of ethyl acetate, where the solvent is used to extract phenolic compounds is very difficult to extract some sugar to pass through, but if other than phenols and phenolic acids reduced the Folin-Ciocalteu method, because in this fraction were relevant concentrations found in all varieties of *Cucurbita spp* with values of 11.29 ± 0.21 to 80.91 ± 1.89 mol GA/g extract. In contrast, the fractions with the lowest values ketones were observed in the quantification of total phenols. According to the spectrophotometric technique all species showed higher concentration of total flavonoids in the ethyl acetate fraction with values that ranged from 54.74 ± 0.46 to 9.27 ± 0.18 (mg of quercetin/g extract) being *C. mixta* Pangalo the variety presented higher content, which in turn is lower than those reported by authors [2] who found values for *Cucurbita argyrosperma* 110 ± 5.32 mg quercetin/g of sample, but in time is greater than that reported [14] for *Cucurbita moschata* (quercetin 17.73 mg/100 g). In all varieties of seed *Cucurbita spp* studied, the highest antiradical activity was detected in the aqueous fractions, highlighting *C. mixta* Pangalo ($IC_{50} 3.83 \pm 0.47$), with higher activity than that reported by [10] for seeds *Cucurbita*, however these values found by [10] are higher than those found for Tetsukabuto, *C. moschata* and *C. maxima* Duchesne in the aqueous fraction. In addition, acetone extracts of hazelnut (0.098 mg/ml) [15] and aqueous extract of walnut (0.15 to 0.22 mg/ml) [16] appear to be more effective DPPH radical inactivating the corresponding pumpkin seed extracts.

6. Conclusion

The results obtained show a clear relationship between the content of total phenols, flavonoids and antioxidant activity, with *C. mixta* Pangalo which showed higher antioxidant activity, this species can be considered a new source of natural antioxidants, to produce a beneficial effect on health.

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