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Article

Artichoke extracts with potential application in chemoprevention and inflammatory processes

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The aim of this work is to study three cultivars of artichoke (*Cynara cardunculus* var. *scolymus*): Gauchito, Guri and Oro Verde in terms of their *in vitro* chemoprevention and anti-inflammatory properties. These cultivars show good productive performance. The phenolic composition of their fresh leaves and edible bracts was analyzed by high performance liquid chromatography and high resolution mass spectrometry (HPLC-HRMS), showing mainly caffeoylquinic acids and flavonoids. Caffeoylquinic acids were quantified and the highest content was found in Gauchito cultivar. In this cultivar, the content of dicaffeoylquinic acids in fresh bracts was six times higher than that in fresh leaves (10064.5 ± 378.3 mg/kg *versus* 1451.0 ± 209.3 mg/kg respectively). Luteolin flavonoids were detected in leaves. The extracts from fresh bracts and leaves were assessed in their *in vitro* bioactivity against human neuroblastoma cells (SH-SY5Y). Inhibition of SH-SY5Y cells proliferation by Gauchito and Guri leaf extracts (8 µg/mL) was higher than 50 %. The leaf extracts of the same cultivars showed an inhibitory effect on human interferon IFN-I, decreasing its activity 50% at 40 µg/mL. Interestingly, the bract extracts did not show *in vitro* bioactivity at these concentrations, nor did the pure compounds chlorogenic acid, cynarin, apigenin and luteolin (at 2 µg/mL). These results suggest that Gauchito and Guri leaf extracts have potential for human neuroblastoma chemoprevention and treatment of inflammatory processes.

Keywords: *Cynara cardunculus* var. *scolymus*. Neuroblastoma. Interferon; Phenolic compounds.

INTRODUCTION

Since remote times *Cynara cardunculus* var. *scolymus* L. (Asteraceae), known as artichoke, plays an important role as food and medicinal plant (Pandino, Lombardo, Mauromicale, 2013; Gebhardt, 2002). Globe artichoke is a perennial herbaceous crop, cultivated mainly for its immature inflorescences called heads, which are marketed either fresh or processed (Lattanzio *et al*., 2009). Its production is concentrated in the Mediterranean area, especially in Italy, Spain and France, while it has also spread to the American continent according to the database of Food and Agriculture Statistics (FAO, 2019).

The Food and Agriculture Organization of the United Nations reported that worldwide production in 2017 was 1,505,328 tons with a yield of 12.30 tons/ha. The main end use of this production is fresh consumption and approximately 65% is industrialized (FAO, 2019). In the last decade, the worldwide demand for fresh vegetables has increased as a result of medical evidence linking their regular consumption with a decrease in

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the development of chronic and degenerative diseases (Liu, 2003).

Natural products are one of the most important sources in the development of new drugs and have been used to prevent several diseases, including cancer (Newman, Cragg, 2016). The term cancer chemoprevention by natural products was introduced in the late 1970s and refers to the prevention of cancer by the selective use of phytochemicals or their analogs (Mehta *et al*., 2010). Some natural agents can prevent and reverse carcinogenic processes in a pleiotropic manner (Guilford, Pezzuto, 2008). Cancer is one of the main concerns in human healthcare, representing the second cause of death worldwide after cardiovascular diseases (WHO, 2018). Natural compounds have attracted special attention since they may contribute in several ways to fight cancer. The use of natural dietary compounds is a promising strategy that is being widely studied to prevent cancer formation or cancer progression (Tan *et al*., 2011). Since oxidative stress, inflammation and the evasion of apoptosis are important biological mechanisms of carcinogenesis, then antioxidant, anti-inflammatory and pro-apoptotic activities represent key properties for prevention, suppression, or reversion of carcinogenesis.

Artichoke can prevent chronic and degenerative diseases (González- Sarrías *et al*., 2017; Pulito *et al*., 2015; Mileo *et al*., 2012; Agarwal, Mukhtar, 1996). Its preventive properties were related to the antioxidant capacity of the phenolic compounds present in the plant. *In vitro* and *in vivo* studies have been reported showing the activity of artichoke leaf extracts in anti-carcinogenic and anti-apoptotic assays. For instance, when malignant mesothelial cell lines were treated with increasing concentrations of artichoke leaf extract (3-200 µg/mL) for 72 hours, the cells showed both decreased growth and apoptosis in a dose dependent manner. At concentrations of 6-12 µg/mL, artichoke leaf extract significantly inhibited cell migration by 50% (Pulito *et al*., 2015). Nevertheless, there is scarce investigation in artichoke edible bracts related with cancer chemoprevention (Miccadei *et al*., 2008; Mileo *et al*., 2012) and interestingly, the Argentinean cultivars had never been studied with this purpose.

Recently, we reported the caffeoylquinic acids content in the three Argentinean artichoke cultivars demonstrating, as other researchers, that the composition in the plants depend on genotype and phenotype (García *et al*., 2016a,b; Lombardo *et al*., 2012; Lombardo *et al*., 2010), used part of the plant, growing conditions (García *et al*., 2016a,b; Martínez-Esplá *et al.,* 2017), etc. The genetic diversity in this crop is vast and the main differences are related with the origin of cultivars (Pagnotta *et al*., 2017). In our ongoing search for bioactive compounds and health-related bibliography, we present in this work the investigation of the Argentinean cultivars regarding their chemical and biological properties. First, extracts from leaves and edible fresh bracts were analyzed by LC-HRMS and the content of caffeoylquinic acids was quantified. Then, the extracts were evaluated by their radical scavenging capacity using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) by TLC. Finally, the extract bioactivity was studied against human neuroblastoma cells (SH-SY5Y), normal cells viability and IFN-I modulation, related to brain cancer and inflammation processes respectively.

MATERIAL AND METHODS

Plant material

The selected cultivars Gauchito (GA), Gurí (GU) and Oro Verde (OV), were grown at the experimental field of the College of Agronomic Sciences, National University of Rosario (UNR), Zavalla, Santa Fe, Argentina (33º01´ S; 60º53´ W). The plant material was collected by R.R. and A.M.E, and the voucher specimens were deposited at UNR Herbarium (*GA*001-UNR10450, *GU*002-UNR10451 *and OV003-UNR10452)*, in December 2016.

Experimental design and evaluated productive variables

The crop was carried out in a soil Vertic Argiudoll. Soil main characteristics in the 0-20 cm surface layer were: organic matter 2.98 %; nitrates 49.89 ppm; P 78 ppm; pH in water (1:2.5) 7.51. In the 20-40 cm surface layer the values were: organic matter 2.09 %; nitrates 54.17 ppm; P 40.01 ppm; pH in water (1:2.5) 7.28.

The plant density was 9125 plants ha^{-1} (1.4 m between rows and 0.8 m within the row). The crop received 102 mm of water by rainfall from May to November. In this period, average temperature was 14.6 ± 4.4 °C and relative air humidity was 79.3 ± 3.6 %. Three plots composed by 160 plants of each cultivar in their second year of production were evaluated. Based on soil analysis and crop requirement, 15 g of urea per plant were applied in June, July and August and the plants were supplemented with 152 mm by dripping between May and November. Control plants do not received urea (T1) and the fertilized plants received urea (T2). Twenty randomly chosen plants from each cultivar were evaluated for total yield per plant (Total weight of heads per plant in g).

Collection of leaves and bracts for chemical and biological analysis

The edible central bracts -between 21st and 30th in centripetal way- were collected from the primary heads. Leaves were collected from the middle layer of 10 random selected plants, once the harvest finished. The base and the apical portion of the leaves were discarded and the rest of the material was mixed. The plant material of each cultivar was washed and stored in freezer (-80 °C) until the extract preparation.

Extract Preparation

The frozen plant material (10 g) was crushed adding liquid nitrogen, macerated with methanol (100mL, 30 min, 200 rpm, 2 x) at room temperature (25 °C). The filtered extracts were stored in amber bottles at 4°C until the analysis. All extracts were pre-filtered through a 0.2 µm syringe filter before biological and HPLC analysis.

Analytical standards

Chlorogenic acid or 5-caffeoylquinic acid (**1**, purity > 95%), cynarin or 1,3-dicaffeoylquinic acid (**2**, purity > 95%), apigenin (**3**, purity > 95%), luteolin (**4**, purity > 98%), and rutin (purity > 95%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Liquid Chromatography- High Resolution Mass Spectrometry analysis

The extracts were analyzed by LC-HRMS using an Agilent 1200 HPLC instruments coupled to UV/Vis detector (Agilent) and a MicroTOF-Q II spectrometer (Bruker Daltonics, MA, USA) equipped with an electrospray ionization (ESI) source, in similar conditions previously reported by us (García *et al*., 2016a,b). The LC gradient elution, given as time (min)/channel B (%), was: 0/8, 21/25, 22/100, 25/100, 27/8, 30/8, used for bract extracts (LC method I); and 0/8, 16/25, 21/25, 23/100, 26/100, 27/8, 30/8, used for leaf extracts (LC method II). HRMS measurements were recorded in negative ionization mode. Nebulizing gas at 0.6 Bar, drying gas at 4.0 L min^{-1} and 180 °C, capillary 2800 V and end plate offset at -500 V. Data Analysis 4.0 SP1 software (Bruker Daltonics GmbH, Germany) was used for the analysis of chromatograms and mass spectra. Monocaffeoylquinic acids were quantified relative to the analytical standard (1) and dicaffeoylquinic acids were quantified relative to the analytical standard (2). All results were mean values ± standard deviation from three independent experiments (n=3) and they were expressed as mg/kg of fresh matter.

2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) ASSAY

This assay was performed using thin layer chromatography (TLC). The extracts (10 µL equivalent to 100 µg) or analytical standards (5 µL equivalent to 5 µg) were applied on Silica gel 60 F_{254} TLC plates purchased from Merck Chemicals Inc., using a CAMAG Automatic TLC Sampler 4 (ATS 4) under nitrogen flux. The plate was developed using an Automatic Developing Chamber (ADC2) with the solvent system ethyl acetate: glacial acetic acid: formic acid: H_2O (100:11:11:26). The chromatogram was captured under white light, UV_{254nm} and UV_{365nm} with CAMAG TLC Visualizer and then, the plates were sprayed with DPPH solution (0.4 mg/mL in EtOH) prepared just before use. After spraying, the plates were placed under an air stream for ethanol removal. The reading of the test was performed after 30 minutes and the image of the TLC was captured

under white light. Free radical scavenging activity was confirmed when the DPPH purple color changed to yellow (Cuendet *et al*., 1997).

Cell culture and proliferation assay

SH-SY5Y cells (human neuroblastoma, ATCC CRL-2266) were used as sample for evaluation of the effect of extracts in cancer and WISH cells (human amnion *cells* ATCC CCL-25) were used as normal cells sample. Cells were cultured in a mixture of DMEM/Ham's F12 1:1(for SH-SY5Y) or MEM (for WISH) medium (Gibco) supplemented with 10% (v/v) FCS and 2 mM glutamine at 37 °C in humidified atmosphere, 95% air and 5% CO_2. Cell proliferation was assessed through MTS assay using a chromogenic kit (CellTiter 96TM AQueous Non-Radioactive Cell Proliferation Assay, Promega) after 72 h of treatment with different extract dilutions: D100 (dilution of extract in MeOH, 1:100), D300 (1:300) and D500 (1:500). Solutions of the analytical standards 1-4 (2 µg/mL) were also evaluated. The vehicle (MeOH) was used as negative control and Doxorubicin was used as positive of anti-proliferative compound. Microplates were read at 492 and 690 nm, and the signal intensity was reported as the mean of the absorbance measured in three wells.

Analysis of extracts effect on the interferon activity

The extracts were analyzed employing a previously developed cell based reporter gene assay (RGA) (Bürgi *et al*., 2016; Bürgi *et al*., 2012). WISH-Mx2/EGFP reporter cells were seeded in 96-well plates (2.0 x 10^4 cells/well) and incubated during 24h at 37ºC with 5% CO_2. Supernatants were discarded and recombinant human interferon (rhIFN-I) was added at a concentration of 60 IU/mL in MEM medium supplemented with 2% FCS, in order to achieve 50% of the EGFP response. At the same time, dilutions 1:100 of extracts or chlorogenic acid were added to the cells. This dilution was non-toxic for the cell line, as previously determined (data not shown). The included controls were: IFN-treated cells (positive control); vehicle- treated cells (MeOH, negative control); CA: chlorogenic acid-treated cells. rhIFN-extract treated cells and controls were incubated during 24h at 37ºC with 5% CO_2. Samples and controls were assayed by triplicates and in three independent experiments. Cells were trypsinized, carefully suspended in 0.2 mL PBS and then EGFP expression was measured by flow cytometer. The percentage of EGFP positive cells was measured using a Guava® EasyCyteTM cytometer (Guava Technology, USA). Data acquisition was performed using Guava CytoSoftTM 3.6.1 software. Data obtained was analyzed using FlowJo version 7.6.5 software. For each sample, 2,000 events were collected gating on the FSC *vs* SCC dot plot and EGFP histogram plot.

Statistical analysis

To analyze the agronomic variables in each cultivar, a t-test was used for variables with normal distribution, while a Kruskal-Wallis Test was used for those variables without normal distribution. The statistical analyses were carried out by INFOSTAT software (Di Rienzo *et al*., 2016). A Chi square was applied to test the differences of caffeoylquinic acids (mono- or di-caffeoylquinic acids) in bracts and leaves within each cultivar and to compare total caffeoylquinic acid content among cultivars. To perform a test of independence, the mean values of mono- and di-caffeoylquinic acids in the three cultivars were transformed to percentages. In vitro cell-based assays were statistically analyzed with one-way ANOVA test followed by Bonferroni's post-test using GraphPad Prism for Windows, version 6 (GraphPad Software Inc.).

RESULTS AND DISCUSSION

The artichoke Gauchito (GA), Guri (GU) and Oro Verde (OV) cultivars (Figure 1), show good productivity and high level of polyphenols.

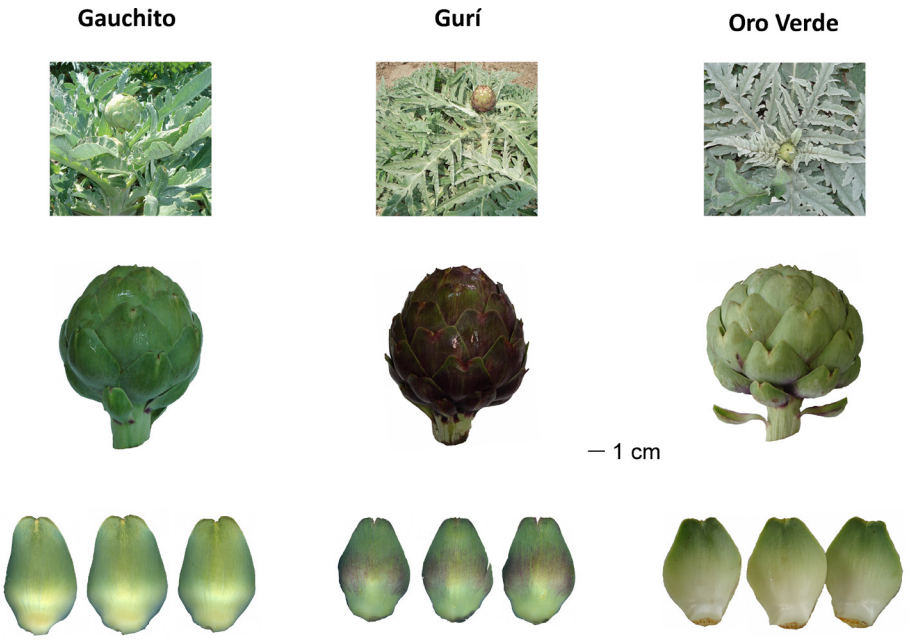

FIGURE 1 - Representative images of plants (first file), primary heads (second file), and edible central bracts (third file) from three artichoke cultivars Gauchito, Guri and Oro Verde.

The total yield per plant in grams is 1344.01 ± 114.35, 1283.87 ± 49.57 and 1521.17 ± 68.38 for GA, GU and OV respectively. No significant difference was found neither among them nor between T1-T2 treatments (p>0.05), and the yield values resulted similar or higher than those of the foreign cultivars grown in the area (Cointry *et al.*, 2005; Lopez Anido, Cointry, Cravero, 2005). Fresh leaves and bracts were selected to prepare the extracts for the chemical and biological characterizations.

All extracts were analyzed by liquid chromatography-high resolution mass spectrometry (LC-HRMS) in negative mode, comparing the retention time (Rt) of the chromatogram peaks and their HRMS spectra with those corresponding to the analytical standards **1** (5-caffeoylquinic acid or chlorogenic acid) and **2** (1,3-dicaffeoylquinic acid or cynarin), or with data reported in literature. The chromatograms of bract extracts show peaks with retention time (Rt) at 6.0 min and 8.9 min, each one with m/z signals 353.0866 and 353.0867 respectively, corresponding to molecular ions $[M-H]^-$ of mono-caffeoylquinic acid (**1'**) and chlorogenic acid assigned by comparison with the analytical standard **1**. Dicaffeoylquinic acids were detected at Rt 17.6 min and 19.2 min according to the m/z signals found by HRMS. These corresponded to the molecular ions $[M-H]^-$ of 1,3-dicaffeoylquinic acid (**2**) and one isomer of this compound (**2'**) respectively, both assigned by comparison with the analytical standard **2**. Additionally, one peak at Rt 20.8 min was detected in the chromatograms. It showed the same m/z signal than Apigenin-7-O-glucuronide (**3'**), suggesting the presence of this compound in the bract extracts. All mentioned compounds have been previously described in other globe artichoke cultivars (Abu-Reidah *et al.*, 2013; Lombardo *et al.*, 2010; Pandino *et al.*, 2011) and were detected in bract extracts of GA, GU and OV (Table I).

TABLE I - Compounds detected by LC-HRMS in bract extracts and leaf extracts of Gauchito (GA), Gurí (GU) and Oro verde (OV) cultivars

Compounds in bract extracts	$R_{t\ (min)}$	Cultivars			Molecular Ion Formula $[M-H]^-$	m/z $[M-H]^-$ Theorical	m/z $[M-H]^-$ Measured	Error (ppm)
		GA	GU	OV				
Monocaffeoylquinic acid (**1'**)	6.0	+	+	+	$C_{16}H_{17}O_9$	353.0878	353.0866	2.9
Chlorogenic acid (**1**)	8.9	+	+	+	$C_{16}H_{17}O_9$	353.0878	353.0867	3.0
1,3-Dicaffeoylquinic acid (**2**)	17.6	+	+	+	$C_{25}H_{23}O_{12}$	515.1195	515.1188	1.4
Dicaffeoylquinic acid (**2'**)	19.2	+	+	+	$C_{25}H_{23}O_{12}$	515.1195	515.1172	4.5
Apigenin-glucuronide (**3'**)	20.8	+	+	+	$C_{21}H_{17}O_{11}$	445.0776	445.0755	4.7
Compounds in leaf extracts								
Chlorogenic acid (**1**)	6.9	+	+	+	$C_{16}H_{17}O_9$	353.0878	353.0867	3.0
1,3-Dicaffeoylquinic acid (**2**)	14.2	+	+	-	$C_{25}H_{23}O_{12}$	515.1195	515.1188	1.4
Luteolin-rutinoside (**4'**)	15.9	+	+	+	$C_{27}H_{29}O_{15}$	593.1512	593.1529	1.7
Luteolin-glucoside (**5'**)	16.9	+	+	+	$C_{21}H_{19}O_{11}$	447.0933	447.0920	2.8
Pinoresinol-glucoside (**6'**)	17.7	+	+	+	$C_{26}H_{30}O_{11}$	519.1872	519.1872	0.0
Apigenin-rutinoside (**7'**)	18.5	+	+	+	$C_{27}H_{29}O_{14}$	577.1563	577.1551	2.1

Retention time (Rt, in minutes). + (detected); – (not detected)

On the other hand, the leaf extract chromatograms showed two peaks with m/z signals corresponding to chlorogenic acid (**1**) and cynarin (**2**) at Rt 6.9 min and 14.2 min respectively. Interestingly, four peaks with retention times of 15.9, 16.9, 17.7 and 18.5 min, corresponding to the molecular ion signals $[M-H]^-$ with m/z values of 593.1529, 447.0920, 519.1872 and 577.1551 respectively, were detected in the extracted ion chromatograms of the leaf extracts (Table I). The m/z values were coincident with those described in literature for the molecular ions $[M-H]^-$ of luteolin-rutinoside (**4'**) and luteolin-glucoside (**5'**) (Pandino *et al*., 2011), pinoresinol-glucoside (**6'**) (Abu-Reidah *et al*., 2013) and apigenin-rutinoside (**7'**) (Lombardo *et al*., 2010; Pandino *et al*., 2011) respectively. All compounds described in the studied extracts were previously reported in other artichoke genotypes.

The content of caffeoylquinic acids was determined in the extracts. The amount of monocaffeoylquinic acids was calculated as chlorogenic acid. The content found in bracts (mg/kg) was 3608.0 ± 286.0 for GA, 604.5 ± 161.9 for GU and 582.7 ± 33.0 for OV. Dicaffeoylquinic acids were calculated as 1,3 dicaffeoylquinic acid and the content in bracts was 10064.5 ± 378.3 for GA, 3482.0 ± 11.5 for GU and 2561.0 ± 89.5 for OV. On the other hand, the amount of monocaffeoylquinic acid in leaves (mg/kg) was 1108.5 ± 41.7 for GA, 1206.5 ± 340.1 for GU and 456.0 ± 212.0 for OV, whereas the total amount of dicaffeoylquinic acids was 1451.0 ± 209.3 for GA and 148.5 ± 70.1 for GU. Dicaffeoylquinic acids could not be quantified in OV leaves. The total amount of caffeoylquinic acids was different among cultivars (χ^2 = 79.85; $p < 0.00001$) and GA showed the highest content.

The phenolic compounds present in artichoke have antioxidant activity (Llorach *et al*., 2002; Perez-Garcia, Adzet, Cañigueral, 2000). These natural antioxidants with redox properties are radical scavengers, reducing

agents or hydrogen donators (Jesionek, Majer-Dziedzic, Chroma, 2015; Kähkönen *et al*., 2005; Proestos *et al*., 2005). Free radical scavenging activity was studied in GA, GU and OV cultivars by TLC-DPPH assay. The leaf and bract extract chromatograms showed several light yellow dots on the purple background in the middle region of the chromatogram, which correlates with the characteristic TLC retention factors of caffeoylquinic acids and flavonoids, indicating the presence of DPPH scavengers in the three cultivars (Figure 2).

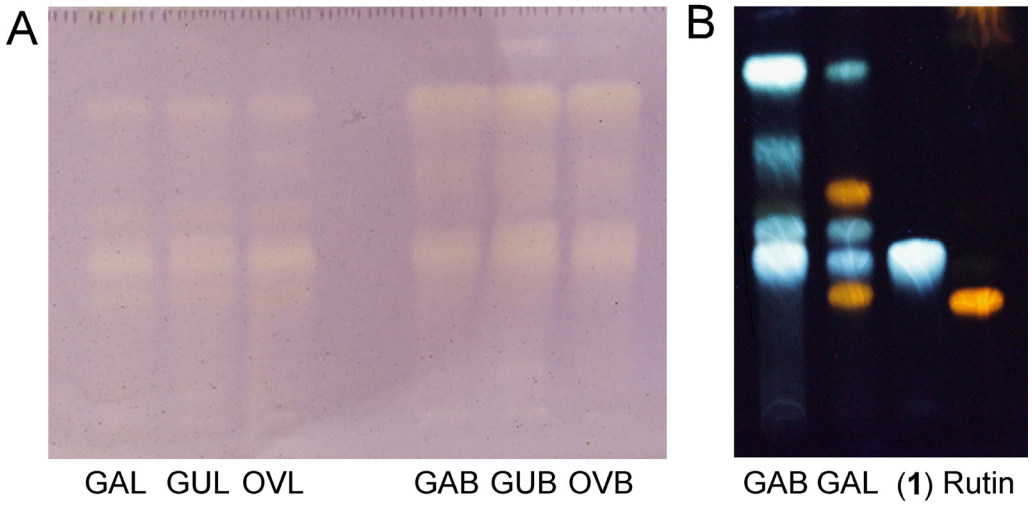

FIGURE 2 - A) DPPH-TLC autographic assay of leaf extracts from Gauchito (GAL), Guri (GUL) and Oro Verde (OVL) (left), and bract extracts from Gauchito (GAB), Guri (GUB) and Oro Verde (OVB) (right). B) TLC chromatogram of GAB and GAL extracts *versus* chlorogenic acid (1) and Rutin, revealed with Natural Product-PEG reagents. System solvent: ethyl acetate: formic acid: acetic acid: water (100:11:11:26).

On the other hand, neuroblastoma cells were treated with different concentrations of bract and leaf extracts of GA and GU cultivars or controls, and the cell viability was measured 72h after treatment. The extracts of OV cultivar were not used in the biological experiments based on the low content of caffeoylquinic acids detected previously. The results revealed that GA and GU leaf extracts (GAL and GUL respectively) significantly reduced the number of live cells. A dose-dependent reduction of cell viability was observed for GAL and GUL, showing positive effect even at the highest dilution evaluated (D500, 8 µg/mL). GAL exerted stronger effect than GUL, producing more than 60% decrease in the viability of SH-SY5Y cells (Figure 3). The concentration of mono- and di-caffeoylquinic acids in the leaf extracts at these dilutions was 0.11 and 0.15 µg/mL i n GA and 0.12 and 0.02 µg/mL in GU, while the bract extract of GA contained 0.36 and 1.01 µg/mL respectively. When these extracts were assessed on normal cell proliferation, none of the extracts showed effect on cell viability (Figure 4). However, Doxorubicin, an antineoplastic drug used in the treatment of several types of cancer, showed a 20% reduction on normal cell viability at a concentration of 0.2 µg /mL. Likewise, this anthracycline antibiotic with antineoplastic activity was reported to decrease more than 60% viability of SH-SY5Y cells when they were exposed to 0.1 µg/mL of drug (Almeida *et al*., 2018). Aimed at understanding if the observed effect on neuroblastoma cells was exerted by the caffeoylquinic acids or the flavonoids present in GA leaf extract, chlorogenic acid (1), 1,3-dicaffeoylquinic acid (2), apigenin (3) and luteolin (4) were tested with a concentration of 2 µg /mL (Figure 5). None of these pure compounds reproduced the activity observed for the leaf extracts of GA and GU cultivars, suggesting that the activity is due to the presence of several

compounds or bioactive non-phenolic compounds that were not identified in the extracts. Even though a more comprehensive study is needed, these results reveal that the Argentinian GA and GU artichokes studied in this work have the potential to inhibit the viability of neuroblastoma cells without affecting viability of normal cells. Therefore, both GA and GU cultivars could be good candidates for chemoprevention.

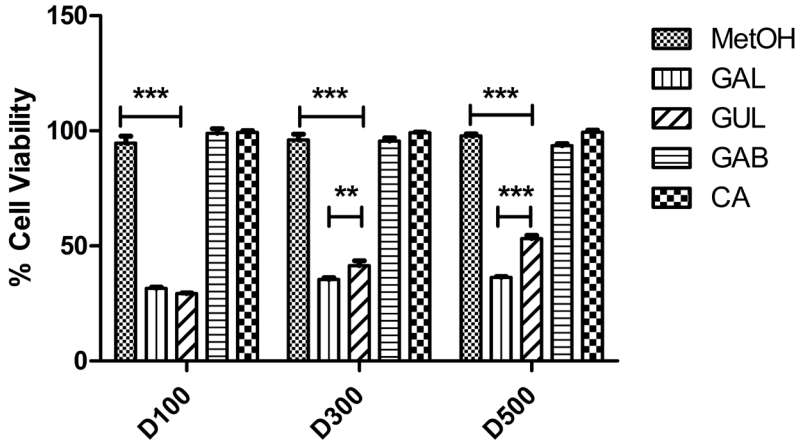

FIGURE 3 - Effect of Gauchito (GA) and Guri (GU) extracts on tumor cell viability. SH-SY5Y human neuroblastoma cells were treated for 72h with dilutions 1:100 (D100), 1:300 (D300) and 1:500 (D500) of vehicle (MeOH, negative Control), GA or GU leaf extracts (GAL and GUL; D100 = 40 µg/mL, D300 = 13 µg/mL, D500 = 8 µg/mL), GA bracts (GAB D100 = 15 µg/mL, D300 = 5 µg/mL, D500 = 3 µg/mL), chlorogenic acid (CA, D100 = 10 µg/mL, D300 = 3.3 µg/mL D500 = 2 µg/mL). Cell viability was evaluated by MTS assay. Mean ± SD is represented for each sample. The asterisks indicate the existence of a significant difference between the extracts and control (** $p < 0.01$ and *** $p < 0.001$ ANOVA statistical test followed by Bonferroni's post-test was applied).

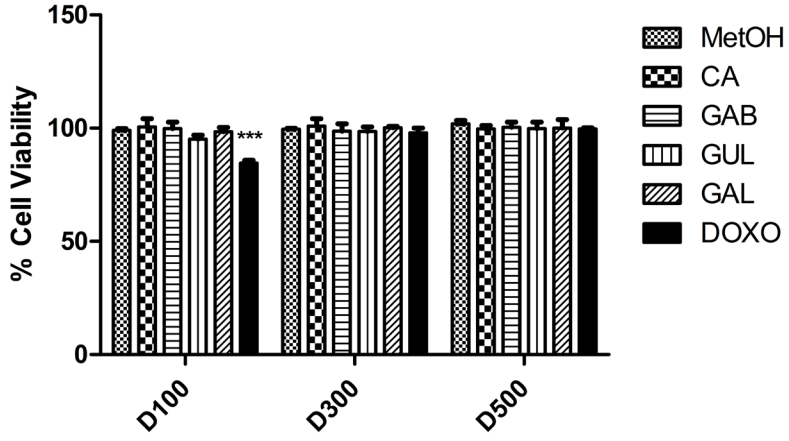

FIGURE 4 - Effect of Gauchito (GA) and Guri (GU) extracts on WISH human amnion cell viability. WISH human amnion cells were treated for 72h with dilutions 1:100 (D100), 1:300 (D300) and 1:500 (D500) of vehicle (MeOH, negative Control), GA or GU leaf extracts (GAL and GUL; D100 = 40 µg/mL, D300 = 13 µg/mL, D500 = 8 µg/mL), GA bracts (GAB D100 = 15 µg/mL, D300 = 5 µg/mL, D500 = 3 µg/mL), chlorogenic acid (CA, D100 = 10 µg/mL, D300 = 3.3 µg/mL D500 = 2 µg/mL). Doxorubicin (DOXO, D100 = 0.2 µg/mL; D300= 0.1 µg/mL and D500 = 0.05 µg/mL), was used as positive control. Cell viability was evaluated by MTS assay. Mean ± SD is represented for each sample. The asterisks indicate the existence of a significant difference between the extracts and control (** $p < 0.01$ and *** $p < 0.001$ ANOVA statistical test followed by Bonferroni's post-test was applied).

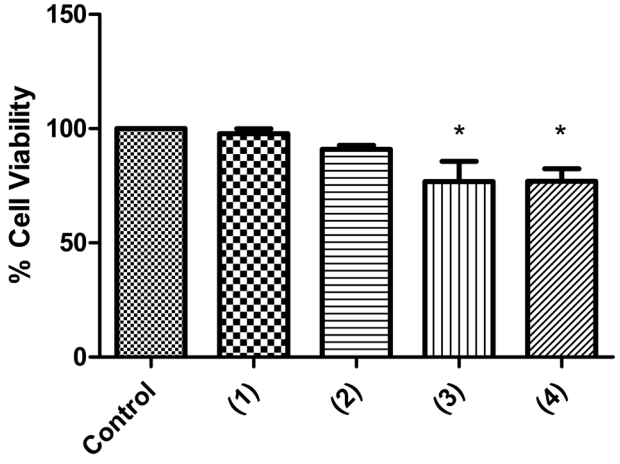

FIGURE 5 - Effect of pure compounds on tumor cell viability. SH-SY5Y human neuroblastoma cells were treated for 72h with analytical standards chlorogenic acid (1), 1,3-dicaffeoylquinic acid (2), apigenin (3) and luteolin (4), at a concentration of 2 µg/mL. Control (MeOH). The viability was evaluated by MTS assay. Mean ± SD is represented for each sample. The asterisks indicate the existence of a significant difference between the compounds and control ($p < 0.01$, ANOVA statistical test followed by Bonferroni's post-hoc test was applied).

Finally, a cell based reporter gene assay (RGA) designed to evaluate compounds that potentially modulate IFN-I activity was used to seek additional biological benefits of the studied artichoke cultivars. IFNs are central molecules of the immune system with pharmacological or pathological actions; finding natural compounds that modulate the action of IFN-I in order to overcome or control different diseases is a challenge that is attracting great attention in the drug discovery field. For this purpose, GA and GU extracts were tested in order to determine its effects on IFN-I response using the WISH-Mx2/EGFP cell line-derived RGA. Then, the percentage of EGFP expressing cells, quantified by flow cytometry, is directly correlated with IFN potency (Bürgi *et al*., 2012). The incubation of GA and GU leaf extracts with WISH-Mx2/EGFP cells for only 24h was enough to register a clear inhibition of the IFN-I activity (Figure **6**). This inhibition is evidenced by the decrease of EGFP fluorescence due to reduced activation of IFN-I activity (Bürgi *et al*., 2012). This effect of the artichoke extracts on IFN-I activity offers a very promising basis to do a more extensive and comprehensive study about Argentinean artichoke extracts as potential natural candidates for treatment of those diseases caused by IFN-I overproduction or hyper-activation. In this regard, it is interesting to note that chronic inflammation has been shown to cooperate with the development of several types of cancer (Ben-Neriah, Karin, 2011).

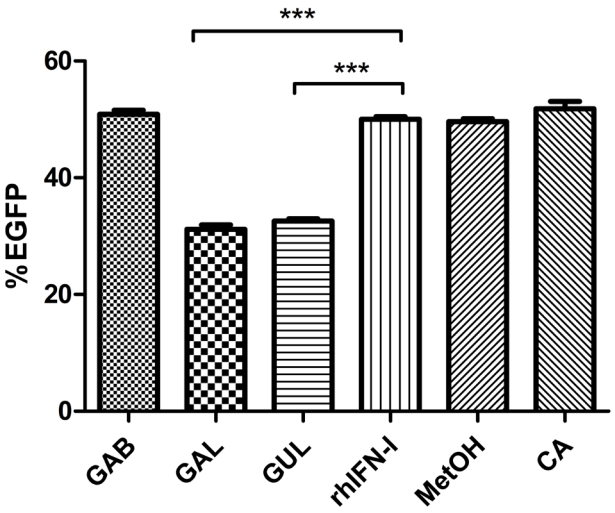

FIGURE 6 - Effect of Gauchito (GA) and Gurí (GU) extracts on the rhIFN-I activity evaluated by WISH-Mx2/EGFP RGA. The effect of each GA and GU extract was evaluated on the rhIFN-I activity trough a specific RGA and their effects were compared with the vehicle (MeOH, Control), chlorogenic acid (CA, 10 µg/mL) and human recombinant IFN type I (rhIFN-I control). Dilution 1:100 of extracts in MeOH (40 µg/mL) of GA or GU leaf extracts (GAL and GUL) and GA bract (GAB). The asterisks indicate the existence of a significant difference between the extracts and the positive control assay (rhIFN-I), (** $p < 0.01$ and *** $p < 0.001$ ANOVA statistical test followed by Bonferroni's post-test was applied).

In short, the three cultivars studied in this work have a good productive performance and present DPPH radical scavengers in their leaf and bract extracts. LC-HRMS results for GA and GU cultivars show mono- and di-caffeoylquinic acids in both edible bracts and leaves, but only the leaves show higher diversity of flavonoids exerting a remarkable cytotoxic effect on SH-SY5Y neuroblastoma cells without affecting the proliferation of normal cells. Moreover, GA and GU leaf extracts inhibit the action of IFN-I on a reporter cell line, suggesting their potential role as negative modulators of IFN-I biological

function. Even though further research is needed, our results suggest that the Argentinean GA and GU artichoke cultivars may well be exploited in pharmaceutical formulations to prevent pathological human signals related to cancer and chronic inflammation.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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