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Characterization of *Persea americana* Mill. peels and leaves extracts and analysis of its potential *in vitro* anti-inflammatory properties

[Caracterización de extractos de piel y hojas de *Persea americana* Mill. y analisis *in vitro* de sus potenciales propiedades anti-inflamatorias]

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Abstract: There is a worldwide trend of increasing prevalence of non-communicable diseases characterized by a chronic inflammatory state. Therefore, it is important to study the relationship between food and health. Avocado (*Persea americana*) stands out in food industry for its nutritional value. Industrials process of avocado generates a large number of by-products, which contain phytochemical compounds with antioxidant properties, such as polyphenols. The objective of the present research was to characterize four aqueous and hydroalcoholic extracts from avocado leaves and peels and analyze it possible anti-inflammatory properties *in vitro*. Total polyphenol content (with the Folin-Ciocalteau method) and antioxidant capacity (by FRAP and DPPH) were determined. Extracts inflammatory features were measured by NO and TNF-α release, and by TNF-α gene expression. Our results indicated that hydroalcoholic extracts present higher total polyphenol content (p<0.001) and antioxidant capacity (p<0.001, by FRAP) than the aqueous ones. Furthermore, we report that hydroalcoholic leaves extract presented greater *in vitro* anti-inflammatory effect, especially the leave hydroalcoholic regarding NO release (p<0.001, against LPS treatment), aqueous and hydroalcoholics regarding TNF-α release (p<0.05), and only the hydroalcoholic in the TNF-α gene expression (p<0.01). In conclusion, the avocado hydroalcoholic extracts, and especially from leaves, present *in vitro* anti-inflammatory features that might be considered for human health improvement applications.

Keywords: *Persea americana*; Avocado; Antioxidant; Polyphenols; Anti-inflammatory features.

Resumen: Existe una tendencia mundial de incremento en prevalencia de enfermedades no transmisibles, que se caracterizan por un estado pro-inflamatorio crónico. Por lo tanto, es importante estudiar la relación entre alimentos y salud. La palta (*Persea americana*), sobresale en la industria por su valor nutricional. El procesamiento de la palta genera gran cantidad de subproductos, que contienen bioactivos con propiedades beneficiosas, como polifenoles. El objetivo del presente trabajo fue caracterizar cuatro extractos de palto (acuoso e hidroalcohólico; de hoja y de cáscara) y analizar sus posibles propiedades anti-inflamatorias *in vitro*. Fueron determinados polifenoles totales (con el método de Folin–Ciocalteau) y capacidad antioxidante (por FRAP y DPPH) de los extractos. Las propiedades anti-inflamatorias de los extractos fueron determinadas por la liberación de NO y de TNF-α, y por la expresión génica de TNF-α. Los resultados indican que los extractos hidroalcohólicos presentan más polifenoles (p<0,001) y capacidad antioxidante (p<0,001, por FRAP) que los acuosos. Mas aún, observamos que los extractos hidroalcohólicos de hojas presentaron mayores efectos anti-inflamatorios *in vitro*, especialmente el hidroalcohólico de hoja en liberación de NO (p<0,001, frente a tratamiento con LPS), acuosos e hidroalcohólicos en liberación de TNF-α (p<0,05), y solo los hidroalcohólicos en la expresión de TNF-α (p<0,01). En conclusión, los extractos hidroalcohólicos de palto, y especialmente el de hoja, presentan propiedades anti-inflamatorias *in vitro* que pueden ser consideradas para aplicaciones en mejoría de salud humana.

Palabras clave: *Persea americana*; Palto; Antioxidante; Polifenoles; Capacidad anti-inflamatoria

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INTRODUCTION

During inflammatory processes, the release of pro-inflammatory mediators that are co-stimulated by signs of endogenous or exogenous origin are triggered. This phenomenon modifies the response of immune cells and can produce a clinic state termed systemic inflammatory response syndrome (Blancas-Flores *et al*., 2010). The increased prevalence of non-communicable diseases (NCDs) (WHO, 2010), which are characterized for present a systemic inflammatory state related with feeding, such as cardiovascular diseases, diabetes mellitus type 2, arterial hypertension and dyslipidemia, has led to a bigger and growing interest in studying the relationships between food properties and health (Araya *et al*., 2006).

Avocado (*Persea americana*), also known as aguacate, is a tropical American fruit native from Central America and Mexico. It is produced by an evergreen tree (15-20 m of height). Avocado it has been a staple dietary component for at least 9.000 years. The genus *Persea* (Clus.) Miller belongs to the family *Lauraceae*. Avocado is a specie with several taxa or subspecies, which include the Mexican (*P. americana* var. drymifolia), West Indian or Antillean (*P. americana* var. americana) and Guatemalan (*P. nubigena* var. nubigena and *P. nubigena* var. guatemalensis) botanical varieties. Each variety presents typical characteristics in terms of leaves, fruits, flowering period, etc. In Chile, it grows from I to VII region, being the world's second largest avocado producer after Mexico (Bravo, 2010). Avocado fruit is rich in monounsaturated fatty acids (especially oleic acid), dietary fiber, vitamins B and E, and other nutrients (Wang *et al*., 2010). From all the avocado varieties, *Persea americana* Mill var. Hass, is the most consumed worldwide (USDA, 2011). Avocado consumption has been associated with several benefits for health, related to improved diet quality and nutrients intake, leading to lower risk of NCDs such as metabolic syndrome (Fulgoni *et al*., 2013; Tabeshpour *et al*., 2017).

In addition to the nutritional values of its fruits, the leaves and other morphological parts of avocado possess therapeutic properties, and are have been widely used for centuries in ancient cultures or folk medicine, generally as infusions (Hurtado-Fernandez *et al*., 2017), for their biological activities relating to control and/or treatment of anemia, diabetes mellitus, gastritis and bronchitis (Ross, 2001). In this context, leaves extract treatments have shown anti-hypertensive (Adeboye *et al*., 1999; Owolabi *et al*., 2005), hypoglycemic (Antia *et al*., 2005), analgesic and also anti-inflammatory effects (Adeyemi *et al*., 2002).

Peels and leaves contain several bioactive compounds, including polyphenols (Torres *et al*., 1987) such as procyanidins (Wang *et al*., 2010; Chavez *et al*., 2011; Kosinska *et al*., 2012), chlorogenic acid, quercetins (Kosinska *et al*., 2012), and epi(catechins) (Hirasawa *et al*., 2008; Chavez *et al*., 2011; Kosinska *et al*., 2012).

Wang *et al.* (2010), reported that peels and pulp from Hass avocado present higher content of polyphenols and antioxidant capacity related to other varieties studied. Similar results found Kosinska *et al.* (2012), regarding antioxidant capacity measured by TEAC, ORAC, DPPH and FRAP assays. Besides, Hass avocado variety shows the highest phenolic compounds content in its seed (64%), followed by peels (23%) and pulp (13%). Therefore, by-products of avocado (leaves and peels) present upper antioxidant activity with respect to pulp (Wang *et al*., 2010) and other fruits already known by its high antioxidant capacity (Wu *et al*., 2004).

The avocado industrial processing generates by-products such as leaves (pruning) and peels, both without commercial value. According to the latter stated, these could be used as potential sources of natural antioxidants or functional food ingredients (Kosinska *et al*., 2012), thus generating new products, granting an added value to avocado industry (Wang *et al*., 2010). Therefore, the objective of the present study was to characterize and analyze the anti-inflammatory properties of aqueous and hydroalcoholic avocado (*Persea americana Mill* var. Hass) extracts from leaves and peels on an *in vitro* model.

MATERIALS AND METHODS

Avocado products

Avocado (*P. americana* cv. Hass) leaves were collected in January 2017 from farm located in Peñaflor (33º35’35’’ latitude; 70º52’48’’ longitude), Valparaiso, Chile. Avocado peels were purchased from a supermarket in Santiago, Region Metropolitana, Chile.

Preparation of avocado extracts var. Hass (aqueous and hydroalcoholic) from leaves and peels

Separately, avocado leaves (50 g) and peels (60 g) were scalded at 95ºC for 4 min and then cooled off

quickly in a cold-water spurt. Then, samples were dried at 45ºC for 18 h on an air forced oven (WTE, Germany) and stored in plastic bags at room temperature in the dark until extract preparation. Dry avocado leaves and peels were pulverized and macerated with ethanol/water (1:1). The prepared extracts were aqueous leaves (AL), aqueous peels (AP), hydroalcoholic leaves (HL) and hydroalcoholic peels (HP) extracts. In order to obtain the hydroalcoholic extracts (HL and HP, from 6,6 and 6,8 g, respectively) or with water to obtain the aqueous extracts (AL and AP, from 5,5 and 5,2 g, respectively), all extracts were dryed for 72 h at room temperature. Then, the extract was separated by filtration. Finally, each extract was carried to a final volume of 250 mL and frozen at -20ºC.

Total polyphenol content of avocado extracts

The total polyphenol content (TPC) was determined by Folin–Ciocalteau colorimetric method (Singleton *et al.*, 1965) and expressed as mg of gallic acid equivalents (GAE) per gram of dry sample (DS), according to a calibration curve (200-600 $\mu g \cdot mL^{-1}$, R^2: 0.993). All experiments were performed in triplicate.

Measurement of the antioxidant activity of avocado extracts

The antioxidant activity was carried out by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Brand-Williams *et al.*, 1995) and Ferric reducing antioxidant power (FRAP) (Benzie *et al.*, 1996), using a spectrophotometer (UV/Vis Lambda 25, Perkin Elmer, Cambridge, UK) at 517 and 593 nm respectively. Results were expressed as half maximal inhibitory concentration (IC_{50}, mg per gram of DS) for DPPH method and mmol Fe^{+2} per 100 g of DS for FRAP. All experiments were performed in triplicate.

HPLC-DAD analysis

The analyses were conducted using an Agilent 1100 HPLC (Agilent Technologies Inc., USA) system coupled with an Esquire 4000 ion trap LC/MS system (Bruker Daltoniks, Germany), using a C18 column (5 μm, 4.6 mm i.d x 25 cm, Spherisorb ODS-2, Waters, Ireland). The mobile phase was formic acid in water (0.34% v/v, solvent A) and acetonitrile (solvent B) at a flow rate of 1 mL/min according to the following elution gradient: 0–3 min, 7.3% B; 3–73, 7.3% B; 73–80 min, 35% B; 80–85 min, 70% B; 85–88 min, 70% B; 88–90 min, 7.3%. The total analysis time was 90 min, and 5 min was required for reestablishing and equilibrating the initial conditions. Phenolic compounds were detected at 280 nm. The mass spectral data were acquired in negative mode; ionization (nebulization) was performed with nitrogen as drying gas at 50 psi, 365ºC and at a flow rate of 10 L/min and capillary voltage 3000 V. All scans were performed in the range 50–1400 m/z. The trap parameters were set in ion charge control using manufacturer default parameters. Collision induced dissociation was performed by collisions with the helium background gas present in the trap. Fragmentation was set with Smart Frag.

Cell culture

All procedures were performed as previously described (Reyes-Farias *et al.*, 2014). The mouse macrophage RAW264.7 cell line (obtained from the Laboratory of Biochemistry, Metabolism and Drug Resistance of Institute of Biomedical Science –ICBM– of University of Chile), was used. Cells were maintained at standard cell culture conditions (37ºC in a humidified atmosphere containing 5% CO_2) and were cultured in DMEM (Invitrogen, Paisley, UK) containing 4.5 $g \cdot L^{-1}$ glucose, 10% fetal bovine serum (Biological Industries, Beit Haemek, Israel) and Penicillin ($1x10^5$ $U \cdot L^{-1}$) and Streptomycin (0,1 $g \cdot L^{-1}$) antibiotics (Corning Life Sciences, USA). For experiments, cells were seeded at 70.000 $cells \cdot [cm^2]^{-1}$ in 12-well plates. Macrophages were pre-treated with each extract at a 100 $mol \cdot L^{-1}$ [total polyphenols] for 1 hour, and then cultured with 5 $mg \cdot L^{-1}$ of lipopolysaccharide (LPS) (Sigma-Aldrich Chemical Co., Saint Louis, USA) for 24 hours. At the end of the trials, culture media were collected inflammatory response evaluation.

Determination of secretion of inflammatory markers

The amount of nitrite in cell-free culture supernatants was measured using Griess reagent according to the manufacturer's protocol (working range 0.43–65 µM nitrite) (Sigma-Aldrich, St. Louis, USA). Briefly, 50 µL of supernatant was mixed with an equivalent volume of the Griess reagent in a 96-well flat bottom plate. After a 30 min incubation in darkness, absorbance at 540 nm was measured using a NanoQuant InfiniteM200 PRO spectrophotometer (Tecan, Männedorf, Switzerland). The amount of nitrite was calculated from a $NaNO_2$ standard curve. On the other hand, TNF-α secretion to culture media

was measured using a MILLIPLEX MAP Mouse Adipokine Magnetic Bead Panel (Merck Millipore, Billerica, MA, USA), according to manufacturer's protocol. This determination was performed using Luminex xMAP technology (Merck Millipore) at the Department of Virology from the Institute of Biomedical Science (ICBM, University of Chile). Results were corrected by cell viability.

Lactate Dehydrogenase assay

Cellular viability was determined according to the activity of lactate dehydrogenase (LDH) enzyme according to a commercial kit (Cayman Chemical Company, Ann Arbor, USA).

Gene expression assay

All procedures were as previously described with modifications (Reyes-Farias *et al*., 2014). Total RNA was isolated from samples using Trizol (Invitrogen, Paisley, United Kingdom), according to the supplier's protocol. Purified RNA was then treated with DNase (DNAfree kit; Ambion, Austin, USA) and used to generate first-strand cDNA with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, USA). The resultant cDNA was amplified with a specific probe for mouse TNF-α (Mn0044326061) in a total volume of 10 μL. Real-time PCR was performed in a Stratagene Mx3000P System (Agilent Technologies) following the manufacturer's recommendations (Applied Biosystems, Foster City, USA). The expression levels of the target gene studied was normalized by the expression of Cyclophilin A (Mn0232430) as the selected internal control (also supplied by Applied Biosystems). Fold change between groups was calculated by the $2^{-\Delta\Delta CT}$ method.

Statistical analyses

Statistical analysis was performed by one-way analysis of variance (one-way ANOVA) followed by Student-Newman-Keuls's post hoc test, and by two-way ANOVA. The results are expressed as mean ± standard deviation (SD). Differences were considered statistically significant at the $p<0.05$ level. Software GraphPad Prism version 6.0 was used for data analysis (GraphPad Prism Inc., San Diego, USA).

RESULTS

Characterization of avocado extracts

Table No. 1 shows the total polyphenols content (TPC) and antioxidant activity (DPPH and FRAP) of avocado extracts (AL, AP, HL and HP) studied. Highest TPC were observed in hydroalcoholic extracts than in aqueous ones ($p<0.001$). Among leaves and peels, higher TPC content was found in the latter ($p<0.01$ on aqueous extracts, $p<0.001$ on hydroalcoholic extracts). The antioxidant capacity measured by DPPH assay is inversely proportional to the value of IC_{50}, since the higher the antioxidant capacity of the extract, the lower concentration it is required to stabilize the radical DPPH. According to this methodology, the hydroalcoholic extracts showed higher antioxidant capacity than the aqueous extracts, and among the latter, peels extract showed the lower IC_{50} values ($p<0.001$ on peels between AP and HP). Finally, the antioxidant capacity measured by FRAP assay showed similar results than the ones obtain by DPPH, but even more higher antioxidant activity on hydroalcoholic extracts ($p<0.01$). Finally, Tables 1S-4S and Figures 1S-4S (supplementary files) shows the identification of phenolic compounds of AP, AL, HP and HL, respectively.

Table No. 1
Total phenolic content and antioxidant capacity of avocado extracts

	Total polyphenolic content	Antioxidant activity	
	Folin-Ciocalteau [mg GAE per gram of DS]	DPPH [IC_{50}: mg per gram of DS]	FRAP [mmol Fe^{+2} per 100 g of DS]
AL	36.03 ± 0.18[a]	3.07 ± 0.27[b]	10.46 ± 0.00[a]
AP	51.60 ± 0.06[b]	4.35 ± 0.16[c]	11.59 ± 0.02[a]
HL	69.09 ± 0.87[c]	2.30 ± 0.30[ab]	20.61 ± 1.14[b]
HP	75.52 ± 0.98[d]	1.73 ± 0.15[a]	18.33 ± 0.18[b]

AL: aqueous leaves extract; AP: aqueous peel extract; HL: hydroalcoholic leaves extract; HP: hydroalcoholic peel extract; n=2; Mean value within each group with different letters (a, b, c and d) indicated significant difference at *p*<0.05. GAE: Gallic Acid Equivalents; DS: Dry Sample

Inflammatory response induced by LPS

The pretreatment of macrophages with AL extract ($p<0.01$), and not with AP, inhibited the release of NO regarding the response induced by LPS (Figure No. 1). HP increased ($p<0.001$) and HL reduced ($p<0.01$) significantly the NO release when compared to LPS only treated cells. On the other hand, TNF-α gene expression was significantly increased by the LPS treatment ($p<0.05$), being this effect partially prevented by the treatment with all the extracts tested (Figure No. 2A). No differences were observed regarding TNF-α protein release (Figure No. 2B). In order to determine if there is a relationship among the type of extraction used and the type of avocado by-product in all the variables analyzed, we performed two-way ANOVA. We observed a significant effect of the by-product used ($p<0.001$) and also an interaction between both factors ($p<0.001$). Thus, we grouped and plot data in terms of type of extraction (hydroalcoholic or aqueous) and by-product (peel or leaves) (Figure No. 3). It was observed a higher anti-inflammatory effect of hydroalcoholic extracts (HA), regarding TNF-α gene expression ($p<0.01$) (Figure No. 3B), and from both types of extraction regarding TNF-α secretion ($p<0.05$) (Figure No. 3C). Finally, it was observed an anti-inflammatory effect of leaves extracts (LV), regarding NO release ($p<0.01$) (Figure No. 3D) and a mild effect from both types of by-products extracts on TNF-α gene expression ($p<0.05$) (Figure No. 3E).

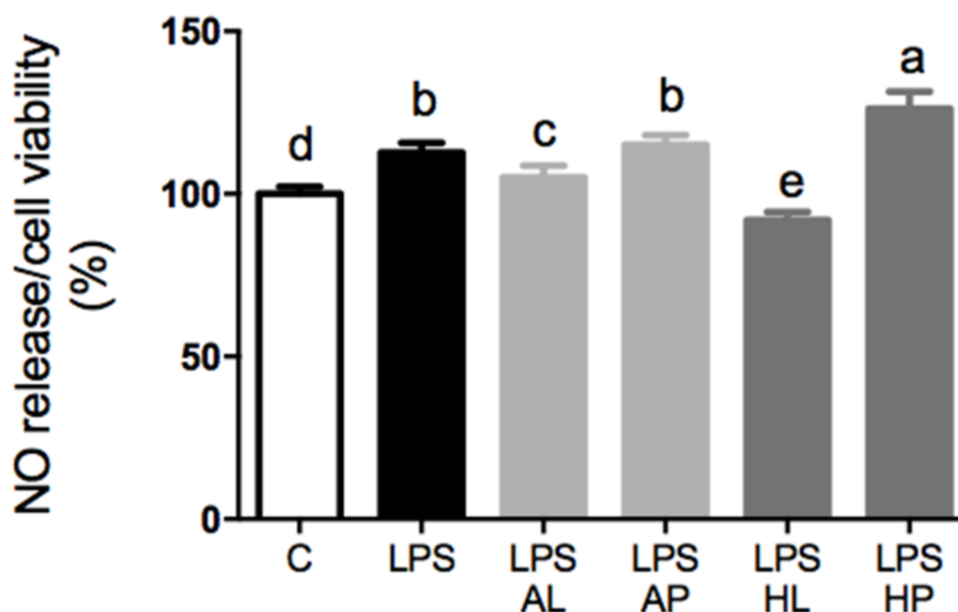

Figure No. 1

Percentage of nitric oxide release from activated mouse macrophages corrected for the percentage of cell viability. Macrophages were pre-treated with each extract 100 mol·L-1 [total polyphenols] for 1 hour, and then activated with 5 mg·L-1 of LPS for 24 hours. Values (n=4) were expressed as mean ± SD. One-way ANOVA followed by Student-Newman-Keuls's post hoc test was performed to determinate statistical differences among groups. NO, nitric oxide; LPS, lipopolysaccharide; AL, aqueous leaves extract; AP, aqueous peel extract; HL, hydroalcoholic leaves extract; HP, hydroalcoholic peel extract. Different letters meant statistical significance of at least *p*<0.05.

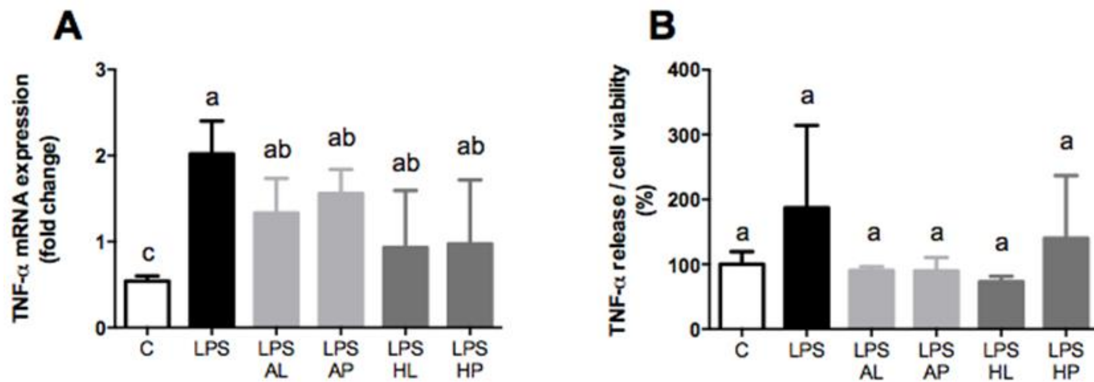

Figure No. 2

(A) TNF-α gene expression and (B) protein release, by activated macrophages. Macrophages were pre-treated with each extract 100 mol·L^{-1} [total polyphenols] for 1 hour, and then activated with 5 mg·L^{-1} of LPS for 24 hours. Values (n=4) were expressed as mean ± SD. One-way ANOVA followed by Student-Newman-Keuls's post hoc test was performed to determinate statistical differences among groups. NO, nitric oxide; LPS, lipopolysaccharide; AL, aqueous leaves extract; AP, aqueous peel extract; HL, hydroalcoholic leaves extract; HP, hydroalcoholic peel extract. Different letters meant statistical significance of at least *p*<0.05.

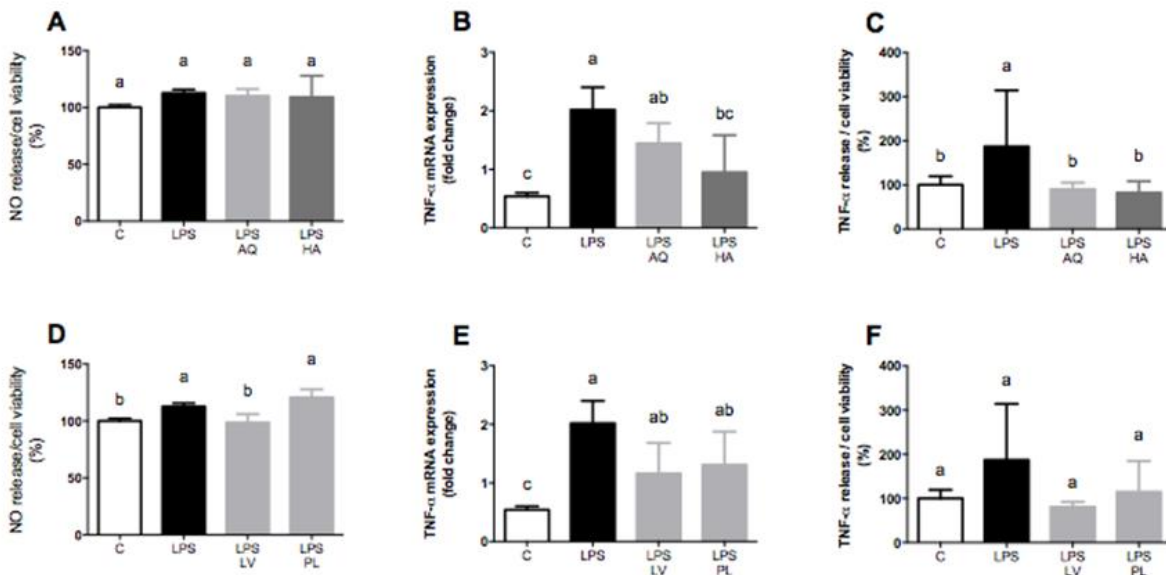

Figure No. 3

Data analysis for NO release, TNF-α gene expression and TNF-α protein grouped by type of extraction (A, B and C; respectively), and by type of by-product (D, E and F, respectively). Macrophages were pre-treated with each extract 100 mol·L^{-1} [total polyphenols] for 1 hour, and then activated with 5 mg·L^{-1} of LPS for 24 hours. Values (n=4) were expressed as mean ± SD. One-way ANOVA followed by Student-Newman-Keuls's post hoc test was performed to determinate statistical differences among groups. NO, nitric oxide; LPS, lipopolysaccharide; AQ, aqueous extracts; HA, hydroalcoholic extracts; LV, leaves extracts; PL, peel extracts. Different letters meant statistical significance of at least *p*<0.05.

DISCUSSION

Avocado by-products have been described to present elevated content of antioxidant molecules, and also, its consumption have been related to better health outcomes. In the present article we aimed to evaluate the possible anti-inflammatory effects of these raw material.

Hydroalcoholic extracts (especially HP), presented total phenolic content significantly higher than aqueous extracts. These differences could be attributed to avocado by-products *per se* (chemical nature of phenolic compounds) and the polarity of solvents used (Naczk *et al*., 2006). Alothman *et al*. (2009) and Turkmen *et al*. (2006), reported in tea and some fruits that 50% ethanol-water mix takes advantage over the phenolic compounds extraction by aqueous solvent, being this reflected in an increase of the total polyphenol content. In the present study, total polyphenol content in peels extracts were higher than those reported by Wang *et al.* (2010), (12,6 mg GAE/g, respectively), but similar to those described by Chávez *et al.* (2011), (32,93 to 89,97 mg GAE/g dry matter). Regarding leaves extracts, Torres *et al.* (1987), found contents of 17,50 to 19,30 GAE mg/g, expressed as fresh weight, in young and mature leaves of Hass variety, being this value lower than the ones observed here. The differences with other authors could be explained by cultivar differences, geographical location, collecting period, extraction method, avocado by-products type (peels or leaves), among others.

As well as in TPC, hydroalcoholic extracts showed the highest antioxidant activity, determined by DPPH and FRAP assays. This activity is higher than those reported for pulp extracts in previous studies (Wang *et al*., 2010). Therefore, it is possible to postulate peels and leaves of this specie as candidates for further investigation regarding disease prevention with a strong oxidative and inflammatory component, such as NCDs. However, it is worth to mention that no differences were observed between peels and leaves extracts, among aqueous and hydroalcoholic extracts. Thus, a differential biological effect by its application could be ascribed to differential compound content. Adeyemi *et al.* (2002), reported anti-inflammatory activity of aqueous extracts from fresh leaves of *Persea americana* on an *in vivo* model with induced sub-plantar tissue inflammation. Thus, as a first approximation, we studied the anti-inflammatory effect from avocado extracts over an inflammation cell model composed by macrophages activated with LPS. We observed that, overall, hydroalcoholic rather than aqueous and leaves rather than peel extracts presented higher anti-inflammatory features. The outcome regarding hydroalcoholic extracts is easy to explain taking into account the polarity of the extraction. The outcome related to type of by-product could be explained by a possible differential composition of compounds in each extract that could have an influence in a biological function basis. Torres *et al.* (2014), evaluated the potential pest control of avocado peels, seed and pulp extracts. These authors found that seed, followed closely by peels extracts, present high larvicidal activity. They described in a seed ethanol extract the presence of alkaloids, tannins, saponins, unsaturated steroids and triterpenoids, 2-deoxysugars, flavonoids (leucoantho-cyanins), fats and oils. Perhaps the peels present some compounds at high concentration that were extracted only in water: ethanol condition, which present pro-inflammatory features (e.g. saponins). Moreover, we observed that hydroalcoholic peels extracts present mainly procyanidins dimers and trimers, and hydroalcoholic leaves extracts present procyanidins trimers and also chlorogenic acid (supplementary data). In this sense, procyanidin dimers and trimers have been reported to impair the inflammatory response in human monocytes (Terra *et al*., 2011). However, procyanidins trimers have been observed to induce higher NO release by rat aortic endothelial cells (Byun *et al*., 2012), and even to activate murine macrophages (Sung *et al*., 2013). Moreover, it has been described that procyanidins monomers and dimers from a french maritime pine bark extract repressed the expression of several anti-inflammatory markers (NO, TNF-α and others), whereas a specific trimer (C2), enhanced these variables in murine macrophages (Park *et al*., 2000). On the other hand, the anti-inflammatory effects of chlorogenic acids have been clearly established before (Liang *et al*., 2016). In fact, it has been reported a strong anti-inflammatory activity over LPS-induced RAW264.7 macrophages, inhibiting the production of NO, IL-1, TNF-α and the expression of COX-2 and iNOS (Hwang *et al*., 2014). Thus, it would be expected an enhanced anti-inflammatory response in extracts that present this compound.

CONCLUSIONS

In conclusion, the avocado hydroalcoholic extracts, especially from leaves, present interesting *in vitro*

anti-inflammatory features that might be considered for human health improvement applications. Thus, the next research step could encompass the study of avocado by-products in greater detail (with special attention in leaves) over different pathological models, in which inflammation plays a central role, in order to evaluate and verify the anti-inflammatory effect here reported. Therefore, could be of value to get a more detailed characterization of polyphenols from these extracts, in order to determine which of them are responsible for the antioxidant and anti-inflammatory response observed, the evaluation of possible synergy, and the optimal doses and routes of administration to enhance health benefits.

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SUPPLEMENTARY MATERIAL

Table No. 1S

Identification of phenolic compounds of aqueous peel extract (AP) by LC–MS

Peak	Precursor m/z $[M+H]^+$	Compound	Precursor m/z $[M+H]^-$	Compound
17	867.2	B-type procyanidin dimer	865.5	B-type procyanidin dimer
			353.1	Caffeoylquinic acid
19	578.9	B-type procyanidin dimer	577.3	B-type procyanidin dimer
			449.7	Eriodictyol-O-hexoside
21	577.8	A-type procyanidin dimer	289.2	Epicatechin
24	867.2	B-type procyanidin trimer	865.5	B-type procyanidin trimer
			561.7	(Epi)afzelechin-(epi)catechin
25	866.5	B-type procyanidin trimer	865.1	B-type procyanidin trimer
	578.3	B-type procyanidin dimer		

Table No. 2S

Identification of phenolic compounds of aqueous leaves extract (AL) by LC–MS

Peak	Precursor m/z $[M+H]^+$	Compound	Precursor m/z $[M+H]^-$	Compound
5			353.3	Caffeoylquinic acid
15	579.1	B-type procyanidin dimer	577.2	B-type procyanidin dimer
			353.1	Chlorogenic acid

Table No. 3S

Identification of phenolic compounds of hydroalcoholic peel extract (HP) extract by LC–MS

Peak	Precursor m/z [M+H]+	Compound	Precursor m/z [M+H]-	Compound
15	578.8	B-type procyanidin dimer	577.4	B-type procyanidin dimer
			353.1	Chlorogenic acid
17	579.0	B-type procyanidin dimer	577.3	B-type procyanidin dimer
			449.6	eriodictyol -O- hexoside
19	866.9	B-type procyanidin dimer	289.2	Epicatechin
	449.8	Kaempferol-O-hexoside		
21	867.1	B-type procyanidin trimer	867.1	B-type procyanidin trimer
23	866.9	B-type procyanidin trimer	865.0	B-type procyanidin trimer
			577.2	B-type procyanidin dimer

Table No. 4S
Identification of phenolic compounds of hydroalcoholic leaves extract (HL) by LC–MS

Peak	Precursor m/z [M+H]+	Compound	Precursor m/z [M+H]-	Compound
6			190.5	caffeoylquinic acid
14	577.9	B-type procyanidin dimer	706.8	Dicaffeoylquinic acid dimer
			577.2	B-type procyanidin dimer
15	867.1	B-type procyanidin trimer	352.8	caffeoylquinic acid dimer
			190.5	caffeoylquinic acid
18	578.8	B-type procyanidin dimer	577.3	B-type procyanidin dimer
21	866.4	B-type procyanidin trimer		
25			865.6	B-type procyanidin trimer
			561.7	Dímero (epi)afzelequina-(epi)catequina
31	627.0	Quercetin-O-dihexoside	625.5	Quercetin-O-dihexoside
41	479.0	Quercetin-O-glucuronide	477.1	Quercetin-O-glucuronide
42	464.9	Quercetin-O-hexoside	515.2	Dicaffeoylquinic acid
			463.3	Quercetin-O-hexoside

Figure No. 1S
HPLC-UV chromatogram at 280 nm of aqueous peel extract (AP)

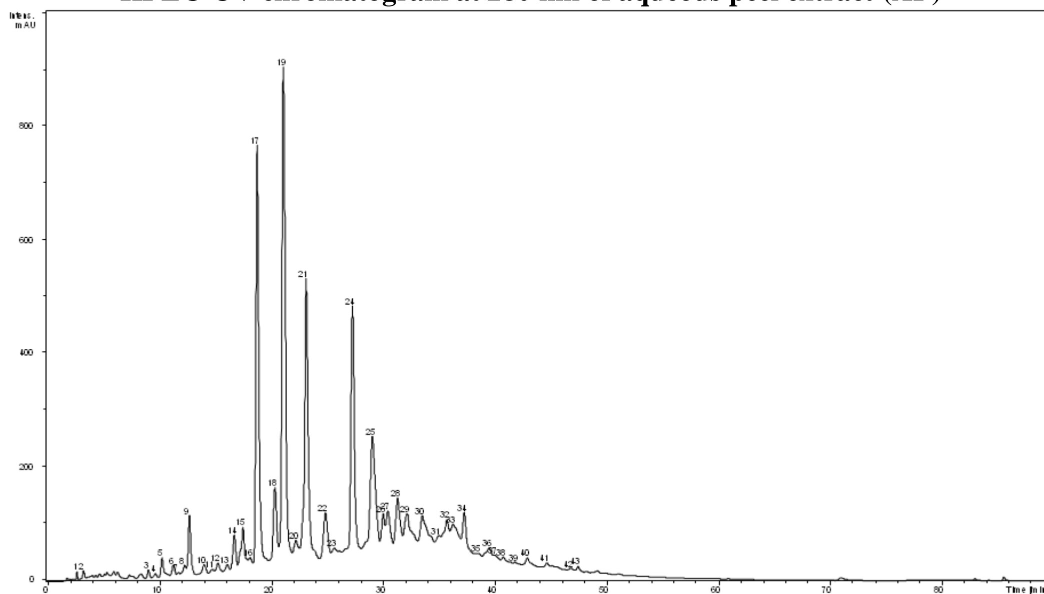

Figure No. 2S
HPLC-UV chromatogram at 280 nm of aqueous leaves extract (AL)

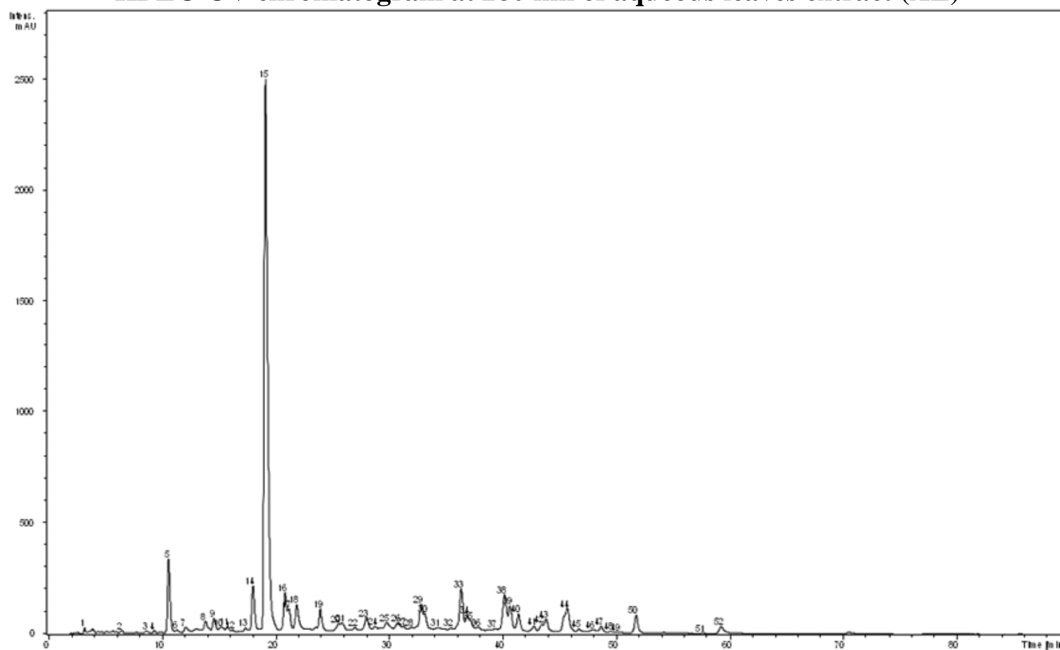

Figure No. 3S
HPLC-UV chromatogram at 280 nm of hydroalcoholic peel extract (HP)

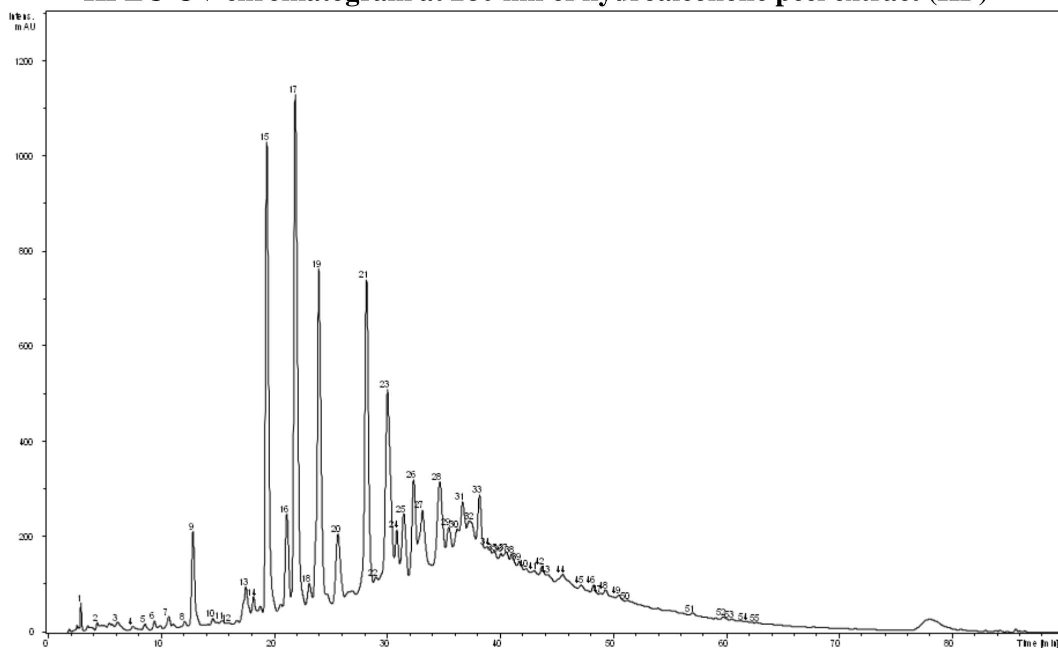

Figure No. 4S
HPLC-UV chromatogram at 280 nm of hydroalcoholic leaves extract (HL)