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Differential biological activity of two extracts of ripe fruits of *Carica candamarcensis* Hook on human neutrophils

[Actividad biologica diferencial de dos extractos de frutos maduros de *Carica candamarcensis* Hook en neutrófilos humanos]

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Abstract: We evaluated the effect of the total macerate (TM) and seed oil (SO) of mature *Carica candamarcensis* fruits, on the release of Matrix metalloproteinase 9 (MMP9) and the phosphorylation of MAPK in neutrophils. The antioxidant capacity of these extracts was evaluated by ABTS assay. Neutrophils stimulated with different dilutions of TM or SO were analyzed for cytotoxicity, MMP9 release, and MAPK phosphorylation, using trypan blue exclusion assays, zymography, and immunoblotting, respectively. Both extracts show antioxidant activity, being higher in TM; none presented cytotoxic effect. The 5% and 2.5% dilutions of TM significantly reduced MMP9 release, and all decreased MAPK phosphorylation. SO significantly increased the release of MMP9 and MAPK phosphorylation, the effect being greater when they were prestimulated with lipopolysaccharide.TM may have anti-inflammatory potential, while SO could have a priming effect that needs to be confirmed.

Keywords: *Carica candamarcensis*; Vegetable extracts; Neutrophils, Matrix metalloproteinase 9, MAPK

Resumen: Evaluamos el efecto del macerado total (MT) y aceite de semillas (AV) de frutos maduros de *Carica candamarcensis*, en la liberación de Matriz metaloproteinasa 9 (MMP9) y la fosforilación de MAPK en neutrófilos. La capacidad antioxidante de estos extractos se evaluó por ensayo ABTS. En neutrófilos estimulados con diferentes diluciones de MT o AV se analizó la citotoxicidad, liberación de MMP9 y fosforilación de MAPK, mediante ensayos de exclusión con azul de tripano, zimografía e inmunotransferencia, respectivamente. Ambos extractos muestran actividad antioxidante, siendo mayor en MT; ninguno presentó efecto citotóxico. Las diluciones 5% y 2,5% de MT redujeron significativamente la liberación de MMP9, y todas disminuyeron la fosforilación de MAPK. El AV incrementó significativamente la liberación de MMP9 y la fosforilación de MAPK, el efecto fue mayor cuando se preestimularon con lipopolisacárido. El MT puede tener potencial antiinflamatorio, mientras que el AV podría tener un efecto "priming" que necesita ser corroborado.

Palabras clave: *Carica candamarcensis*; Extractos vegetales; Neutrofilos, Matriz metaloproteinasa 9, MAPK

ABBREVIATIONS

ABTS+: 2.2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ACD: Trisodium Citrate 13.2g/L, Citric Acid 4.8g/L and Dextrose 14.7g/L, 1.0mL tubes
ANOVA: Analysis of variance
AU: Arbitrary Units
COX2:Cyclooxygenase 2
HBSS: Hank's equilibrated solution
HUVEC: Umbilical cord entothelial cells
IL: Interleukin
LPS: Lipopolysaccharide
MAPK: Mitogen-Activated Protein Kinases
MMP9: Matrix Metalloproteinase 9
PAF: Platelet aggregation factor
PMN: Polymorphonuclear cells
PVDF: polyvinylidene fluoride
RA: Rheumatoid Arthritis
ROS: Reactive oxygen species
SEM: Standard error of the mean
SO: Seed oil
TEAC: Trolox equivalent antioxidant capacity
TM: total maceration extract
TNFα: Tumor necrosis factor alpha

INTRODUCTION

Neutrophils, or polymorphonuclear cells (PMN), belong to the immune system, constitute the first defense line, and are the most abundant white blood cells in humans (Borregaard, 2010). They contribute to the development of the inflammatory response and are the effector cells of the adaptive immunity (Futosi *et al*., 2013). Diverse stimuli induce neutrophil activation, triggering various responses such as chemotaxis, degranulation, as well as reactive oxygen species (ROS) and cytokine release, all these processes being regulated by complex signaling pathways. Nevertheless, a defective activation of these cells causes tissue damage and inflammatory degenerative diseases (Futosi *et al*., 2013; Cowland & Borregaard, 2016; Németh *et al*., 2020).

Neutrophils secrete a gelatinase known as Matrix Metalloproteinase 9 (MMP9) to move from the capillary endothelium to the damage site. MMP9 is a 92 kDa proenzyme that is responsible for degrading proteins of the extracellular matrix such as laminin and elastin. Furthermore, MMP9 modulates mediators of inflammation (cytokines and chemokines) and contributes to tissue remodeling by promoting the formation of new blood vessels (Mócsai *et al*., 2015; Cowland & Borregaard, 2016). Sometimes, the exacerbated release of MMP9 can cause tissue damage during the inflammatory response, which is why it has been associated with diverse diseases such as aneurysm, thrombosis, arthritis, and atherosclerosis. Likewise, it favors angiogenesis and, consequently, the formation of tumors due to uncontrolled cellular growth. Thus, it is important to study the regulation of MMP9 release during inflammatory processes (Häger *et al*., 2010; Mócsai, 2013; Nauseef & Borregaard, 2014). However, in healthy people, some stimuli may exert a "priming" effect that prepares the neutrophil to generate a faster and more effective response, which is demonstrated by the release of MMP9 granules and activation of some signaling pathways, among others (El-Benna *et al*., 2016; Liew & Kubes, 2019).

MAPK (Mitogen-Activated Protein Kinases) pathways are widely studied in neutrophils because they play an important role in signaling transduction mechanisms that regulate degranulation, survival, and apoptosis, among others. This is the reason why MAPK proteins are potential therapeutic targets in the treatment of different diseases caused by inflammatory processes where neutrophils are involved (Chu *et al*., 2018; Németh *et al*., 2020).

Recently, the study of natural therapeutic alternatives for the treatment of diseases related to the inflammatory process control has received great attention, making use of the properties of vegetable extracts to regulate pathological processe (Figueira *et al*., 2016). *Carica candamarcensis* Hook, (sin. *Vasconcella pubescens* or *V. candamarcensis*), commonly known as "chilacuán" or "papayuela de la montaña", is native to the Andean region and is distributed from Panamá to northern Chile. This plant is characterized by the production of latex with a high Papain content, which is a proteolytic enzyme with applications in the pharmaceutical industry. Also, *C. candamarcensis* has traditionally been used by local communities as a medicinal alternative to curing tonsillitis, gastritis, and flu. Normally, the fruit of the plant is consumed directly or in homemade preparations (Tonaco *et al*., 2018; Letelier *et al*., 2020).

Different studies carried out with *C. candamarcensis* have shown *in vitro* bactericidal activity without having noticeable mutagenic, genotoxic or cytotoxic effects on eukaryotic cells (Mena-Huertas *et al*., 2011). Mello *et al*. (2008), revealed that enzymes from the P1G10 proteolytic fraction of the fruit exert a protective and reconstructive role in gastric tissue, which is mediated by an increase in mucus content and a stimulation of mitosis to recover gastric ulcer lesions. Alburquerque *et al*. (2020), found that the same

fraction has an anti-inflammatory effect, and Lemos *et al*. (2018), showed that it is able to inhibit metastasis in melanoma and helps to modulate the expression of proteins related to cell proliferation, migration, and differentiation.

Plants have been used for therapeutic, cosmetic, and nutritional purposes since ancient times. Recently, the number of studies on composition and characterization of the biological properties of several herbaceous plant species has increased, and they have demonstrated the rationality of their use as a therapeutic alternative to treating several health problems and disorders (Fabricant & Farnsworth, 2001). Several reports have presented promising results regarding the treatment of inflammatory diseases with vegetable extracts, using neutrophils responses as study models (Bedouhene *et al*., 2017; Kłeczek *et al*., 2019; Schepetkin *et al*., 2019; Chniguir *et al*., 2019a; Chniguir *et al*., 2019a; Khajah *et al*., 2020). Even though several potential biological activities of this plant have been characterized, the effect of *C. candamarcensis* fruits on the regulation of neutrophil responses during inflammatory processes is not very well understood currently. Therefore, the objective of this study is to assess the effect of total maceration and seed oil extracted from ripe fruits of *C. candamarcensis* on MMP9 release and MAPK phosphorylation in neutrophils isolated from human peripheral blood, which were previously stimulated with Lipopolysaccharide (LPS) (*Escherichia coli* O55:B5).

MATERIALS AND METHODS

Collection of biological material

Ripe fruits of *C. candamarcensis* were collected applying a random sampling method in the village of El Encano, which is located 27 km from the city of San Juan de Pasto in the Department of Nariño. The town is located at 2820 masl and its coordinates are: 1°10.365' North Latitude and 77°08.496' West Latitude (Morillo *et al*., 2015). The classification of the plant was validated by an expert from the PSO Herbarium of the University of Nariño. A total of 20 ripe and free from pathogen fruits were selected from different individuals of wild species. Fruits were kept in hermetic bags and were transferred to laboratory refrigerators, where they were externally washed with a 2% sodium hypochlorite solution (NaClO). Then, they were kept at -20°C in hermetic clean plastic bags.

Preparation of extracts from C. candamarcensis Hook fruits

In order to obtain the total maceration (TM), 100 g of *C. candamarcensis* ripe fruits were crushed and macerated, followed by a 10 min centrifugation at 1000 *x g* in 50 mL Falcon tubes. The aqueous supernatant was collected and filtered through a 0.45 µm polyvinylidene fluoride (PDVF) membranes (Santa Cruz Biotechology, INC).

The seed oil from *C. candamarsencis* ripe fruits was extracted through the supercritical fluid method using the protocol by Dorado *et al*. (2017). Extracts were kept in sterile 1.5 ml amber Eppendorf tubes at -20°C until being used.

Innocuity was assessed by seeding 10 µL of extracts on nutrient agar using a disposable calibrated inoculation loop (Biologix Group LTD) and incubating plates at 37°C for 24 h. unseeded agar plates were used as controls.

Isolation of neutrophils from human peripheral blood

Neutrophils were purified from peripheral blood withdrawn from clinically healthy adult volunteer donors according to guidelines approved by the research ethics committee of the University of Nariño. Blood was collected in Trisodium Citrate 13.2 g/L, Citric Acid 4.8 g/L and Dextrose 14.7g/L, 1.0 mL tubes (ACD), and neutrophils were purified in Percoll's discontinuous centrifugation gradients (Hidalgo *et al*., 2015). Then, neutrophils were suspended in Hank's equilibrated solution (HBSS) (5.33 mM KCl, 0.441 mM KH_2PO_4, 138 mM NaCl, 0.34 mM Na_2HPO_4 and 5.56 mM D-glucosa). Purity and viability were greater than 95% as determined by May-Grunwald Giemsa staining and trypan blue exclusion assay, respectively.

Cytotoxicity test of extracts

$5x10^5$ neutrophils were suspended in 500 µL HBSS Ca^{2+} medium and were incubated at 37°C with various working dilutions of extracts, as follows: (i) 5%, 2.5% or 1.25% of total macerate (TM); and (ii) 2%, 1% or 0.5% of seed oil (SO), taking into account their solubility in the HBSS Ca^{+2} and using 10% H_2O_2 as a positive control. After a 5 min incubation period, initial viability was measured through the trypan blue (0.4%) exclusion method (Sigma-Aldrich), according to the protocol by Cakmak *et al*. (2004). Neutrophils were incubated, and the same procedure was repeated each hour for a total of 3 hours.

Assessment of extract antioxidant activity

A discoloration test was carried out with the cationic radical 2.2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+), following the method suggested by Kleczek *et al*. (2019). This method is based on the capacity of ABTS+ to capture long-lived anions. Initially, a cationic radical stock solution was prepared dissolving 50 mg ABTS+ (Sigma-Aldrich) in 50 ml deionized water. Subsequently, 2.45 potassium persulfate ($K_2S_2O_8$) were added and the reaction was kept in darkness for 48 hour at 37°C. From this solution, working solutions with an absorbance of 0.750 ± 0.050 nm at 754 nm wavelength were prepared for all assays (Chao *et al*., 2014; Nowak *et al*., 2018).

Assessment of MMP9 activity

$1x10^6$ neutrophils growing in 500 µL HBSS Ca^{+2} medium were used as negative controls, whereas LPS (*E. coli* O55:B5 5 µg/mL) was added to the cells used as positive controls (Milara *et al*., 2019). Various dilutions of total maceration (TM: 5%, 2.5%, and 1.25%) or seed vegetable oil (SO: 2%, 1%, and 0.5%) were added to the assays. All samples and controls were incubated at 37°C for 15 minutes. Different dilutions of SO were used according to their solubility in the medium. After incubation, cells were centrifuged at 600 × *g* for 6 minutes and supernatants were analyzed for gelatinase activity by zymography (Manosalva *et al*., 2020).

An assay similar to the ones described above was conducted in order to determine a possible priming effect. Specifically, after a 5 minutes of incubation in the medium, PMNs were treated for 15 minutes with the different working concentrations (TM or SO) under conditions similar to the previous test, then with LPS (5 µg/mL) for 5 minutes.

Gel electrophoresis of the substrate was performed as described by Mena *et al*. (2016). Briefly, 10 µL supernatant was loaded into 10% polyacrylamide gels 0.75 mm thickness that contained 0.28% gelatin. Gels were first processed at 200 V for 1 h within a Bio-Rad Mini Protean II (Bio-Rad Laboratories, Richmond, CA) and subsequently washed twice in 2.5% Triton X-100 in distilled water on a shaker at room temperature for 30 minutes. Then, gels were incubated in reaction buffer (100 mM Tris at pH 7.5 and 10 mM $CaCl_2$) at 37°C overnight. Gels where stained with 0.5% Coomassie Brilliant Blue R-250 in acetic acid:methanol:water (1:3:6). Colorless areas in which the gelatin was degraded are evidence of enzymatic activity. Gelatinolytic bands were compared to a recombinant MMP9 standard (Sigma Aldrich, USA) and a standard molecular mass marker (Fermentas International Inc., Canada) (Mena *et al*., 2016). In order to quantify MMP9 activity, gels were digitized and the intensity of the bands was measured using Image J 1.35s.

Assessment of MAPK phosphorylation through immunoblot

Neutrophils (5×10^6) were incubated for 10 minutes in medium containing different dilutions of the (TM: 5%, 2.5%, and 1.25%) or seed vegetable oil (SO: 2%, 1%, and 0.5%), using LPS (5 µg/mL) as positive control. Neutrophils growing only in HBSS + Ca^{+2} during 10 minutes at 37°C were used as negative control.

To evaluate a possible priming effect, a test similar to the previous one was carried out, initially incubating the neutrophils with the different working dilutions of TM and SO for 10 minutes, and subsequently stimulating them with LPS (5 µg/mL) for 5 minutes.

Total proteins were extracted according to the protocol by Hidalgo *et al*. (2015), but, in the case of TM stimulated neutrophils it was necessary to use twice the concentration of protease inhibitors and PMFS. Protein extracts were quantified using the Bradford method and 100 g total proteins were analyzed by 10% SDS-PAGE. Immunoblotting was performed according to a protocol previously described by Mena *et al*. (2016). Primary antibodies against phospho-p38 MAPK [Phospho-p38 MAPK (Thr180/Tyr182) (3D7) Rabbit mAb Cell Signaling, Beverly, MA, EE. UU] and phospho-ERK1/2 [Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Rabbit mAb Cell Signaling, Beverly, MA, USA] were used according to the manufacturers' instructions.

Anti-rabbit HRP-conjugated antibody [Anti-rabbit IgG HRP-linked Cell Signaling, Beverly, MA, USA] was used as secondary antibody and bands were visualized using a chemiluminescence system (Perkin-Elmer, USA). Membranes were stripped and reblotted with either anti-actin antibody [Beta-actin (D6A8) Rabbit mAb Cell Signaling, Beverly, MA, USA] or anti-total ERK1/2 antibody [p44/42 MAPK (Erk1/2). Antibody #9102 Cell Signaling] and the re-probed signal was detected as previously described. The intensity of each band was quantified by the ImageJ Software and was normalized to β-actin for p-p38 MAPK, whereas p-ERK1/2 was normalized to total ERK1/2.

Data Analysis

Each test was conducted in triplicate (N=3) (results obtained from different blood donors). Five assays were carried out in cases where the standard deviation was very large (N=5). Data were processed through one-way analysis of variance (ANOVA) and Dunett's mean comparison test using GraphPad Prism v8.0 (GraphPad Software Inc. CA, USA) with a 5% significance level and are presented in the graphs as mean ± SEM.

Ethical Considerations

This research study was developed in accordance with all the ethical and bioethical provisions required for scientific research at the international level by the Declaration of Helsinki and resolution number 8430 of 1993 of the Colombian Ministry of Health. It was approved by the research ethics committee of the University of Nariño and considered risk-free (VIIS-CEI-004). The participation of the donors was voluntary and they signed an informed consent.

The plant material was collected in accordance with the guidelines established in Decree 3016 of December 2013 of the Ministry of the Environment and Development of Colombia, and resolution 126 of February 23, 2015 of Corponariño (Regional Autonomous Corporation of Nariño).

RESULTS

Cytotoxicity and antioxidant activity

Neither the concentrations of the analyzed extracts nor 5 µg/µL LPS showed evident cytotoxicity during the three hour incubation period. In all cases, viability greater than 80% was maintained and there were statistically significant differences with respect to the 10% H_2O_2 positive control (Figure No. 1).

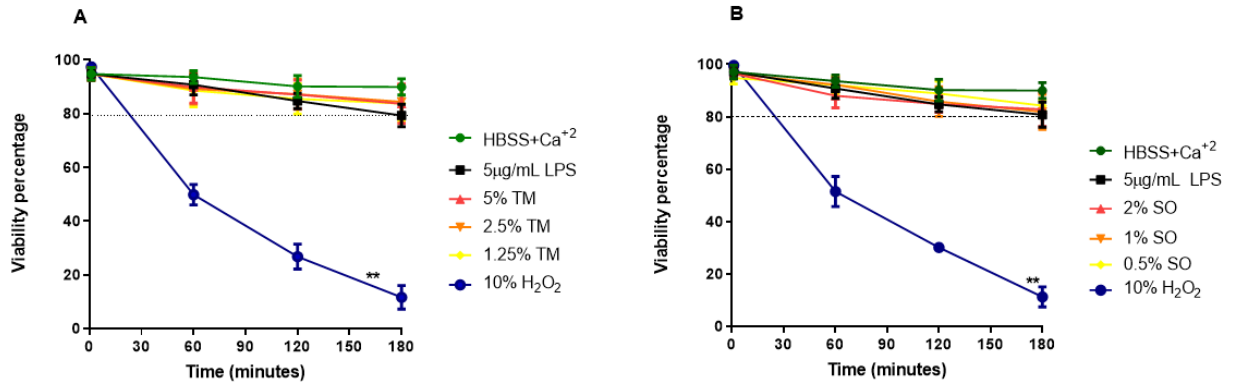

Figure No. 1

Survival of human neutrophils treated with extracts of *C. candamarcensis*. (A) Total maceration. **(B)** Vegetable seed oil. Results were obtained from three independent assays. One-way ANOVA was performed. The Dunnet test demonstrates significant differences between treatments and 10% H_2O_2 positive control at 60, 120 and 180 minutes (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

The ABTS+ analysis of the evaluated extracts demonstrates a low antiradical activity, reaching an antioxidant activity equivalent to 0.6975 µMol/mL and 0.3875 µMol/mL of Trolox equivalent antioxidant capacity (TEAC) for total maceration and seed oil of *C. candamarcensis*, respectively (Figure No. 2).

MMP9 release regulation capacity

The LPS working concentration for the zymography analysis was initially established because various authors have shown different values, including 1 µg/mL (Tang *et al*., 2016), 5 µg/mL (Milara *et al*., 2019) and 10 µg/mL (Trentini *et al*., 2014). Thus, the 5 µg/mL LPS working concentration was selected since samples displayed highly significant differences in the Dunnet test (p=0.0034) with respect to the HBSS Ca^{+2} control (Figure No. 3A).

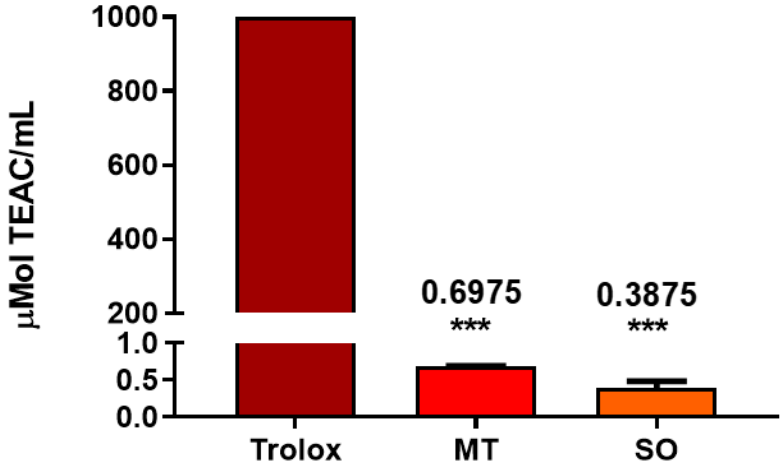

Figure No. 2
Antioxidant activity of extracts prepared from ripe fruits of *C. candamarcensis*
Antioxidant activity is presented as units equivalent to Trolox µMolTEAC/mL. TM (Total Maceration), SO (Seed Oil). n=3. Statistical analysis was performed with One-way ANOVA and Bonferroni post-test, ***$p \leq 0.0001$.

The TM from ripe fruits triggers a significant decrease in the MMP9 release compared to the positive control (LPS). Likewise, there is a significant reduction when neutrophils are pre-treated with 5% and 2.5% TM extracts and then stimulated with LPS with respect to positive control (cells treated with LPS only) (Figures No. 3B and No. 3C).

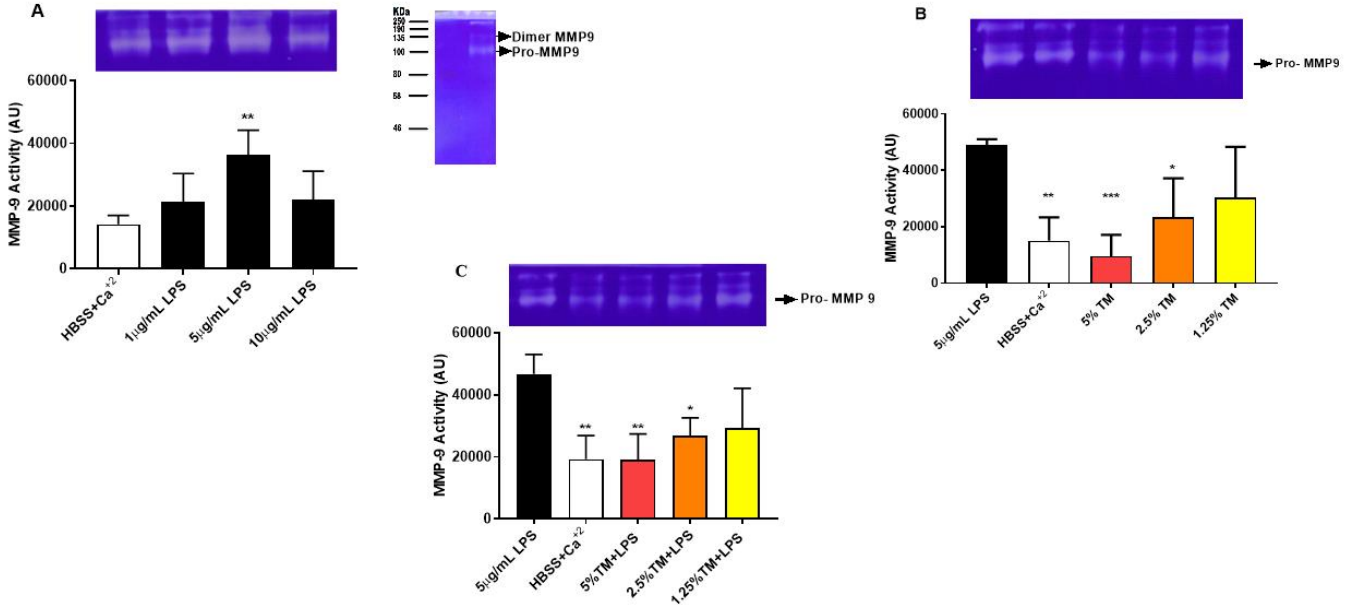

Figure No. 3
Total maceration (TM) of ripe fruits of *C. candamarcensis* reduces MMP9 release from neutrophils isolated from human peripheral blood. $1x10^6$ neutrophils were grown in 500 µL HBSS Ca^{+2} and stimulated during 15 min at 37°C with different concentrations of TM extracted from *C. candamarcensis*. (**A**) Concentration curve of *E. coli* O55:B5 (1, 5 and 10µg/mL) LPS to stimulate MMP9 release. (**B**) Effect of different concentrations of TM from ripe fruits on MMP9 release. (**C**) Effect on the release of MMP9 from neutrophils initially stimulated with different

concentrations of TM from ripe fruits for 15 min and later for 5 min with 5 µg/mL LPS. Bars represent arithmetic mean ± SEM, n=3 to 5. One-way variance analysis and subsequent Dunnet test were performed using HBSS Ca^{+2} as control for (A) and LPS for (B and C). *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. The upper image of each panel is representative of the zymography results obtained in one of the experiments. AU=Arbitrary Units

Neutrophils stimulated with different SO dilutions showed a significant increment in MMP9 release compared to the negative control (HBSS Ca^{+2}), and this increase is similar to that generated by positive control (LPS). MMP9 release is also significantly higher when neutrophils were first incubated with SO and later with LPS in comparison to the positive control (LPS only) (Figure No. 4A and No. 4B).

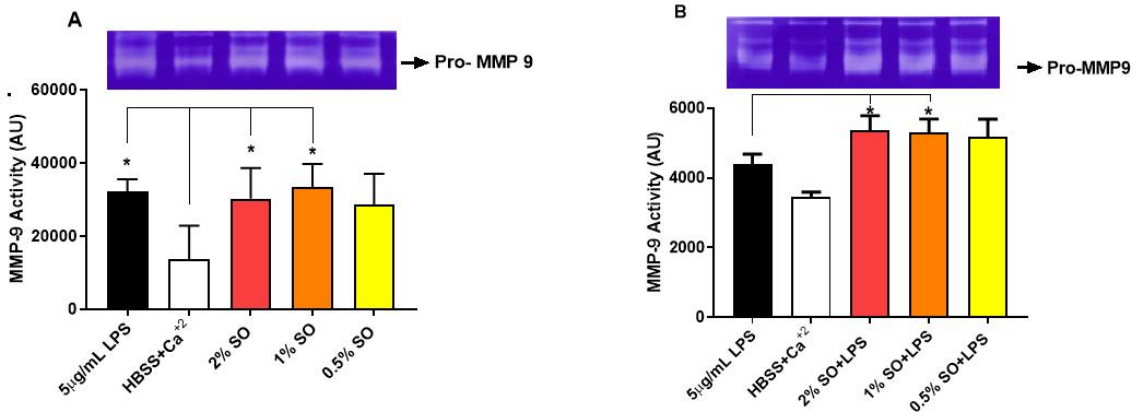

Figure No. 4
Effect of vegetable oil extracted from fruit seeds (SO) of *C. candamarcensis* on MMP9 release
$1x10^6$ neutrophils growing in 500 µL HBSS Ca^{+2} were stimulated for 15 min at 37°C with different SO concentrations obtained from *C. candamarcensis* Hook. **(A)** Effect of different concentrations of SO collected from ripe fruits on MMP9 release. **(B)** Effect on the release of MMP9 from neutrophils initially stimulated with different concentrations of SO from ripe fruits for 15 min and later for 5 min with 5 µg/mL LPS. Each bar represents arithmetic mean± SEM, n=3 to 5. One-way variance analysis followed by Dunnet test were carried out using HBSS Ca^{+2} and LPS as controls for (A) and (B), respectively. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. The upper image of each panel is representative of the zymography results obtained in one of the experiments. AU=Arbitrary Units

MAPK phosphorylation

The ERK1/2 and p38(MAPK)phosphorylation analyses of neutrophils stimulated with TM working concentrations shows that both proteins have low phosphorylation levels, which are similar to those seen in the negative control (HBSS Ca^{+2}). Nevertheless, there is a significant increase in protein phosphorylation when cells were incubated with SO compared to negative control (HBSS Ca^{+2}) (Figure No. 5).

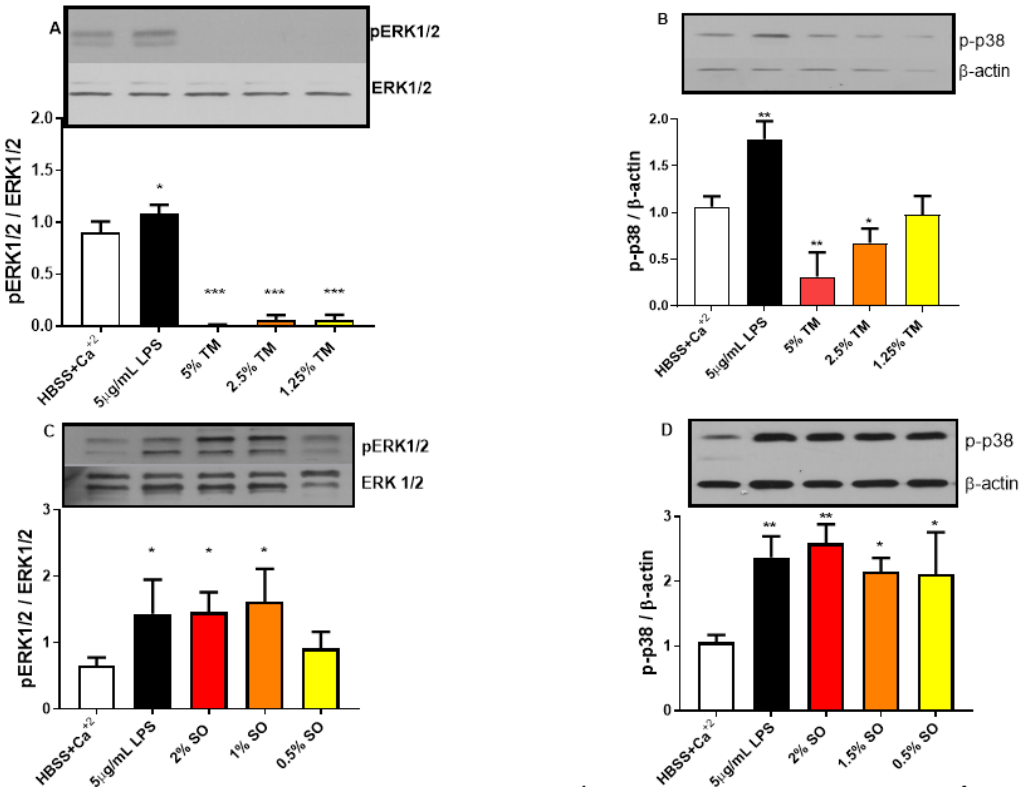

Figure No. 5

Effect of fruit extracts of *C. candamarcensis* on ERK1/2 and p38 (MAPK) phosphorylation
$4x10^6$ neutrophils were seeded in 500 µL HBSS Ca^{+2}. Then, they were stimulated for 15 min at 37°C with different concentrations of MT or SO obtained from fruits of *C. candamarcensis* Hook. Effect of different concentrations of TM from ripe fruits on the phosphorylation of ERK1/2 **(A)** and p38 **(B).** Effect of differ-ent concentrations of SO from seeds of ripe fruits on the phosphorylation of ERK1/2 **(C)** and p38 **(D**). Each bar represents arithmetic mean ± SEM, n =3. One-way variance analysis followed by Dunnet test were car-ried out using HBSS Ca^{+2} as control. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. The upper image of each panel is representative of the immunoblot results obtained in one of the experiments

Cells behaved differently when they were first subjected to a five minutes' pre-incubation with either oil or aqueous extracts followed by LPS stimulation. For instance, the TM+LPS treatment elicited a statistically significant reduction in the phosphorylated forms of ERK1/2 and p38 with respect to the treatment with LPS only. On the other hand, cells incubated with SO+LPS exhibited a statistically significant increase in the phosphorylated levels of ERK1/2 and p38 MAPK compared to cells treated with LPS only (Figure No. 6).

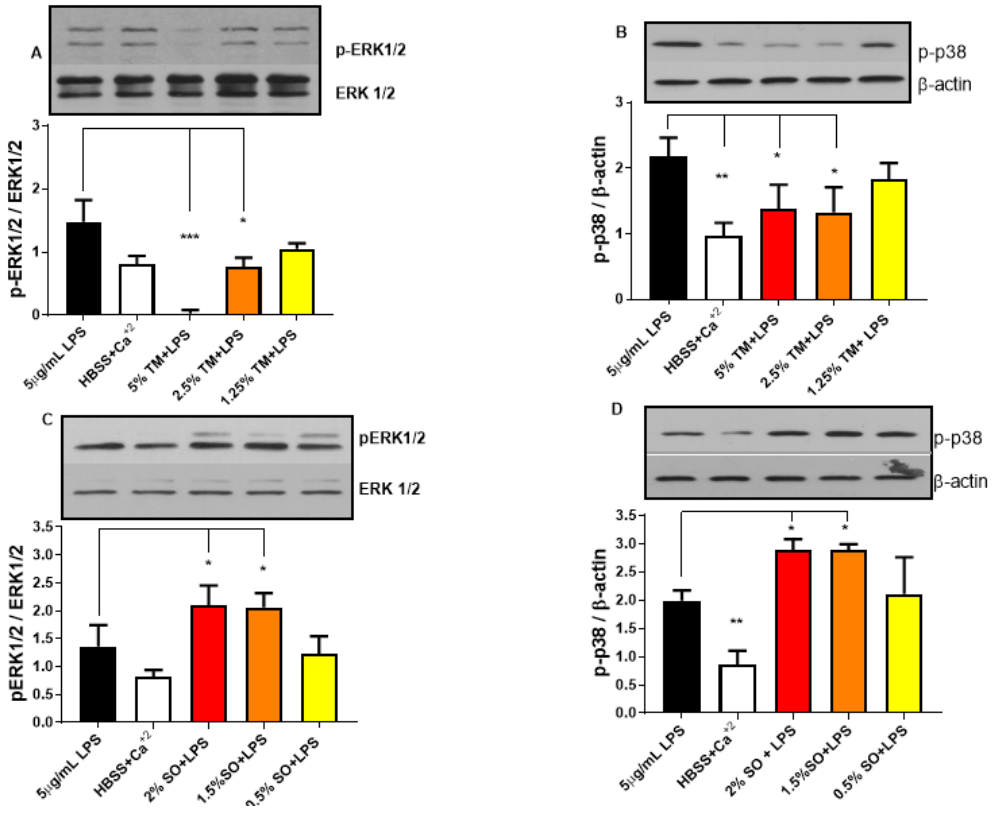

Figure No. 6

Effect of pre-stimulation with fruit extracts of *C. candamarcensis* followed by LPS treatment on ERK1/2 and p38 (MAPK) phosphorylation. 4x10^6 neutrophils growing in HBSS Ca^{+2} were stimulated for 10 min with different concentrations of TM or SO extracted from fruits and seeds of *C. candamarcensis* Hook respectively, and subsequently with 5µg/mL LPS for 5 min at 37°C. Effect of different concentrations of TM from ripe fruits + LPS on the phosphorylation of ERK1/2 (A) and p38 (B). Effect of different con-centrations of SO from seeds of ripe fruits + LPS on the phosphorylation of ERK1/2 (C) and p38 (D). Each bar represents arithmetic mean ± SEM, n =3. One-way variance analysis followed by Dunnet test were car-ried out using 5 µg/mL LPS as positive control. *$p\leq0.05$, **$p\leq0.01$, ***$p\leq0.001$. The upper image of each panel is representative of the immunoblot results obtained in one of the experiments.

DISCUSSION

C. candamarcensis is a plant native to the Andes that is characterized by its therapeutic properties, which is why it is used traditionally to treat burns, flu, gastritis, among other pathologies (Vidal *et al.*, 2009). In addition, studies carried out by Mena-Huertas *et al.* (2011), Ralph *et al.* (2014), and Tonaco *et al.* (2018), suggest that this plant has a therapeutic potential. Previous evidence by Ralph *et al.* (2014), also revealed an anti-inflammatory potential of the P1G10 fraction of the latex of *C. candamarcensis* in murine models. They demonstrated that these extracts are able to reduce the expression of pro-inflammatory mediators such as interleukin 1 (IL1) and cyclooxygenase 2 (COX2) when mice are infected with enteric Salmonella.

Likewise, Araujo e Silva *et al.* (2014), revealed a decrease in the production of pro-inflammatory mediator prostaglandin E2 in Wistar rats subjected to gastric lesions (Araujo e Silva *et al.*, 2014). Similar results were obtained in this study, which indicate that total maceration of ripe fruits of the plant not only does not show cytotoxicity, but it also has high antioxidant activity and significantly reduces MMP9 release and ERK1/2 and p38 phosphorylation with respect to LPS positive control. Also, the TM extracts reduce the effect induced by LPS on MMP9 release and MAPK phosphorylation, which demonstrates a potential anti-inflammatory capacity.

Several studies demonstrate that leaves of *C. papaya*, another member of the Caricaceae family, have phenolic compounds (caffeic acid) and flavonoids (micricetin) with antioxidant and antimicrobial properties (Hernandez-Varela *et al*., 2013; Jagtap *et al*., 2019), and that the presence of polyphenols in the fruit is associated with their antioxidant properties and anti-inflammatory activity (Dorado *et al*., 2017). Particularly, the aqueous extract of fruits of *C. candamarcensis* has hydrocarbon compounds (heicosan) and monoterpenes (linalool) that have antimicrobial properties as well as sesquiterpenes (famesol) that have been shown to have anti-inflammatory properties (Mena-Huertas *et al*., 2005; Dorado *et al*., 2017). Carrasco & Zelada (2008), studied *C. candamarcensis* fruits and demonstrated that 167 mg/100 g of fruit is composed of phenolic compounds with a vitamin C content equivalent to 31.41 mg/100 g of fruit and a small amount of carotenoids (0.71 mg/100 g fruit). All these compounds may be associated with the antioxidant activity found in this study.

Similar to our results obtained with TM of *C. candamarcensis*, there is evidence that natural substances with antioxidant capacity are able to reduce MMP9 (Granica *et al*., 2015; Kim-Park *et al*., 2016; Chniguir *et al*., 2019b) release and ERK1/2 phosphorylation (Chniguir *et al*., 2019a) in neutrophils as well as p38 MAPK phosphorylation in umbilical cord entothelial cells (HUVEC) and thus are characterized as having anti-inflammatory potential (Ma *et al*., 2015). Yang *et al*. (2018), carried out a study with resveratrol to treat rheumatoid arthritis (RA), demonstrating that this anti-oxidant compound decreases both phosphorylation of the p38 MAPK and the inflammatory process. Bedouhene *et al*. (2017), showed that polyphenols with antioxidant capacity from *Olea europaea* (olive) fruits have the capacity to reduce p38 and ERK1/2 MAPK phosphorylation, degranulation, and chemotaxis of neutrophils isolated from human peripheral blood. All these findings support the results of our study showing that the antioxidant activity of TM can be associated with a decline in p38 and ERK1/2 MAPK phosphorylation.

Salacia impressifolia (Miers) extracts have potential apoptotic and anti-metastatic activity in melanoma cells due to a reduction in MAPK phosphorylation and MMP9 release (Aranha *et al*., 2021). The role of p38 and ERK1/2 pathways in the regulation of the release of MMP9 granules has been demonstrated (Futosi *et al*., 2013; Mócsai, 2013). TM is a complex mixture of pulp and peel of ripe fruits of *C. candamarcensis*. The synergic activity of its compounds demonstrates an adequate antioxidant activity that may be associated with the ability to decrease phosphorylation of the MAPK pathways and, consequently, to reduce MMP9 release in neutrophils. These responses can help to reduce the effects of chronic inflammatory processes associated with various pathologies such as cancer(Yang *et al*., 2018).

Vegetable oil derived from the seeds (SO) of *C. candamarcensis* has a different effect from the one observed with TM. Although this extract does not display a cytotoxic effect and has a TROLOX equivalent antioxidant activity lower than that of TM, an increase in both MMP9 release as well as p38 and ERK1/2 phosphorylation can be seen with respect to the negative control (HBSS Ca^{+2}) . In addition, these effects are significantly higher when a subsequent stimulus with LPS (5 µg/mL) is performed, suggesting a possible priming (pre-activation) effect in neutrophils (Miralda *et al*., 2017). Neutrophils have been described as being in a resting state before going into a fully activated state. However, a priming state implies that there must be some intermediate activation states before complete activation (Deniset & Kubes, 2016; Vogt *et al*., 2018).

The role of plant extracts on the priming effect is still unknown since their function have been evidenced mainly by the stimulation of some agents such as TNFα (Tumor necrosis factor alpha) and PAF (Platelet aggregation factor) in human neutrophils (El-Benna *et al.*, 2016). Neutrophils in priming and activated stages show a significantly improved bactericidal capacity that is associated with their increase in degranulation, ROS, and longevity. However, this enhancement also confers the ability to cause significant indirect tissue injury (Vogt *et al*., 2018).

The vegetable oil extracted from *C. candamarcensis* seeds was characterized by Masson *et al.* (2012), who found that it contains a high percentage of oleic acid (71%), followed by linoleic (13.16%), palmitic (9.53%), stearic (3.46%), and linolenic (0.7%) acids. Although the anti-inflammatory properties of oleic acid (omega 9 acid) have been demonstrated, there is also evidence indicating its potential participation in the enhancement of both MAPK phosphorylation and MMP9 release (Hidalgo *et al*., 2011; Matoba *et al*., 2018; Marcial-Medina *et al*., 2019). In addition, it has been reported that this seed vegetable oil has compounds such as linoleic (Mena *et al*., 2016;

Manosalva *et al*., 2020), stearic and palmitic acids (Tull *et al*., 2009; Rodrigues *et al*., 2016) with potential pro-inflammatory activity, which may be associated with MAPK phosphorylation and MMP9 release.

The CMS2MS2 fraction of the latex of *C. candamarcensis* fruits has validated function in incrementing proliferation of fibroblasts that depends on ERK phosphorylation (Tonaco *et al*., 2018). The complex mixture and conjugated activity of these components may be inducing an increase in MMP9 release and p38 and ERK1/2 phosphorylation observed with SO of *C. candamarcensis* seeds, and may generate a potential priming effect in neutrophils. These responses may be important for the progress of cells from a basal state (where response capacities of neutrophils are limited) to an improved pre-activation and alertness state that helps neutrophils to respond more effectively to stimuli and activate a subset of functions including degranulation and production of NET's and ROS (Deniset & Kubes, 2016; Miralda *et al*., 2017; Liew & Kubes, 2019).

Natural products have been used as sources of biological active ingredients and even new drugs, hence the importance of studying plant extracts in depth, checking the potential of fractions or formulations obtained from different components from the same source (Schepetkin *et al*., 2019; Khajah *et al*., 2020; Benvenutti *et al*., 2021). Our results highlight the need to carry out differential studies on plant extracts since each of their fractions show variability in terms of components and concentrations, which can lead to differential effects and biological responses (Kłeczek *et al*., 2019; Chuang *et al*., 2021).

CONCLUSION

Extracts obtained from *C. candamarcensis* ripe fruits have a high biological potential with therapeutic and functional food applications. The TM extracted from ripe fruits has an antioxidant and anti-inflammatory capacity through a reduction in gelatinase granule release and ERK1/2 and p38 MAPK phosphorylation. Thus, its consumption could be applied in the treatment of chronic diseases associated with inflammatory processes such as arthritis and different types of cancer. On the other hand, the SO obtained from seeds of *C. candamarcensis* has an opposite effect as it increases both MMP9 release and p38 and ERK1/2 MAPK phosphorylation after stimulation with LPS, suggesting a priming effect and a protective role against acute inflammatory processes. Since this phenomenon has been reported to be related to NADPH oxidase activity, it is necessary for future studies to assess ROS and NET's levels in order to rule out a possible pro-inflammatory effect.

REFERENCES

Albuquerque RM, Pizzitola MP, Araújo e Silva AC, Dittz D, Freitas KM, Ferreira E, Salas CE, Lopes MTP. 2020. The proteolytic fraction from *Vasconcellea cundinamarcensis* latex displays anti-inflammatory effect in a mouse model of acute TNBS-induced colitis. **Sci Rep** 10: 3074. **https://doi.org/10.1038/s41598-020-59895-3**

Aranha ESP, Portilho AJS, Bentes de Sousa L, da Silva EL, Mesquita FP, Rocha WC, Araújo da Silva FM, Lima ES, Alves APNN, Koolen HHF, Montenegro RC, Vasconcellos MC. 2021. 22β-hydroxytingenone induces apoptosis and suppresses invasiveness of melanoma cells by inhibiting MMP-9 activity and MAPK signaling. **J Ethnopharmacol** 267: 113605. **https://doi.org/10.1016/j.jep.2020.113605**

Araujo e Silva AC, Lemos FO, Gomes MTR, Salas CE, Lopes MTP. 2014. Role of gastric acid inhibition, prostaglandins and endogenous-free thiol groups on the gastroprotective effect of a proteolytic fraction from *Vasconcellea cundinamarcensis* latex. **J Pharm Pharmacol** 67: 133 - 141. **https://doi.org/10.1111/jphp.12318**

Bedouhene S, Moulti-Mati F, Dang PMC, El-Benna J. 2017. Oleuropein and hydroxytyrosol inhibit the N-formyl-methionyl-leucyl-phenylalanine-induced neutrophil degranulation and chemotaxis via AKT, p38, and ERK1/2 MAP-Kinase inhibition. **Inflammopharmacology** 25: 673 - 680. **https://doi.org/10.1007/s10787-017-0367-7**

Benvenutti L, Nunes R, Venturi I, Ramos SA, Broering MF, Goldoni FC, Pavan SE, Pastor MVD, Malheiros A, Quintão NLM, Fernandes ES, Santin JR. 2021. Anti-inflammatory and healing activity of the hydroalcoholic fruit extract of *Solanum diploconos* (Mart.) Bohs. **J Immunol Res** 2021. **https://doi.org/10.1155/2021/9957451**

Borregaard N. 2010. Neutrophils, from marrow to microbes. **Immunity** 33: 657 - 670. **https://doi.org/10.1016/j.immuni.2010.11.011**

Cakmak GD, Schins RPF, Shi T, Fenoglio I, Fubini B, Borm PJA. 2004. *In vitro* genotoxicity assessment of commercial quartz flours in comparison to standard DQ12 quartz. **Int J Hyg Environ Health** 207: 105 - 113. **https://doi.org/10.1078/1438-4639-00276**

Carrasco RR, Zelada CRE. 2008. Determinación de la capacidad antioxidante y compuestos bioactivos de frutas nativas peruanas. **Rev Soc Quím Perú** 2: 108 - 124.

Chao PY, Lin SY, Lin KH, Liu YF, Hsu JI, Yang CM, Lai JY. 2014. Antioxidant activity in extracts of 27 indigenous taiwanese vegetables. **Nutrients** 6: 2115 - 2130. **https://doi.org/10.3390/nu6052115**

Chniguir A, Pintard C, Liu D, Dang PMC, El-Benna J, Bachoual R. 2019a. Eugenol prevents fMLF-induced superoxide anion production in human neutrophils by inhibiting ERK1/2 signaling pathway and p47phox phosphorylation. **Sci Rep** 9: 1 - 11. **https://doi.org/10.1038/s41598-019-55043-8**

Chniguir A, Zioud F, Marzaioli V, El-Benna J, Bachoual R. 2019b. *Syzygium aromaticum* aqueous extract inhibits human neutrophils myeloperoxidase and protects mice from LPS-induced lung inflammation. **Pharm Biol** 57: 55 - 63. **https://doi.org/10.1080/13880209.2018.1557697**

Chu D, Dong X, Shi X, Zhang C, Wang Z. 2018. Neutrophil-based drug delivery systems. **Adv Mater** 30: 1706245. **https://doi.org/10.1002/adma.201706245**

Chuang LT, Huang WC, Hou YC, Chyuan JH, Chang H, Chang CI, Tsai TH, Tsai PJ. 2021. Suppressive effect of two cucurbitane-type triterpenoids from *Momordica charantia* on cutibacterium acnes-induced inflammatory responses in human THP-1 monocytic cell and mouse models. **Molecules** 26: 579. **https://doi.org/10.3390/molecules26030579**

Cowland JB, Borregaard N. 2016. Granulopoiesis and granules of human neutrophils. **Immunol Rev** 273: 11 - 28. **https://doi.org/10.1111/imr.12440**

Deniset JF, Kubes P. 2016. Recent advances in understanding neutrophils. **F1000Research** 5: 2912. **https://doi.org/10.12688/f1000research.9691.1**

Dorado DJ, Hurtado AM, Martínez-Correa HA. 2017. Extracción supercrítica de aceite de semillas de papaya (*Carica papaya*): composición y propiedades fisicoquímicas. **Vitae** 2: 35 - 45. **https://doi.org/10.17533/udea.vitae.v24n2(2)a05**

El-Benna J, Hurtado-Nedelec M, Marzaioli V, Marie JC, Gougerot-Pocidalo MA, Dang PMC. 2016. Priming of the neutrophil respiratory burst: role in host defense and inflammation. **Immunol Rev** 273: 180 - 193. **https://doi.org/10.1111/imr.12447**

Fabricant DS, Farnsworth NR. 2001. The value of plants used in traditional medicine for drug discovery. **Environ Health Perspect** 109: 69 - 75. **https://doi.org/10.1289/ehp.01109s169**

Figueira ME, Oliveira M, Direito R, Rocha J, Alves P, Serra AT, Duarte C, Bronze R, Fernandes A, Brites D, Freitas M, Fernandes E, Sepodes B. 2016. Protective effects of a blueberry extract in acute inflammation and collagen-induced arthritis in the rat. **Biomed Pharmacother** 83: 1191 - 1202. **https://doi.org/10.1016/j.biopha.2016.08.040**

Futosi K, Fodor S, Mócsai A. 2013. Reprint of neutrophil cell surface receptors and their intracellular signal transduction pathways. **Int Immunopharmacol** 17: 1185 - 1197. **https://doi.org/10.1016/j.intimp.2013.11.010**

Granica S, Piwowarski JP, Kiss AK. 2015. Ellagitannins modulate the inflammatory response of human neutrophils *ex vivo*. **Phytomedicine** 22: 1215 - 1222. **https://doi.org/10.1016/j.phymed.2015.10.004**

Häger M, Cowland JB, Borregaard N. 2010. Neutrophil granules in health and disease. **J Intern Med** 268: 25 - 35. **https://doi.org/10.1111/j.1365-2796.2010.02237.x**

Hernandez-Varela J, Fernandez V, Sulbaran B. 2013. Caracterización fisicoquímica, actividad antioxidante y contenido de polifenoles totales de pulpa lechosa de *Carica papaya*. **Rev Obs Conoc** 2: 195.

Hidalgo MA, Nahuelpan C, Manosalva C, Jara E, Carretta MD, Conejeros I, Loaiza A, Chihuailaf R, Burgos RA. 2011. Oleic acid induces intracellular calcium mobilization, MAPK phosphorylation, superoxide production and granule release in bovine neutrophils. **Biochem Biophys Res Commun** 409: 280 - 286. **https://doi.org/10.1016/j.bbrc.2011.04.144**

Hidalgo MA, Carretta MD, Teuber SE, Zárate C, Cárcamo L, Concha II, Burgos RA. 2015. fMLP-induced IL-8 release is dependent on NADPH oxidase in human neutrophils. **J Immunol Res** 2015: 1 - 14. **https://doi.org/10.1155/2015/120348**

Jagtap NS, Wagh RV, Chatli MK, Kumar P, Malav OP, Mehta N. 2019. Optimisation of extraction protocol for *Carica papaya* L. to obtain phenolic rich phyto-extract with prospective application in chevon emulsion

system. **J Food Sci Technol** 56: 71 - 82. **https://doi.org/10.1007/s13197-018-3456-8**

Khajah MA, EL-Hashim AZ, Orabi KY, Hawai S, Sary HG. 2020. Onion bulb extract can both reverse and prevent colitis in mice via inhibition of pro-inflammatory signaling molecules and neutrophill activity. **Plos One** 15: e0233938. **https://doi.org/10.1371/journal.pone.0233938**

Kim-Park WK, Allam ES, Palasuk J, Kowolik M, Park KK, Windsor LJ. 2016. Green tea catechin inhibits the activity and neutrophil release of matrix metalloproteinase-9. **J Tradit Complement Med** 6: 343 - 346. **https://doi.org/10.1016/j.jtcme.2015.02.002**

Kłeczek N, Michalak B, Malarz J, Kiss AK, Stojakowska A. 2019. *Carpesium divaricatum* Sieb. & Zucc. revisited: Newly identified constituents from aerial parts of the plant and their possible contribution to the biological activity of the plant. **Molecules** 24. **https://doi.org/10.3390/molecules24081614**

Lemos FO, Dittz D, Santos VG, Pires SF, Andrade HM, Salas CE, Lopes MTP. 2018. Cysteine proteases from *V. cundinamarcensis* (*C. candamarcensis*) inhibit melanoma metastasis and modulate expression of proteins related to proliferation, migration and differentiation. **Int J Mol Sci** 19. **https://doi.org/10.3390/ijms19102846**

Letelier L, Gaete-Eastman C, Peñailillo P, Moya-León MA, Herrera R. 2020. Southern species from the biodiversity hotspot of Central Chile: A source of color, aroma, and metabolites for global agriculture and food industry in a scenario of climate change. **Front Plant Sci** 11. **https://doi.org/10.3389/fpls.2020.01002**

Liew PX, Kubes P. 2019. The neutrophil's role during health and disease. **Physiol Rev** 99: 1223 - 1248. **https://doi.org/10.1152/physrev.00012.2018**

Ma MM, Li Y, Liu XY, Zhu WW, Ren X, Kong GQ, Huang X, Wang LP, Luo LQ, Wang XZ. 2015. Cyanidin-3-O-glucoside ameliorates lipopolysaccharide-induced injury both *in vivo* and *in vitro* suppression of NF-κB and MAPK pathways. **Inflammation** 38: 1669 - 1682. **https://doi.org/10.1007/s10753-015-0144-y**

Manosalva C, Alarcón P, González K, Soto J, Igor K, Peña F, Medina G, Burgos RA, Hidalgo MA. 2020. Free fatty acid receptor 1 signaling contributes to migration, MMP-9 activity, and expression of IL-8 induced by linoleic acid in HaCaT cells. **Front Pharmacol** 11. **https://doi.org/10.3389/fphar.2020.00595**

Marcial-Medina C, Ordoñez-Moreno A, Gonzalez-Reyes C, Cortes-Reynosa P, Perez Salazar E. 2019. Oleic acid induces migration through a FFAR1/4, EGFR and AKT-dependent pathway in breast cancer cells. **Endocrinol Connect** 8: 252 - 265. **https://doi.org/10.1530/EC-18-0543**

Masson L. 2012. **Semillas de frutos nativos y cultivados en Chile: su aceite como fuente de compuestos nacionales**. Tesis, Universidad Complutense de Madrid, Madrid, España.

Matoba A, Matsuyama N, Shibata S, Masaki E, Emala CW, Mizuta K. 2018. The free fatty acid receptor 1 promotes airway smooth muscle cell proliferation through MEK/ERK and PI3K/Akt signaling pathways. **Am J Physiol Cell Mol Physiol** 314: L333 - L348. **https://doi.org/10.1152/ajplung.00129.2017**

Mello VJ, Gomes MTR, Lemos FO, Delfino JL, Andrade SP, Lopes MTP, Salas CE. 2008. The gastric ulcer protective and healing role of cysteine proteinases from *Carica candamarcensis*. **Phytomedicine** 15: 237 - 244. **https://doi.org/10.1016/j.phymed.2007.06.004**

Mena-Huertas J, Yépez-Chamorro MC, Santacruz A, Noguera E. 2005. Actividad antibacteriana *in vitro* de extractos de plantas medicinales regionales promisorias sobre aislamientos de Helicobacter pylori. **Rev Cent Est Salud** 1: 13 - 21.

Mena-Huertas J, Embus-Córdoba C, Rosero-Ruiz VL, Navarro-Yépez J, Ortiz-Trujillo IC, Yépez-Chamorro MC. 2011. Ausencia de efecto citotóxico, mutagénico y genotoxicode extracto acuoso y aceite esencial de *Carica candamarcensis* Hook (Plantae: Caricacease). **Actual Biol** 33: 153 - 164.

Mena SJ, Manosalva C, Carretta MD, Teuber S, Olmo I, Burgos RA, Hidalgo MA. 2016. Differential free fatty acid receptor-1 (FFAR1/GPR40) signalling is associated with gene expression or gelatinase granule release in bovine neutrophils. **Innate Immun** 22: 479 - 489. **https://doi.org/10.1177/1753425916656765**

Milara J, Contreras S, de Diego A, Calbet M, Aparici M, Morcillo E, Miralpeix M, Cortijo J. 2019. *In vitro* anti-inflammatory effects of AZD8999, a novel bifunctional muscarinic acetylcholine receptor antagonist /β2-adrenoceptor agonist (MABA) compound in neutrophils from COPD patients. **Plos One** 14: e0210188. **https://doi.org/10.1371/journal.pone.0210188**

Miralda I, Uriarte SM, McLeish KR. 2017. Multiple phenotypic changes define neutrophil priming. **Front Cell Infect Microbiol** 7. **https://doi.org/10.3389/fcimb.2017.00217**

Mócsai A. 2013. Diverse novel functions of neutrophils in immunity, inflammation, and beyond. **J Exp Med** 210: 1283 - 1299. **https://doi.org/10.1084/jem.20122220**

Mócsai A, Walzog B, Lowell CA. 2015. Intracellular signalling during neutrophil recruitment. **Cardiovasc Res** 107: 373 - 385. **https://doi.org/10.1093/cvr/cvv159**

Morillo A, Villota D, Lagos T, Ordóñez H. 2015. Caracterización morfológica y molecular de 18 introducciones de uchuva *Physalis peruviana* L. de la colección de la Universidad de Nariño. **Rev Fac Nac Agron** 64: 6043 - 6053.

Nauseef WM, Borregaard N. 2014. Neutrophils at work. **Nat Immunol** 15: 602 - 611. **https://doi.org/10.1038/ni.2921**

Németh T, Sperandio M, Mócsai A. 2020. Neutrophils as emerging therapeutic targets. **Nat Rev Drug Discov** 19: 253 - 275. **https://doi.org/10.1038/s41573-019-0054-z**

Nowak D, Gośliński M, Wojtowicz E, Przygoński K. 2018. Antioxidant properties and phenolic compounds of vitamin C-rich juices. **J Food Sci** 83: 2237 - 2246. **https://doi.org/10.1111/1750-3841.14284**

Ralph MT, Silva AFB, da Silva DL, do Nascimento DCO, da Silva DMF, Gomes-Filho MA, Souza PRE, Evêncio-Neto J, Ramos MV, Salas CE, Lima-Filho JV. 2014. Peptidases from latex of *Carica candamarcensis* upregulate COX-2 and IL-1 mRNA transcripts against *Salmonella enterica* Ser. typhimurium-mediated inflammation. **Mediators Inflamm** 2014: 1 - 8. **https://doi.org/10.1155/2014/819731**

Rodrigues HG, Sato FT, Curi R, Vinolo MAR. 2016. Fatty acids as modulators of neutrophil recruitment, function and survival. **Eur J Pharmacol** 785: 50 - 58. **https://doi.org/10.1016/j.ejphar.2015.03.098**

Schepetkin IA, Kirpotina LN, Khlebnikov AI, Balasubramanian N, Quinn MT. 2019. Neutrophil immunomodulatory activity of natural organosulfur compounds. **Molecules** 24: 1809. **https://doi.org/10.3390/molecules24091809**

Tang FSM, Van Ly D, Spann K, Reading PC, Burgess JK, Hartl D, Baines KJ, Oliver BG. 2016. Differential neutrophil activation in viral infections: Enhanced TLR-7/8-mediated CXCL8 release in asthma. **Respirology** 21: 172 - 179. **https://doi.org/10.1111/resp.12657**

Tonaco LAB, Gomes FL, Velasquez-Melendez G, Lopes MTP, Salas CE. 2018. The proteolytic fraction from latex of *Vasconcellea cundinamarcensis* (P1G10) enhances wound healing of diabetic foot ulcers: A double-blind randomized pilot study. **Adv Ther** 35: 494 - 502. **https://doi.org/10.1007/s12325-018-0684-2**

Trentini A, Bellini T, Manfrinato MC, Dallocchio F, Fainardi E, Alvisi R, Alvisi V, Volta CA. 2014. Balanced and unbalanced solutions modulate the release of matrix metalloproteinase-9 (MMP-9) from neutrophils in response to inflammatory stimuli: an *in vitro* study. **Inflamm Res** 63: 325 - 328. **https://doi.org/10.1007/s00011-014-0709-5**

Tull SP, Yates CM, Maskrey BH, O'Donnell VB, Madden J, Grimble RF, Calder PC, Nash GB, Rainger GE. 2009. Omega-3 fatty acids and inflammation: Novel interactions reveal a new step in neutrophil recruitment. **Plos Biol** 7: e1000177. **https://doi.org/10.1371/journal.pbio.1000177**

Vidal LV, Finot VL, Mora KC, Venegas FA. 2009. Características físico-químicas del látex de papayuelo (*Vasconcellea cundinamarcensis* Badillo, Caricaceae). **Inform Tecnol** 20. **https://doi.org/10.4067/S0718-07642009000600012**

Vogt KL, Summers C, Chilvers ER, Condliffe AM. 2018. Priming and de-priming of neutrophil responses *in vitro* and *in vivo*. **Eur J Clin Invest** 48: e12967. **https://doi.org/10.1111/eci.12967**

Yang G, Chang CC, Yang Y, Yuan L, Xu L, Ho CT, Li S. 2018. Resveratrol alleviates rheumatoid arthritis via reducing ROS and inflammation, inhibiting MAPK signaling pathways, and suppressing angiogenesis. **J Agric Food Chem** 66: 12953 - 12960. **https://doi.org/10.1021/acs.jafc.8b05047**