Free Radical Biology and Medicine 167 (2021) 335–347

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original article

Phytoprostanes and phytofurans modulate COX-2-linked inflammation markers in LPS-stimulated THP-1 monocytes by lipidomics workflow

María Campillo [a,1], Sonia Medina [b,1,**], Federico Fanti [b,c], Juana I. Gallego-Gómez [a], Agustín Simonelli-Muñoz [a], Valérie Bultel-Poncé [d], Thierry Durand [d], Jean Marie Galano [d], Francisco A. Tomás-Barberán [b,e], Ángel Gil-Izquierdo [b,*], Raúl Domínguez-Perles [b]

[a] Departamento de Enfermería, Universidad Católica de Murcia, UCAM, 3010, Murcia, Spain
[b] Research Group on Quality, Safety and Bioactivity of Plant Foods. Department of Food Science and Technology, CEBAS-CSIC, University Campus of Espinardo – Edif. 25, 30100, Espinardo, Spain
[c] University of Teramo, Faculty of Bioscience and Technology for Food, Agriculture and Environment, 64100, TE, Italy
[d] Institut des Biomolécules Max Mousseron (IBMM), UMR, 5247, CNRS, University of Montpellier, ENSCM, Faculty of Pharmacy, Montpellier, France
[e] Department of Biotechnology, College of Science, Taif University, Saudi Arabia

ARTICLE INFO

Keywords:
Monocytes
Phytoprostanes
Phytofurans
Inflammation
Prostaglandins
UHPLC-QqQ-ESI-MS/MS

ABSTRACT

Inflammation is a fundamental pathophysiological process which occurs in the course of several diseases. The present work describes the capacity of phytoprostanes (PhytoPs) and phytofurans (PhytoFs) (plant oxylipins), present in plant-based foods, to modulate inflammatory processes mediated by prostaglandins (PGs, human oxylipins) in lipopolysaccharide (LPS)-stimulated THP-1 monocytic cells, through a panel of 21 PGs and PG's metabolites, analyzed by UHPLC-QqQ-ESI-MS/MS. Also, the assessment of the cytotoxicity of PhytoPs and PhytoFs on THP-1 cells evidenced percentages of cell viability higher than 90% when treated with up to 100 μM. Accordingly, 50 μM of the individual PhytoPs and PhytoFs 9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, 9-L_1-PhytoP, *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF were evaluated on their capacity to modulate the expression of inflammatory markers. The results obtained demonstrated the presence of 7 metabolites (15-keto-$PGF_{2\alpha}$, $PGF_{2\alpha}$, 11β-$PGF_{2\alpha}$, PGE_2, PGD_2, PGDM, and $PGF_{1\alpha}$) in THP-1 monocytic cells, which expression was significantly modulated when exposed to LPS. The evaluation of the capacity of the individual PhytoPs and PhytoFs to revert the modification of the quantitative profile of PGs induced by LPS revealed the anti-inflammatory ability of 9-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, 9-L_1-PhytoP, and *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, as evidenced by their capacity to prevent the up-regulation of 15-keto-$PGF_{2\alpha}$, $PGF_{2\alpha}$, PGE_2, $PGF_{1\alpha}$, PGDM, and PGD_2 induced by LPS. These results indicated that specific plant oxylipins can protect against inflammatory events, encouraging further investigations using plant-based foods rich in these oxylipins or enriched extracts, to identify specific bioactivities of the diverse individual molecules, which can be useful for nutrition and health in the frame of well-defined pathophysiological processes.

Abbreviations: BHA, butylated hydroxyanisole; COX, cyclooxygenase; ECACC, European Collection of Cell Culture; ESI, electrospray ionization; FBS, fetal bovine serum; IL-12, interleukine-12; LOX, lipoxygenase; LPS, lipopolysaccharide; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cell; PLA2, phospholipase A_2; PG, prostaglandin; PhytoFs, phytofurans; PhytoPs, phytoprostanes; PMS, phenazine methosulphate; PPAR-γ, peroxisome proliferator-activated receptor-gamma; SPE, solid-phase extraction; TH2, type 2 helper immune response; THP-1, human monocytic cell line; UHPLC, ultra-high pressure liquid chromatography; XTT, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide salt.

* Corresponding author.
** Corresponding author.
E-mail addresses: smescudero@cebas.csic.es (S. Medina), angelgil@cebas.csic.es (Á. Gil-Izquierdo).
[1] These two authors contributed equally to the present work.

https://doi.org/10.1016/j.freeradbiomed.2021.03.002
Received 13 November 2020; Received in revised form 24 February 2021; Accepted 1 March 2021
Available online 17 March 2021

1. Introduction

Inflammation is the first biological response of the immune system to an array of factors that disturb the homeostasis of cells and tissues, and is triggered to restore the physiological conditions [1]. In this scenario, the innate immune system plays a pivotal role in the evolution of inflammation, with its functioning mainly based on receptors that recognize conserved molecular structures of pathogens, such lipopolysaccharide (LPS). When these are recognized, the immune system initiates a variety of host responses by activating different molecular pathways involved in systemic inflammation. During this process, cells secrete cyclooxygenase (COX) products, such as prostaglandins (PGs) E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$, respectively), and their metabolites, responsible for the activation of the inflammatory cascade [2–5].

In the search for new compounds with the capacity to prevent inflammation, various studies aimed at unraveling the mechanism/s of action of secondary metabolites of higher plants. These studies revealed the valuable capability to avoid PG secretion in response to the LPS challenge through the inhibition of the enzymes involved in the production of PGs, such as phospholipase A_2 (PA2), cyclooxygenase (COX), and lipoxygenase (LOX). Therefore, they modulate the inflammatory process [6–9] due to the inhibition of specific enzymes [10–12], which has been associated with various health benefits.

In this regard, the presence of a new type of secondary metabolite, belonging to the family of plant oxylipins, and represented by phytoprostanes (PhytoPs) and phytofurans (PhytoFs) has been reported in plant-based foods [13–15]. To the present date, these compounds have been characterized in a wide diversity of plant-based foods, namely nuts, hazelnuts, almonds, vegetable oils, cereals, olives, wine, peas, rice, several tropical fruits, cocoa, macroalgae, chocolate, pistachio, date, and especially in legumes, at different concentrations [16–40]. They have been suggested to be bioactive compounds, mainly based on their structural analogy with isoprostanoids [41]. However, their capacity to modulate inflammation with respect to their ability to influence the isoprostanoid profile in a pro-inflammatory environment, has not been addressed as of yet. Hence, the pro- and anti-inflammatory activity of individual PhytoPs and PhytoFs merits to be explored, as PGs play a central role in diseases coursing with inflammation [34].

In this scenario, the present article aims to uncover the capacity of individual PhytoPs and PhytoFs to modulate the inflammatory response triggered by LPS in the THP-1 human monocytic cell line, *in vitro*. The inflammatory modulation foreseen will be monitored by assessing the prostanoid profile through a panel of 21 human oxylipins analyzed in cells and growth media by UHPLC-QqQ-ESI-MS/MS.

2. Material and methods

2.1. Chemicals and reagents

The PhytoPs, 9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, and 9-L_1-PhytoP, and the PhytoFs *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF, were synthesized according to published procedures [42–47], and provided by the *Institut des Biomolécules Max Mousseron* (IBMM) (Montpellier, France). A total of 21 PGs were analyzed in the current study (Table 1). The authentic standards corresponding to this range of PGs were purchased from Cayman Chemicals (Ann Arbor, Michigan, USA). Hexane was obtained from Panreac (Castellar del Valles, Barcelona, Spain), and butylated hydroxyanisole (BHA) and Bis-Tris (bis-(2-hydroxyethyl)-amino-tris (hydroxymethyl) methane) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All LC-MS grade solvents (deionized water, acetonitrile, methanol, and formic acid) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Water was treated in a Milli-Q water purification system from Millipore (Bedford, MA, USA). The solid-phase extraction (SPE) cartridges used were Strata cartridges (Strata X-AW, 100 mg/3 mL), and these were acquired from Phenomenex (Torrance, CA, USA).

Table 1
Retention time and MRM transitions of prostaglandins assessed in LPS-stimulated and non-stimulated THP-1 cells and growth media.

Prostaglandin	R_t (min.)	Parent ion (m/z [M − H]$^-$)	Fragment (m/z MS2 [M − H]$^-$)
Prostaglandin generated from AA			
Prostaglandin D-pathway			
Tetranor-PGDM	3.17	327.1	108.9
PGDM	3.20	327.1	309.1
Tetranor-PGDM lactone	3.50	309.1	142.9
Tetranor-PGJM	3.60	609.0	155.0
2,3-dinor-11β-$PGF_{2\alpha}$	10.57	325.2	237.1
PGD_2	13.22	350.9	315.0
11-*β*-$PGF_{2\alpha}$	13.61	353.0	309.1
Prostaglandin E-pathway			
Tetranor-PGEM	3.17	327.0	308.8
Tetranor-PGAM	3.58	309.0	2900.9
20-OH-PGE_2	4.66	367.1	349.2
PGE_2	13.07	351.2	333.1
Prostaglandin F-pathway			
Tetranor-PGFM	3.14	329.4	311.1
ent-20-OH-$PGF_{2\alpha}$	5.10	369.2	325.1
19(R)–OH–$PGF_{2\alpha}$	5.15	369.2	325.1
15-keto-$PGF_{2\alpha}$	12.90	351.1	314.9
$PGF_{2\alpha}$	13.60	353.1	309.0
Prostaglandin I-pathway			
2,3-dinor-6-keto $PGF_{1\alpha}$	10.80	341.0	134.9
6-keto-$PGF_{1\alpha}$	12.69	369.0	245.1
Prostaglandin generated from DGLA			
PGE_1	12.84	353.2	317.2
$PGF_{1\alpha}$	13.67	355.2	311.0
Prostaglandin generated from EPA			
17-*trans*-$PGF_{3\alpha}$	12.47	350.9	307.0

AA, arachidonic acid; DGLA, dihomo-ɣ-linolenic acid; EPA, eicosapentanoic acid.

2.2. Cell lines and culture conditions

The monocytic human (THP-1) cell line (ECACC® General Cell Collection-88081201) was obtained from the European Collection of Cell Culture (ECACC, Public Health England, Porton Down, Salisbury, UK). Cells were grown in RPMI 1640, supplemented with 2 mM glutamine and 10% fetal bovine serum (FBS), in 75 cm^2 cell culture flasks to the logarithmic growth phase (0.6–1.4 × 10^6 cells/mL), at 37 °C in a humidified atmosphere containing 5% CO_2. The passage number of the cells used in this study was between 17 and 20.

2.3. XTT reduction-based cell viability test

The toxicity of individual PhytoPs and PhytoFs was tested in THP-1 cells, resorting to the XTT methodology. For this, exponentially-growing cells were seeded into a 96-well plate at a density of 10^4 cells/well. After incubation for 24 and 48-h with decreasing concentrations of the PhytoPs and PhytoFs (100.000, 50.000, 25.000, 5.000, 1.000, 0.200, 0.020, and 0.002 μM), the cells were incubated with the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt (XTT) solution at a final concentration of 1 mg/mL (7.65 μg phenazine methosulphate (PMS)/mL) for 4-h, at 37 °C. The absorbance was measured at 450 nm, using 690 nm as a reference wavelength, using a microtiter plate reader (Victor 3, PerkinElmer Las, Jügesheim, Germany). Cell viability was calculated as average "*(optical density (OD) of wells – OD blank)/average OD of control wells*," and expressed as a percentage (%).

2.4. LPS-induced inflammatory model

Cells were seeded into 24-well plates at 7 × 10^5 cells/well in RPMI 1640, supplemented with 2 mM glutamine and 10% fetal bovine serum (FBS) to study the modulatory effects of PhytoPs and PhytoFs on the PG profile of the human monocytic cell line THP-1. Cells were treated with 50 μM of the individual PhytoPs and PhytoFs in triplicate (n = 3) for 24-h. This concentration was selected based on previous studies that detected plant oxylipins at micromolar quantities in several food matrices, such as flax oil or pea [16,31]. In this regard, although this concentration could exceed the theoretical concentration achieved in target cells (which has not already been described), especially because of the ongoing breakdown of dietary PhytoPs and PhytoFs during gastrointestinal digestion, this experimental design set-up in the present work was utilized to understand the biological potential of the plant oxylipins under consideration. An inflammatory stimulus (100 ng/mL LPS) was then added, and cells were maintained at 37 °C and 5% CO_2 for another 24-h.

2.5. Extraction of prostaglandins from cells and culture medium

The PG profile was determined in the THP-1 cell lysates (5 × 10^5) and their growth medium. Once the cells and supernatants exposed to 100 ng/mL LPS from *Escherichia coli* 0127:B8 for 24 h were collected, 0.005% BHA (final concentration) was added, and the medium was conserved at −80 °C until the PGs were extracted. Methanol/HCl 200 mM (0.5 mL) was added to 0.5 mL THP-1 growth medium and centrifuged at 10000×*g* for 5 min to precipitate the serum proteins.

After removing the growth medium, the cells were lysed by incubation with lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% Triton X-100, containing 0.005% BHA) for 1-h, on ice. Lysates were centrifuged at 8000×*g* for 5 min. The supernatants were collected and stored at −80 °C until the extraction of PGs.

Both pre-processed growth medium and cell lysate extracts were first enzymatically hydrolyzed (β-glucuronidase Type HP-2 from *Helix pomatia*), and PGs were extracted according to the procedure already described [48,49]. After hydrolysis, the samples were subjected to a clean-up procedure by solid-phase extraction (SPE) using Strata X-AW cartridges (100 mg/3 mL), following the method described in the literature [48]. Target compounds were eluted with 1 mL of methanol and dried using a SpeedVac concentrator. The concentration of PGs was determined in cell lysates and growth media, and described in both biological matrices and as an addition of both concentrations that constitutes the total PGs synthesized by THP-1 cells.

2.6. UHPLC-QqQ-MS/MS analyses of prostaglandins

The chromatographic resolution of PGs in cell lysates and growth medium was performed by UHPLC coupled to a 6460 QqQ-MS/MS (Agilent Technologies, Waldbronn, Germany), using the set up previously described [48,50], and an ACQUITY BEH C_{18} column (2.1 × 150 mm, 1.7 μm pore size) (Waters, MA, USA). The mobile phases used in the chromatographic separation were deionized water/formic acid (99.9:0.1, *v/v*) (solvent A) and methanol (solvent B) through the gradient scheme (t, %B): (0.00; 60), (7.00; 60), (7.01; 73), (10.00; 73), (10.01; 80), (18.00; 100), (19.00; 100), and (19.01; 60). The flow rate and injection volume were 0.150 mL/min and 20 μL, respectively. The identification and quantification of PGs were achieved through the analysis of the parent masses and specific fragmentation patterns of 21 PGs (Table 1), which was performed through a mass spectrometry analysis by multiple reaction monitoring (MRM) mode, and the application of electrospray ionization (ESI) in the negative mode and the ion optics settings previously described for these compounds [48,50]. Also, the identification and quantification of the PGs monitored were performed using authentic standards of the PGs referred to in Table 1. The 8-iso prostaglandin $F_{2\alpha}$-d_4 (contains 4 deuterium atoms at positions 3, 3′, 4, and 4') was used as an internal standard because of its similar ionization pattern relative to the PGs. Data acquisition and processing were performed using the MassHunter software version B.08.00 (Agilent Technologies, Walbronn, Germany). The concentration of the PGs were calculated according to standard curves that were freshly prepared each day of analysis. Additionally, the limit of quantification (LOQ), calculated as a signal/noise ratio of 10, was set up at 8 pM, which agrees with the LOQ previously reported by Labuschagne et al. for quantifying lipid peroxidation in cellular systems [51].

2.7. Statistical analysis

All treatments and extractions were performed in triplicate (n = 3), and the data were expressed as the mean ± standard deviation (SD). Statistical tests were performed at a 5% significance level using the SPSS 24.0 software package (LEAD Technologies, Inc., Chicago, USA). Data were subjected to a one-way analysis of variance (ANOVA), confirming that the ANOVA requirements were met, especially regarding the normal distribution of the residuals and the homogeneity of variance, through the Kolmogorov–Smirnov (with Lilliefors correction) and Levene tests, respectively. When statistical differences were identified, the variables were compared using Tukey's multiple range test.

3. Results

As mentioned before, this present work aimed at evaluating individual PhytoPs and PhytoFs on their capacity to modulate the PGs response triggered during LPS-induced inflammation in THP-1 monocytic cells. On this aspect, the starting hypothesis was that plant oxylipins are capable of modulating the PGs profile in the course of an inflammatory process.

Presently, in association with this objective, it is well-known that monocytes and macrophages are key elements of the innate immune system and the inflammatory process [52]. Hence, the so-called "THP-1" is a human leukemia monocytic cell line, characterized by a strong correspondence with the monocytic fraction of peripheral blood mononuclear cells, which preserves a robust capacity to synthesize many molecules related to the inflammatory process [53]. Although THP-1 cells may not express matching features relative to primary monocytes and their regulatory mechanisms, they are characterized by their physiological properties, which are characteristic of primary monocytes *in vivo* [54]. Because of this, the THP-1 cell line has widely been used as a model to study the molecular pathways involved in the inflammatory response, and the capacity of food compounds to modulate such pathways, which are strongly linked with the course of many pathophysiological situations [7,55].

3.1. THP-1 human monocytic cell line viability

Before evaluating the capacity of PhytoPs and PhytoFs to modulate inflammation and avoid non-addressed harmful effects of these plant oxylipins on THP-1 cells during the inflammation assay, the cytotoxic effect of PhytoPs and PhytoFs against the human THP-1 cells was assessed. With this objective, an XTT assay was carried out after 24 and 48-h in the presence of decreasing concentrations of the individual PhytoPs, 9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, and 9-L_1-PhytoP, and the individual PhytoFs *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF (100.000, 50.000, 25.000, 5.000, 1.000, 0.200, 0.020, and 0.002 μM) (Fig. 1).

The results obtained evidenced a minimal modification of the THP-1 cell viability when exposed to high concentrations of individual PhytoPs and PhytoFs. In this regard, the range of concentrations tested for the ten plant oxylipins evaluated did not decrease the viability of THP-1 cells significantly after 24-h, relative to the untreated control, except for *ent*-

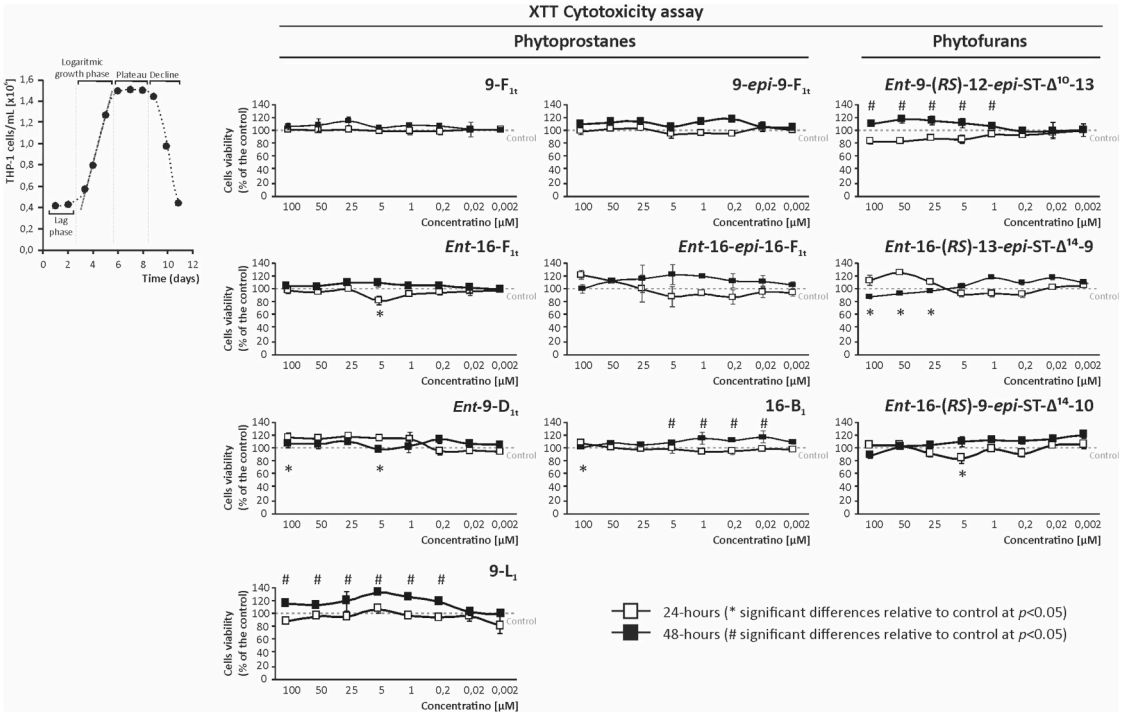

Fig. 1. Cytotoxic activity of phytoprostanes (9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, and 9-L_1-PhytoP) and phytofurans (*ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF). Cytotoxicity on THP-1 cells (monocyte cell line) of decreasing concentrations of phytoprostanes and phytofurans (100.000, 50.000, 25.000, 5.000, 1.000, 0.200, 0.020, and 0.002 μM) were measured by an XTT assay during the logarithmic grow phase of THP-1 cells. Cytotoxicity is expressed as mean ± SD of the percentage of viability relative to the control at 24 and 48 h. Significant decreases in cell viability relative to control conditions after 24 h were set at $p < 0.05$ (*) and after 48 h at $p < 0.05$ (#).

16-F_{1t}-PhytoPs and *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, which induced a significant reduction in the viability at 5 μM (15.3% lower than the control, on average). When evaluating the cytotoxicity after 48-h, again, no cytotoxic effect was observed. On the contrary, the exposure to high concentrations of PhytoPs and PhytoFs, in general, caused a significant increase in the cells' viability, regarding 16-B_1-PhytoP (5.00–0.02 μM caused an increase of 13.5%, on average), 9-L_1-PhytoP (100.00–0.20 μM caused a rise of 20.5%, on average), and *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF (100.00–1.00 μM caused an increase of 14.2%, on average) (Fig. 1).

Accordingly, the limited cytotoxic effect attributable to PhytoPs and PhytoFs after 24 and 48-h could be dependent on the structural similarities with the mammal's oxylipins (isoprostanoids) already described at diverse concentrations in human cells, without a deleterious effect at homeostatic concentrations. On the other hand, the promotion of viability induced by a select group of PhytoPs and PhytoFs, mainly after a 48-h exposure for most concentrations, could be related to a diverse capacity of the diverse PhytoPs and PhytoFs to penetrate cells, according to their chemical structure [56]. Moreover, the increase in cell viability could also be influenced by the capacity of PhytoPs and PhytoFs to provide additional biochemical advantages to cells (*i.e.*, contributing to the redox-balance in cells) [56]. In this aspect, regarding PhytoPs and PhytoFs, this hypothesis is in good agreement with previous descriptions on the cytotoxicity of PhytoPs and PhytoFs extracts obtained from *Gracilaria longissima* (edible red algae) by Martínez-Sánchez et al., who reported no cytotoxicity and no effect of cell proliferation nor induction of cell death of intestinal epithelial cells by PhytoPs and PhytoFs at concentrations of up to 50 ng/mL [34].

3.2. Modulation of the inflammatory response-mediated through intracellular prostaglandins by plant oxylipins after LPS-induced inflammation

Once the safe conditions of individual PhytoPs and PhytoFs regarding THP-1 cells were found, the study of their ability to modulate LPS-induced inflammation by monitoring changes in the quantitative PGs profile was initiated. Thus, the capacity to modulate the inflammatory response was monitored by assessing a panel of 21 PGs (Table 1), analyzed in cells and growth medium, and also measured as the sum of intra- and extra-cellular PGs, as both are secreted by cells and provide the complete picture of the PG response. This experimental design allowed providing further insights into the specific inflammatory modulation of individual PhytoPs and PhytoFs by evaluating changes in the intracellular and extracellular concentration of the 7 PGs found in quantifiable amounts in THP-1 cells (15-keto-$PGF_{2\alpha}$, $PGF_{2\alpha}$, 11β-$PGF_{2\alpha}$, PGE_2, PGD_2, PGDM, and $PGF_{1\alpha}$). Also, this work provides new complementary information with respect to previous descriptions of the biological functions of PhytoPs and PhytoFs, focused on the cytokines profile and/or the phenotype of immune-cells through earlier works which focused on a single plant oxylipin or complete plant extracts of PhytoPs and PhytoFs [30,34,57–61]. In this regard, the use of whole extracts constitutes an approach that allows retrieving valuable results, but is limited in scope. There is still a need for additional experimental inputs for a sound comparison of the anti-inflammatory contribution of the diverse individual plant oxylipins. Indeed, unraveling the biological relevance of the various individual PhytoPs and PhytoFs would allow selecting those plant-based foods that have the adequate quantitative profile for these compounds, according to the diverse pathophysiological conditions, in this case, inflammation. Because of this, it is not surprising that the resulting information on the biological functions, not just regarding plant oxylipins but other bioactive phytochemicals, remains confusing, while a sound scientific proof of the many different

protective features attributed to plant-based foods is still mostly incomplete [25,62].

The concentration of the PGs was measured in cell lysates of THP-1 monocytes treated with individual PhytoPs and PhytoFs, as agents that could prevent the inflammation triggered by LPS. Six PGs (15-keto-$PGF_{2\alpha}$, PGE_2, $PGF_{1\alpha}$, PGD_2, 11β-$PGF_{2\alpha}$, and PGDM) were quantified in control (untreated) cells, as well as in cells exposed to LPS, while in LPS-stimulated cells, the presence of $PGF_{2\alpha}$ was also detected at a concentration of 27.76 ng/mL (Fig. 2).

When assessing the effect of the diverse PhytoPs and PhytoFs on the modulation of the concentration of $PGF_{2\alpha}$ induced by LPS, it was observed that almost all compounds (9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF) significantly mitigated the upregulation caused by LPS, providing values ranging between 3.50 and 11.56 ng/mL. On the contrary, 9-L_1-PhytoP caused a significant increase in the intracellular concentration of $PGF_{2\alpha}$, up to 32.51 ng/mL (Fig. 2).

The THP-1 cells level of 15-keto-$PGF_{2\alpha}$ and PGE_2 (0.018 and 0.033 ng/mL, respectively) increased in LPS-stimulated cells 2.1-fold, on average, to reach 0.038 and 0.062 ng/mL, respectively. The increase of 15-keto-$PGF_{2\alpha}$ (a metabolite of $PGF_{2\alpha}$) in LPS-stimulated cells was significantly prevented by *ent*-9-D_{1t}-PhytoP, as it diminished its concentration to levels that were not significantly different ($p > 0.05$) than untreated control cells (0.014 ng/mL). However, no PhytoPs or PhytoFs significantly reduced the LPS-induced concentration of PGE_2 (although lowering trends were identified in cells treated with *ent*-9-D_{1t}-PhytoP, 9-F_{1t}-PhytoP, *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF) (Fig. 2). On the other hand, THP-1 cells treated with 9-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, or *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF before exposure to LPS exhibited a significantly higher concentration of 15-keto-$PGF_{2\alpha}$ (0.057, 0.082, and 0.101 ng/mL, respectively) relative to LPS-treated cells, while for PGE_2, *ent*-16-*epi*-16-F_{1t}-PhytoP, and 16-B_1-PhytoP increased its concentration in THP-1 cells by 45.4%, on average (Fig. 2).

In THP-1 cells, it was also observed that $PGF_{1\alpha}$ concentration increased from 0.013 ng/mL (control cells) to 0.063 ng/mL (LPS-exposed cells). With respect to the capacity of PhytoPs and PhytoFs to protect cells against the pro-inflammatory effect of LPS regarding $PGF_{1\alpha}$ concentration, it was found that none of the tested compounds reduced the effect of LPS significantly. However, the presence of 9-*epi*-9-F_{1t}-PhytoP gave rise to a (non-statistically significant) lower concentration (0.040 ng/mL) (Fig. 2). In contrast, some PhytoPs and PhytoFs boosted the LPS-induced increase in the $PGF_{1\alpha}$ concentration, allowing for the detection of significantly augmented amounts (0.112, 0.115, and 0.106 ng/mL for 9-F_{1t}-PhytoP, 16-B_1-PhytoP, and *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, respectively) (Fig. 2).

Regarding the presence of PGD_2, whose concentration in control cells (0.024 ng/mL) experienced a significant increase after LPS exposure (0.037 ng/mL), PhytoP 9-D1t-PhytoP diminished the intracellular concentration of PGD_2 induced by LPS and allowed recording a concentration that was even lower than that observed in untreated cells (0.017 ng/mL). Also, 9-F_{1t}-PhytoP, *ent*-16-*epi*-F_{1t}-PhytoP, 16-B_1-PhytoP, and *ent*-9(*RS*)-12-*epi*-ST-Δ10-13-PhytoF boosted the LPS effect in regard to PGD_2, giving rise to concentrations that were significantly higher than that recorded in LPS exposed cells (0.064, 0.079, 0.051, and 0.103 ng/mL, respectively) (Fig. 2). The trend recorded was related to 11β-$PGF_{2\alpha}$, the primary metabolite of PGD_2, which was significantly increased in THP-1 cells exposed to LPS up to 0.335 ng/mL (3.3-fold higher concentration than untreated control cells). Again, this polyclonal activator's pro-inflammatory effect on 11β-$PGF_{2\alpha}$ was not prevented by any of the individual PhytoPs and PhytoFs under evaluation. In contrast, 9-F_{1t}-PhytoP, 16-B_1-PhytoP, and *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF reinforced the capacity of LPS to increase the cellular concentration of 11β-$PGF_{2\alpha}$ (0.593 ng/mL, on average, 5.9-fold higher than control cells)

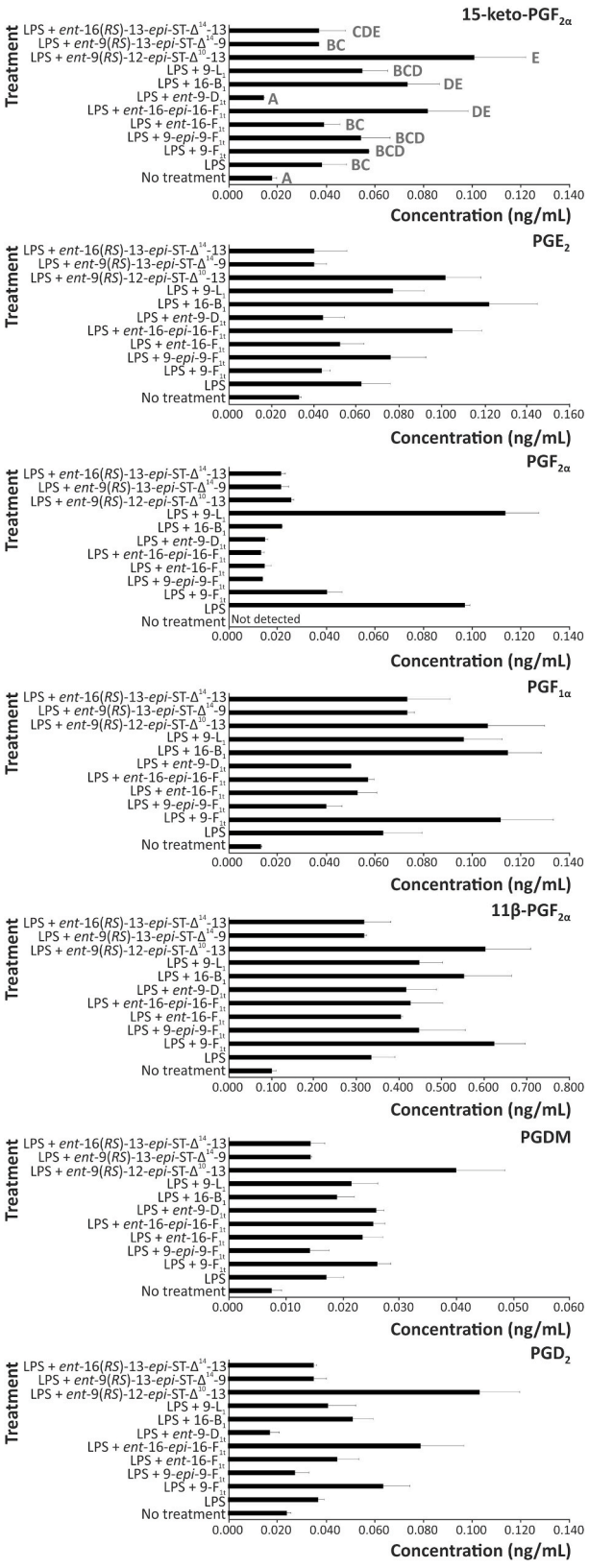

Fig. 2. Effect of individual phytoprostanes and phytofurans treatment of LPS-stimulated THP-1 monocytic cells in intracellular quantitative profile of prostaglandins. Prostaglandins were determined in the whole cell extracts of THP-1 cells pre-exposed to 50 μM of the individual plant oxylipins for 24 h followed by 24 h co-exposure with 100 ng/mL of LPS. Values show mean ± SD (n = 3). Bars with distinct capital letter in each bar-plot were significantly different at $p < 0.05$, according to one-way ANOVA and Tukey's multiple range test.

relative to LPS treated cells (Fig. 2).

Moreover, PGDM, a major urinary metabolite of PGD_2 and a biomarker used to assess the endogenous production of PGD_2, was found in untreated THP-1 monocytes, and its concentration increased when treating cells with LPS (0.008 and 0.017 ng/mL, respectively). Once again, most PhytoPs and PhytoFs appeared to be irrelevant in terms of modifying the concentration of PGDM. Still, some of them (9-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, and *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF) worked alongside LPS, contributing to the achievement of significantly higher concentrations (0.026, 0.025, 0.026, and 0.040 ng/mL, respectively).

3.3. Modulation of the inflammatory response-mediated through prostaglandins excreted in the cells' supernatant by plant oxylipins after LPS-induced inflammation

The PG profile in the culture medium of THP-1 monocytes is shown in Fig. 3. The seven PGs found in the cell lysates were also present in the supernatants.

Two out of the seven PGs detected were not found in untreated control cells ($PGF_{2\alpha}$ and its metabolite 15-keto-$PGF_{2\alpha}$). On the one hand, $PGF_{2\alpha}$ released to the medium was significantly decreased by 9-F_{1t}-PhytoP, 9-D_{1t}-PhytoP, and 16-B_1-PhytoP, and *ent*-9(*RS*)-12-*epi*-ST-Δ10-13-PhytoF, which once added to the growth medium as a preventive treatment against the pro-inflammatory effect of LPS, mitigated the $PGF_{2\alpha}$ concentration 1.6-fold, on average, in comparison with LPS treated cells (10.263 ng/mL) (Fig. 3); and, on the other, the release of 15-keto-$PGF_{2\alpha}$ in cells treated with almost all individual PhytoPs and PhytoFs was higher than in cells exposed only to LPS, with the exception of *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, which did not show significant differences regarding LPS-treated cells. Therefore, all experimental conditions (LPS plus individual PhytoPs or PhytoFs or LPS alone) allowed describing concentrations in the growth medium (0.021–0.056 ng/mL) that were higher than those found for untreated control cells, in which 15-keto-$PGF_{2\alpha}$ was not detected (Fig. 3).

With respect to the PGE_2 released to the medium, its concentration increase 1.8-fold as a result of LPS exposure (0.309 ng/mL), relative to untreated control cells (0.169 ng/mL) (Fig. 3). All individual PhytoPs and PhytoFs decreased the concentration of PGE_2 in the growth media, with 9-F_{1t}-PhytoP and 9-L_1-PhytoP being the most efficient plant oxylipins regarding this effect, which resulted in concentrations that were even lower than the described in control cells (Fig. 3).

As for $PGF_{1\alpha}$, the release into the growth media was also increased 10-fold in THP-1 monocytes exposed to LPS (0.091 ng/mL) relative to untreated cells (0.009 ng/mL) (Fig. 3). However, interestingly, some PhytoPs and PhytoFs (*ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF) were competent enough to prevent the increased levels induced by LPS, giving rise to concentrations of $PGF_{1\alpha}$ that were significantly lower than those recorded in monocytes exposed to LPS (0.057 ng/mL, on average). Moreover, three PhytoPs enhanced the increasing effect of LPS regarding $PGF_{1\alpha}$, 9-*epi*-9-F_{1t}-PhytoP (0.117 ng/mL), *ent*-16-F_{1t}-PhytoP (0.117 ng/mL), and especially 9-F_{1t}-PhytoP (0.247 ng/mL) (Fig. 3).

Additionally, the concentration of PGD_2 released into the growth medium strongly increased by LPS exposure (Fig. 3), resulting in a 9.8-fold increase. Four PhytoPs (9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, and 16-B_1-PhytoP) mitigated the PGD_2 raising-effect of LPS, which increased the amount of PGD_2 released into the medium, giving rise to the average concentration of 0.031 ng/mL. However, for *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, a higher amount of PGD_2 was released into the growth medium (0.163 ng/mL) than the one found in LPS-only exposed cells. *Ent*-16-F_{1t}-PhytoP and *ent*-16-*epi*-16-F_{1t}-PhytoP significantly reduced the concentration of the metabolite of PGD_2 (11β-PGF2α) in THP-1 monocytes growth medium induced by LPS (0.284 ng/mL) and 16-B_1-PhytoP (0.202 ng/mL, on average). At the same time, all three PhytoFs (*ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-

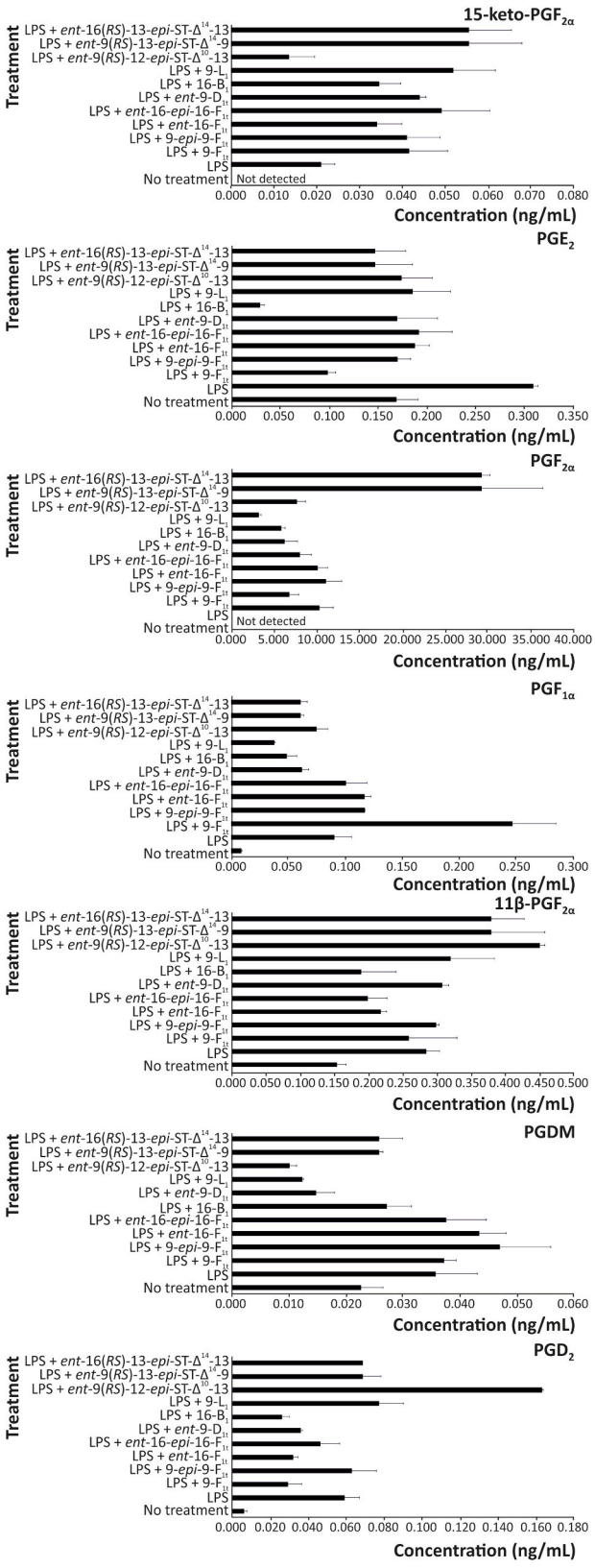

Fig. 3. Effect of individual phytoprostanes and phytofurans treatment of LPS-stimulated THP-1 monocytic cells in prostaglandin release into the growth medium. Prostaglandins were determined in the whole cell extracts of THP-1 cells pre-exposed to 50 μM of the individual plant oxylipins for 24 h followed by 24 h co-exposure with 100 ng/mL of LPS. Values show mean ± SD (n = 3). Bars with distinct capital letter in each bar-plot were significantly different at $p < 0.05$, according to one-way ANOVA and Tukey's multiple range test.

10-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF) seemed to provide a collaborative stimulus to LPS, augmenting the concentration of 11β-$PGF_{2\alpha}$ significantly (0.403 ng/mL, on average) (Fig. 3). When evaluating the evolution of another PGD_2 metabolite (PGDM) due to the treatments applied to THP-1 cells, it was observed that its concentration in the growth medium increased 1.6-fold after exposure to LPS (Fig. 3), although the increase observed was not statistically significant. Two PhytoPs (9-L_1-PhytoP and 16-B_1-PhytoP), and one PhytoF (*ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF) decreased the amount of PGDM released into the medium, providing final concentrations of 0.015, 0.012, and 0.010 ng/mL, respectively (Fig. 3).

4. Discussion

4.1. Understanding the role of phytoprostanes and phytofurans as modulators of the prostaglandin profile of LPS-stimulated THP-1 monocytes

Although the changes in the intra- and extra-cellular concentrations of PGs as a result of the application of individual PhytoPs and PhytoFs as preventive agents of inflammation could indicate controversial biological activities of the separate compounds, it has to be taken into consideration that both of them are the result of the THP-cells metabolism. Accordingly, this would entail that the diverse human oxylipins monitored in the present work could be characterized by distinct kinetics, not only referring to their synthesis but also their excretion. In this regard, considering the level of these oxylipins as a whole (intra- and extra-cellular accumulative concentrations) would help with the understanding of the actual capacity of individual PhytoPs and PhytoFs to modulate the inflammatory response.

Thus, applying this analytical approach, it was observed that the concentration of the PGs PGE_2 and PGDM were not modified significantly by neither the pro-inflammatory stimulus applied nor by the treatment with individual PhytoPs or PhytoFs (Fig. 4). Also, with respect to the capacity of these compounds to prevent the modulation of the synthesis of PGs induced by LPS, the increase detected for the PGs 15-keto-$PGF_{2\alpha}$, $PGF_{1\alpha}$, and 11-β-$PGF_{2\alpha}$ was not reverted by individual PhytoPs or PhytoFs. Indeed, regarding specific PhytoPs and PhytoFs, a biological activity reinforces the increasing trend recorded when applying the pro-inflammatory stimulus. Hence, this was observed for 15-keto-$PGF_{2\alpha}$ for almost all the PhytoPs and PhytoFs which, although in a non-significant manner, exhibited the capacity to increase the concentration recorded when treating THP-1 cells with LPS by 1.7-fold, on average. A similar trend was observed for $PGF_{1\alpha}$ and 11-β-$PGF_{2\alpha}$ for which individual PhytoPs and PhytoFs increased their concentration by up to 2.3 and 1.7-fold, on average, relative to LPS-treated cells (0.077 and 0.310 ng/mL, respectively). Moreover, for these two PGs, the increase was statistically significant ($p < 0.01$) for 9-F_{1t}-PhytoP (0.179 ng/mL) and *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF (0.527 ng/mL) (Fig. 4). On the contrary, individual PhytoPs and PhytoFs were competent for mitigating the increase of specific PGs induced by LPS. In this regard, the increased induced on the $PGF_{2\alpha}$ concentration was significantly prevented by *ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF, which restored values that were not significantly different relative to untreated controls (Fig. 4). Similarly, concerning PGD_2, although most of the PhytoPs evaluated did not provide a significant mitigation of the pro-inflammatory changes induced by LPS, *ent*-9-D_{1t}-PhytoP reduced its level down to values found in the untreated control. Regarding PGD_2, it is important to notice that again *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF reinforced the increase induced by LPS, giving rise to even significantly higher concentrations (0.133 ng/mL) (Fig. 4).

The biological activities of PhytoPs and PhytoFs have been suggested based on their structural similarity with mammal oxylipins derived from arachidonic acid (C20:4, n-6, AA) (isoprostanes and prostaglandins) that have important biological functions [63]. In this regard, to date, the bioactivity of PhytoPs and, to a lower extent, of PhytoFs, has been described mainly for their immunomodulatory power [57–59,61,64–67], anti-cancer activity [68], and anti-inflammatory potential [34,61,69–72]. However, despite these studies, the current knowledge on these compounds' biological functions is still scarce, being restricted to assessing whole plant-extracts and, in marginal cases, some individual PhytoPs (*e.g.*, 16-E_1-PhytoP). At the same time, there continues to be a gap of information concerning the biological interest of most PhytoPs and PhytoFs described so far.

In this scenario, the current availability of a wide range of synthesized individual PhytoPs and PhytoFs [42–47] has allowed new experimental designs that will somehow contribute to clarify the biological functions of these plant oxylipins, providing valuable information that can complete the current knowledge generated. Regarding the diverse aspects raised, one that needs to be addressed is the capacity of PhytoPs and PhytoFs to modulate the concentration of mammal isoprostanoids. This is of particular relevance because of the role of mammal isoprostanoids in an array of pathophysiological events, namely immunomodulation, vasoconstriction, platelet activation, and anti-aggregation, smooth muscle contraction of bronchi, and anti-inflammatory and apoptosis-inducing properties [73]. Therefore, describing the capacity of PhytoPs and PhytoFs to modulate the PG level during inflammation would allow for a better understanding of the current biological interest of plant oxylipins.

As for the immunomodulatory capacity already demonstrated by PhytoPs, this has been attributed to the structural analogy with human isoprostanoids (*e.g.*, 16-E_1-PhytoP *vs* PGE_2) (Traidl-Hoffmann et al., 2005). This structural similarity allows 16-E_1-PhytoP to mimic the functionality of PGE_2, thus inhibiting the production of interleukin (IL)-12 by dendritic cells and the activation of the peroxisome proliferator-activated receptor-gamma (PPAR-γ) dependent mechanisms, that in turn cause the inhibition of the nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) [57,58]. Interestingly, these pathways are also involved in inflammation, and could indicate that the modulation of PG level achieved when exposed to a pro-inflammatory stimulus (Figs. 2 and 3) could be related to an anti-inflammatory effect of PhytoPs and PhytoFs.

Indeed, aside from the immunomodulatory competences, human isoprostanoids have also been explicitly associated to anti-inflammatory activity due to the presence of an A-, J-, or deoxy-J-ring systems in their structure [70]. Thus, the presence of these reactive groups (hydroxyl and keto groups) on the cyclopentenone ring, and additional electrophilic properties enclosed in the chemical structure of PhytoPs and PhytoFs, have prompted the evaluation of coincident bioactivities on the modulation of inflammation by these plant oxylipins. In this respect, the assessment of diverse PhytoPs on anti-inflammatory functions has allowed pointing out the 9 and 16 series of the PhytoPs A_1, B_1, and deoxy-J_1, as those with the anti-inflammatory potential [69], which has been attributed to their structural analogy with the mammal's PGA_1 and deoxy-PGJ_2 [71].

In the sequence of these previous characterizations, the present work describes the capacity of individual PhytoPs and PhytoFs to modulate the PG profile in a pro-inflammatory environment *in vitro*, using LPS-stimulated THP-1 monocytes. Inflammatory mediators derived from COX-2 (Table 1) were measured through a targeted metabolomic approach. In this regard, the PG profile of LPS-stimulated monocytes was characterized by an increased concentration of seven PGs and their metabolites (15-keto-$PGF_{2\alpha}$, $PGF_{2\alpha}$, 11β-$PGF_{2\alpha}$, PGE_2, PGD_2, PGDM, and $PGF_{1\alpha}$), tentatively as a result of COX-2 up-regulation [74]. Considering to the short half-life of primary PGs, in the present study, some PG conjugates were also monitored after an enzymatic hydrolysis that breaks down the glucuronide moieties present in PGs [75], thus determining all isoprostanoids present in cells lysates and supernatants and avoiding underestimations of their concentration [48].

The infection of cells by microorganisms activates the inflammatory response due to the recognition of the LPS present in the microbial wall

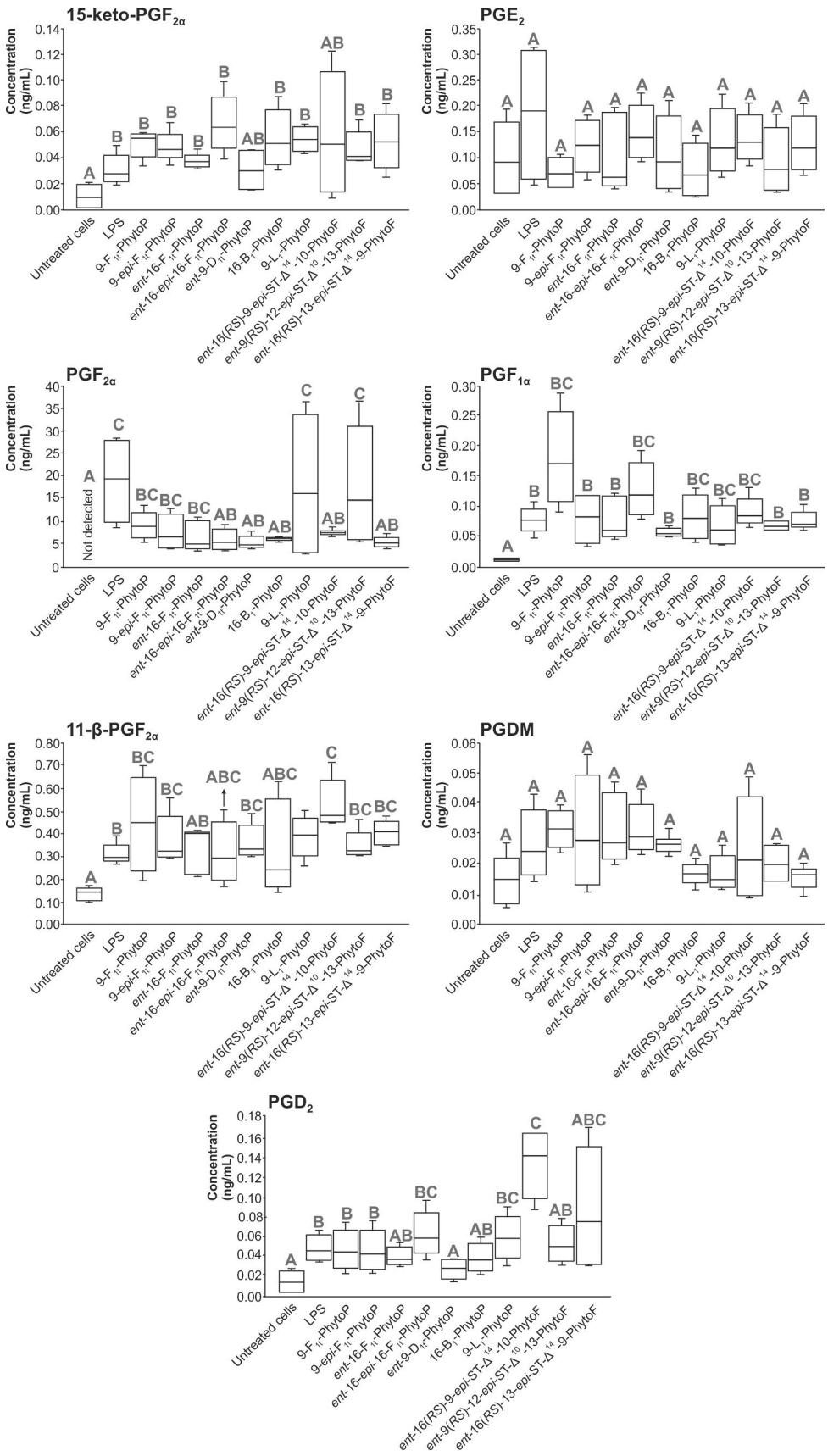

Fig. 4. Box plots with quartiles (upper values 75%, median 50%, and lower values 25%) of the effect of phytoprostanes and phytofurans on total (intracellular plus extracellular) prostaglandins THP-1 cells *in vitro* pre-exposed to 50 μM of individual plant oxylipins for 24-h, followed by 24-h treatment with 100 ng/mL of LPS. Boxes with a different capital letter within each plot are statistically different at $p < 0.05$ according to the analysis of variance (ANOVA) and Tukey's multiple range test.

by receptors of immune cells [2], which results in an overproduction of inflammatory mediators. Namely, PGE_2, which is synthesized from PGH_2 (formed from AA by COX-2), with the participation of various PG synthases [74] (Fig. 5), has versatile biological activities related to the inflammation process depending on binding to different E-prostanoid (EP) receptors (EP1, EP2, EP3, and EP4) [76], and specifically in monocytes [77]. The participation of PGE_2 in inflammation has been described as having a multifaceted role. This involves pro-inflammatory biological effects during the initial phases of the inflammatory process, including cell recruiting, activation, and proliferation, apoptosis, angiogenesis, and enhancement of immune cell surveillance, and augmenting sensory nerves to increase the pain response [78]. Besides, PGE_2 also controls mechanisms related to the resolution of inflammation in subsequent phases by acting as an immunosuppressive agent with respect to the cytokines and chemokines profiles expressed by immune cells (IL-12, IL-12p40, IFN-γ, and TNF-α, among others), as well as the expression level of their cognate receptors [79]. This anti-inflammatory effect is of special relevance in monocytes. In this cell population, PGE_2 triggers the inhibitory production of the chemokines responsible for the chemo-attraction of naïve T cells (CCL19), thus interfering with the activation of effector T cells [80].

The results obtained in the present work evidenced a multidimensional modulation of intracellular PGE_2 and release into the growth medium. Thus, in the presence of specific PhytoPs and PhytoFs (9-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, and *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF) in the medium, found a non-significant decrease ($p > 0.05$), of this PG (Fig. 2). In this respect, diverse *in vivo* studies have demonstrated that this effect could be due to an inhibition of COX-2, which entails the subsequent decrease of the PGE_2 concentration [74], preventing the pro-inflammatory signaling cascade triggered by this PG [57,58]. Alternatively, some PhytoPs induced a significant increase in PGE_2 in THP-1 cells (*ent*-16-F_{1t}-PhytoP and 16-B_1-PhytoP), which suggests specific, or even reverse, activities of the diverse plant oxylipins on the PGE_2 metabolism, probably affecting the activity of PGE_2 synthase and COX-2 in many ways [81]. Also, this modulatory activity was not precisely equal when considering intracellular levels and the concentration in the growth medium as a result of PGE_2, which could be due to the time window required for the biological activity of the diverse PhytoPs and PhytoFs, depending on the signaling route or molecular targets [82]. This result would suggest that PhytoPs and PhytoFs, through a multifaceted capacity to modulate of PGE_2, could be involved in the modification of the blood vessels wall permeability and thus, on the formation of edemas and the infiltration of leukocytes, during the different phases of the inflammatory process [83]. Interestingly the analysis of the additive (intra- and extra-cellular concentrations of PGE_2) did not reproduce the significant differences observed when analyzing both fractions separately, which reinforces the idea on the relevance of the kinetics of synthesis of this PG and the effect of the separate PhytoPs and PhytoFs in specific phases of the inflammatory process.

Mammal PGE_2, through the action of PGE_2 9-keto-reductase, is reduced to obtain $PGF_{2\alpha}$, which in turn, can also be synthesized from PGH_2 as a result of a reduction catalyzed by PGH_2 endoperoxide-reductase [84] (Fig. 5). Therefore, $PGF_{2\alpha}$ is involved in creating the inflammatory environment by influencing the synthesis and secretion of IL1β, IL6, IL8, and TNFα [85]. $PGF_{2\alpha}$ was neither detected in THP-1 cells nor released to the growth medium under control conditions, while after exposure to LPS, it was found in quantifiable concentrations in both cases (Figs. 2 and 3). The decreased concentration of $PGF_{2\alpha}$ induced by specific PhytoPs and PhytoFs (9-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, 9-L_1-PhytoP, and *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF) could be attributed to a decreased synthesis of PGE_2 according to the above referred potential mechanisms of action [81] or even to the inhibition of the enzymatic routes responsible for its formation from PGE_2 or PGH_2 upon their capacity to interact with the complex network of substrates or enzymes characterized by a high sequence homology such as the PGF synthases AKR1C2 and AKR1C3, among others [86]. This trend was even stronger when considering the $PGF_{2\alpha}$ released into the growth media, where almost all PhytoPs and PhytoFs mitigated the concentration of this PG. This is of particular relevance concerning 9-L_1-PhytoP, which surprisingly exhibited the most potent capacity to increase the intracellular level of $PGF_{2\alpha}$, while regarding the amount of this PG released to the growth medium, it appeared as the most potent compound in decreasing the concentration of $PGF_{2\alpha}$ in the THP-1 growth medium. This finding reinforces the idea of existing specific time windows for the separate biological action of the plant oxylipins.

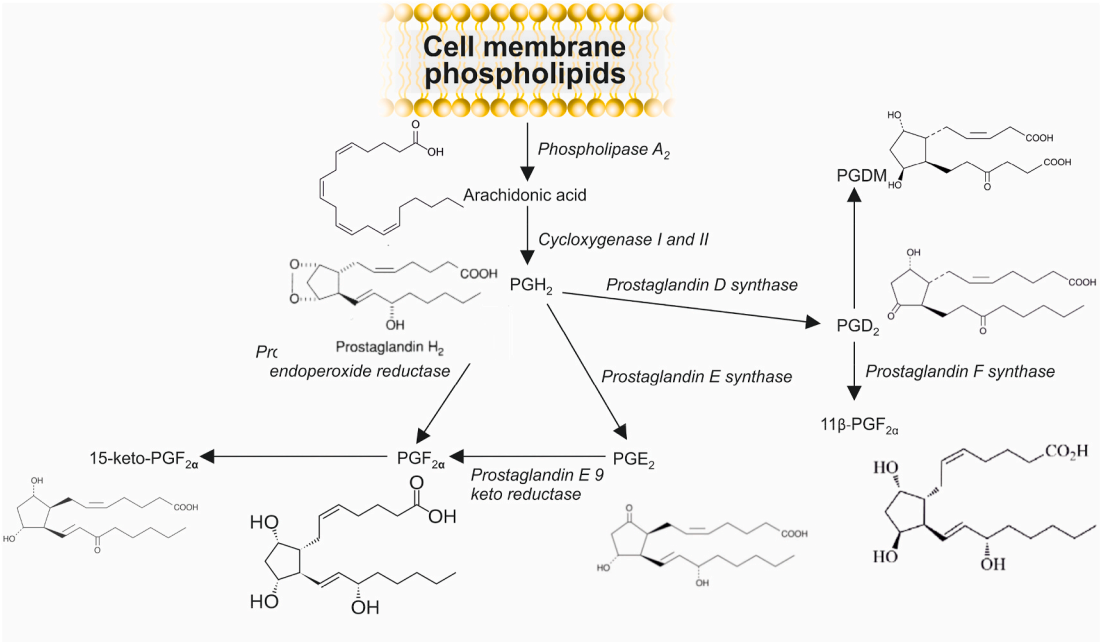

Fig. 5. Metabolic pathways affecting the prostaglandins-profile assessed in LPS-stimulated monocytes (THP-1 cell line) pre-exposed to phytoprostanes (9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, and 9-L_1-PhytoP) and phytofurans (*ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF).

$PGF_{2\alpha}$ is, in turn, metabolized to 15-keto-$PGF_{2\alpha}$ (Fig. 5), which is characterized by an anti-inflammatory activity developed by wounding-activated chemical defense molecules [87]. The upregulation of the intracellular concentration of 15-keto-$PGF_{2\alpha}$ induced by specific PhytoPs and PhytoFs (*ent*-16-*epi*-16-F_{1t}-PhytoP, 16-B_1-PhytoP, and *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF), or downregulation (*ent*-9-D_{1t}-PhytoP), contrasted with the almost constant increase of 15-keto-$PGF_{2\alpha}$ released caused by nearly all plant oxylipins, which is in good agreement with the cumulative significance of this result. Hence, this outcome strongly suggests the anti-inflammatory potential of PhytoPs and PhytoFs, according to the biological functions described for 15-keto-$PGF_{2\alpha}$ [87].

$PGF_{1\alpha}$, a PG derived from dihomo-γ-linolenic acid, was found in THP-1 cells lysate, and the growth medium of LPS stimulated monocytes. Although neither individual PhytoPs nor PhytoFs exhibited the capacity to mitigate the increase in the intracellular concentration of $PGF_{1\alpha}$, its release into the growth medium was significantly diminished by 9-L_1-PhytoP, 16-B_1-PhytoP, *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF, and *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF (Figs. 2 and 3). However, the joint analysis of the intra- and extracellular levels performed to provide information on the total synthesis of this PG as a result of the treatments monitored, did not confirm the capacity of PhytoPs and PhytoFs to modulate its status, which again, could be related to the ability of these compounds to take part in specific phases of the inflammatory process.

PGD_2, which was also detected in THP-1 monocytes, is closely involved in the course and resolution of inflammation. In this regard, PGD_2 is synthesized from PGH_2 by prostaglandin D-synthase and metabolized into several metabolites, including 11β-$PGF_{2\alpha}$ and PGDM, which maintain the pro- and anti-inflammatory characteristics described above for PGE_2 in the diverse phases of the inflammatory process [88]. The biological interest of PGD_2 regarding inflammation is found in its capacity to activate the PPAR-γ receptor by itself and by its metabolites, thus contributing to the resolution of inflammation [88]. A similar response of PGD_2 and 11β-$PGF_{2\alpha}$ was observed in both THP-1 cells and growth medium treated with individual PhytoPs and PhytoFs as modulatory agents of the inflammation induced by LPS (Figs. 2 and 3). PGD_2 is a structural isomer of PGE_2, and this could be responsible for the opposite response of PGD_2 and its metabolites relative to PGE_2, within the frame of the specific PG profile and metabolism of monocytes [89], as a specific modulatory action of the separate PhytoPs and PhytoFs.

5. Conclusions

As natural modulators of biological processes, the phytochemical compounds present in plant-based foods could be responsible for their anti-inflammatory properties. However, at present, there is still an open discussion on their competence to improve human health, in this case, regarding inflammation. Thus, concerning the newly-described class of secondary metabolites (PhytoPs and PhytoFs), specific compounds within this family (*ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF) showed, in addition to an absence of cytotoxicity on THP-1 monocytic cells, a valuable anti-inflammatory potential by modulating, *in vitro*, at 50 μM, the intracellular level of key PGs (15-keto-$PGF_{2\alpha}$, $PGF_{2\alpha}$, PGE_2, $PGF_{1\alpha}$, PGD_2, and PGDM in THP-1 cells), mediators of the inflammatory process, as well as their release into the growth medium. Although this concentration could exceed the level obtained in target cells (almost unexplored) this preliminary study will enable the future discovery of the biological potential of the bioaccessible and bioavailable fraction of the target compounds, in respect to specific pathophysiological conditions. However, both intra- and extra-cellular PGs in an additive form restrict the significant capacity to modulate the quantitative PG profile to $PGF_{2\alpha}$ (*ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, and 16-B_1-PhytoP), PGD_2 (9-D_{1t}-PhytoP and *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF), and 15-*epi*-15-F_{2t} (*ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, and 9-L_1-PhytoP), suggesting that the different individual PhytoPs and PhytoFs could act in diverse phases of the inflammatory process, characterized by the diverse quantitative profile of PGs according to their custom synthesis kinetic. Indeed, according to these results, the capacity of PhytoPs and PhytoFs to modulate the synthesis and overall level of PGs in the diverse phases of the inflammatory process needs to be further explored to understand their actual relevance as modulators of this pathological event. Also, as these plant oxylipins are present in a wide variety of plant-based foods, according to current knowledge, the unraveling of their anti-inflammatory potential is of high value in order to shed some light on the relative contribution of the separate bioactive components of these foods, *in vivo*, that would allow selecting the most beneficial compounds according to their quantitative profile of PhytoPs and PhytoFs. The findings described in the present work, regarding the capacity of individual PhytoPs and PhytoFs to restore the homeostasis of PGs in a pro-inflammatory environment, contribute to overcoming a critical limitation of studies with vegetable extracts that do not allow identifying the specific molecule/s displaying anti-inflammatory activities. On the other hand, the identification of the individual PhytoPs and PhytoFs responsible for specific bioactivities would allow extracting conclusions on the structure-activity relationship for this family of compounds and making suggestions on the possible mechanism of action of the individual PhytoPs and PhytoFs. These outcomes demonstrate the potential anti-inflammatory effects of specific PhytoPs and PhytoFs; however, based on these results, further mechanistic *in vitro* studies and *in vivo* experiments are needed to elucidate the operative character of the bioaccessible and bioavailable fractions of these compounds, and the involvement of the diverse cytokines and chemokines that co-participate in the inflammatory response.

Acknowledgments

This work was partially funded by the "Fundación Séneca de la Región de Murcia" Grupo de Excelencia 19900/GERM/15, and the Spanish project AGL2017-83386-R from the Spanish Ministry of Science, Innovation, and Universities. This work is included in the framework of the collaboration between the Spanish Research Council (CEBAS-CSIC) and CNRS-University of Montpellier, as "Projets Internationaux de Cooperation Scientifique" (PICS-2015-261141). The authors declare no conflict of interest.

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