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Original Research

In Vitro Bioactivities of Extracts from Tomato Pomace

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Abstract

Background: Tomato pomace (TP) is a coproduct generated by the extraction of tomato pulp, and is a potential source of bioactive molecules. In this study, we isolated several fractions from TP and evaluated their biological properties. **Materials and Methods**: TP was treated by maceration at room temperature with green solvents (ethanol, ethyl acetate, ethanol:water and ethanol:ethyl acetate) or supercritical CO_2 (SC-CO_2). The extracts were analyzed by HPLC-DAD to determine their composition, and their antioxidant activity was assessed. The potential therapeutic effects of the isolated fractions were assessed *in vitro*. **Results**: We identified 30 molecules on chromatography profiles, which revealed an abundance in phenolic acids, carotenoids, flavonoids and tannins, with differences in selectivity according to the solvent and pretreatment used. The highest radical scavenging activities were measured at 64–72% inhibition, corresponding to the ethanol or ethanol:water extracts with the highest polyphenol or flavonoid contents. Carotenoid content was increased by chemical pretreatment, to attain levels of 161 mg β-carotene/g ethyl acetate extract. This level of carotenoids seemed to have anti-inflammatory effects, with an IC_{50} of 9.3 μg/mL. In terms of anti-diabetic effects, the activities of α-glucosidase and α-amylase were best inhibited by extraction in an ethanol-to-water mixture (50:50). Cytotoxicity in a tumor cell line were highest for SC-CO_2 extracts (64.5% inhibition) and for ethanol extracts obtained after the enzymatic pretreatment of TP (37% inhibition). Some extracts also had dose-dependent activity against Zika virus. **Conclusions**: New fractions obtained from TP with ecocompatible solvents in mild conditions are rich in bioactive molecules. A comparison of the chromatographic profiles of the extracts led to the identification of several key molecules with therapeutic properties. The chemical pretreatment of TP is justified as a mean of increasing the carotenoid content of ethyl acetate fractions, whereas enzymatic pretreatment can increase the antioxidant activity of ethyl acetate fractions and increase the cytotoxicity of ethanol fractions. The SC-CO_2 fraction contained a smaller number of metabolites detectable on HPLC, but it had high levels of cytotoxicity and antioxidant activity. Finally, the fractions obtained appeared to be suitable for use to target one or several of the biological activities studied.

Keywords: tomato pomace; green extraction; biological activity; HPLC-DAD

1. Introduction

Fresh and processed tomatoes for human consumption represent a major market in Europe. Moreover, a number of the compounds present in tomatoes are now recognized to have beneficial effects on human health [1]. During the processing of fresh tomatoes to recover the pulp, large amounts of a coproduct called tomato pomace (TP) are generated. This coproduct accounts for 3 to 5% of the fresh mass of tomatoes and a world production of 5.4 to 9.0 Mt [2]. The valorization of tomato pomace constitutes a technical, economic and environmental challenge for improving crop competitiveness through the development of new production chains. Indeed, the recycling of this residual biomass provides an opportunity to recover molecules of interest and to meet market demands.

TP consists principally of primary cell wall polysaccharides, forming fibers that were characterized by Lenucci *et al*. [3], who identified these biopolymers as a useful source for bioethanol production. However, TP is a mixture of tomato skin, seeds and a small amount of pulp, and it contains a number of different metabolites found in fresh tomatoes. The skin contains a cuticle layer consisting of polyesters (cutin) and polysaccharides (cellulose, hemicelluloses, and pectins) [4]. The seeds are rich in lipids and proteins and are also a source of tocochromanols (tocopherols and tocotrienols) [5]. Tomato pomace has been described as a source of carotenoids (including lycopene, carotenes and lutein), phenolic compounds (such as chlorogenic acid, coumaric acid, and caffeic acid) and flavonoids, including flavones and flavonols (such as rutin, quercetin and kaempferol), and flavanols (mainly epicatechin), which are particularly abundant in the skin [6]. TP also contains

molecules from other families, such as tannins, vitamins, glucoside derivatives, the concentrations of which depend on environmental factors [7]. Some of these compounds, particularly lycopene, the principal carotenoid in tomatoes, and β-carotene and lutein [8], are recognized as strong antioxidants. Lycopene has an ability to inhibit reactive forms of oxygen to combat oxidative stress ten times greater than that of α-tocopherol [9]. Lycopene content is highly dependent on plant variety and month of harvest.

Carotenoids have already been extracted from TP with pectinolytic and cellulolytic enzyme treatment, followed by extraction with solvent. The hydrolysis mediated by enzyme treatments leads to a breakdown of cell wall structure [2]. In these conditions, 11.5 mg lycopene/g tomato waste can be extracted with ethyl acetate [10]. Another study [11] showed that lycopene can be recovered efficiently from tomato skin by treatment with a combination of enzymes and a surfactant (Span 20), which was retained in the final formulation. In the absence of pretreatment, tomato pomace has been placed in contact with hexane:acetone mixture at room temperature, to recover carotenoids [12,13]. Extractions have also been performed by sonication (SAE) or with microwaves (MAE), to generate fractions with antioxidant properties [14]. Sonication was applied to media containing ethanol 95° (50 min) [15], sunflower oil (10 min, 70 W) [16] or n-hexane:acetone (100 W, 30 min) [17]. Micro-waves (300 W) were used with ethanol 95° (60 s) for the extraction of several metabolites from TP [18]. These two modes of activation made it possible to decrease contact times and the amount of solvent used, as they facilitated the diffusion of solvents into the biopolymer network. However, these conditions led to an increase in the temperature of the extraction medium, which may cause the degradation of some molecules and a lower selectivity of extraction. Moreover, it was not possible to exclude the possibility of changes to the chemical structure of target compounds [14].

Carotenoids were also extracted from tomato pomace or tomato skin with SC-CO_2 above a pressure of 300 bars and at temperatures between 60 and 80 °C [19–22]. The fractions obtained were particularly rich in lycopene, with an extraction yield of up to 82%. These conditions constitute an interesting alternative to conventional extractions for recovering substances with a low polarity. CO_2 is not toxic or flammable, and its solvating power is enhanced above its critical point. Nevertheless, these conditions require investment in safe equipment. Metabolites from TP have frequently been reported to be beneficial to health. Lycopene, for example, has been reported to decrease the risks of developing chronic diseases, such as cancer or heart diseases [23]. Carotenoids extracted from purple tomato skins (in an acetone:hexane mixture) have been shown to have antitumor effects against various cancer cell lines [24]. The phenolic compound content of a methane extract of TP has been shown to have antimutagenic activity [25]. Biological properties have generally been studied after isolating a particular category of compounds, such as lycopene, carotenoids or polyphenols from TP.

In this study, we assessed the value of whole extracts obtained from TP for use in the fields of cosmetics and nutraceuticals. Our methodology was based on the selection of eco-compatible processes for treating tomato pomace and generating cocktails of molecules, the synergic effects of which could result in interesting therapeutic properties. The effects of pretreatment of the raw material before solvent extraction, particularly for oxidative treatment, have never before been evaluated. The fractions isolated, characterized by the presence of key molecules, were analyzed to assess their antioxidant properties (DPPH inhibition), anti-inflammatory activities (15-lipoxygenase inhibition), anti-proliferative effects on 116 HCT cancer cell lines, anti-diabetic potential (inhibition of α-amylase and α-glucosidase) and antiviral activities against Zika virus.

2. Materials and Methods

Fig. 1 describes the strategy used for extract preparation. Half the tomato pomace samples were subjected to chemical or enzymatic pretreatment in an aqueous medium. The other samples were treated directly by maceration in an organic solvent or placed in an SC-CO_2 reactor.

Quantitative spectrophotometric analyses and qualitative analyses (HPLC-DAD) analyses were performed to identify the categories of metabolites present in the extracts.

2.1 Materials and Reagents

All the chemicals used were of analytical grade. Ethanol, ethyl acetate, acetic acid, oxalic acid and ammonium oxalate were purchased from VWR (France). The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), rutin, lycopene, β-carotene and standard compounds used for identification and quantification were purchased from Sigma Aldrich and Extrasynthese.

Cellulase, pectinase, 15-lipoxygenase, α-amylase and α-glucosidase were obtained from Sigma Aldrich (France). Acetonitrile (ACN), Dulbecco's modified Eagle's medium (DMEM), dimethylsulfoxide (DMSO) and Roswell Park Memorial Institute medium (RPMI) were also obtained from Sigma Aldrich (France). The reagents 1,1-diphenyl-2-picrylhydrazyl (DPPH), aluminum trichloride (6-hydroxy-2,5,7,8-tetramethyl chroma-2-carboxylic acid), and Trolox were obtained from Alfa Aesar (France).

2.2 Preparation and Pretreatment of the Tomato Pomace

Fresh tomato pomace was supplied by the "Les Jus du Soleil" cooperative (Marmande, France). Tomato pomace was oven-dried at 50 °C during 8 hours to obtain dried tomato waste (DTW). The residual moisture content of the raw material after drying was 2%. Before extraction, the DTW was ground with an electric grinder to obtain particles between 1 and 2 mm in diameter (measured on optical microscopy, magnification 10×). Some samples were

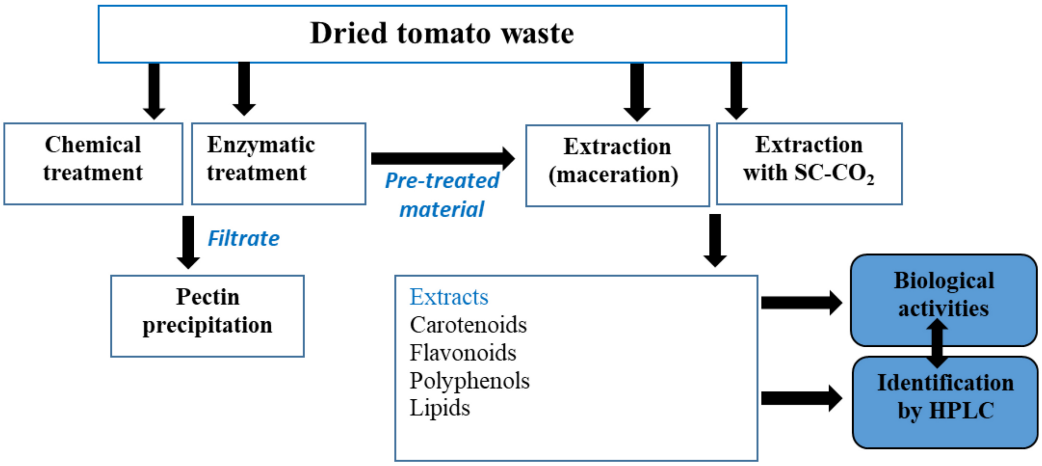

Fig. 1. Strategy for the preparation of extracts from dried tomato waste.

subjected to a pretreatment before the mixing of the DTW with a solvent. The pretreatment was designed to hydrolyze polyesters and polysaccharides, so as to facilitate the release of metabolites during extraction.

For chemical pretreatment, we used the oxidizing solution described by Grassino *et al*. [26]. We added 16 g f DTW to a mixture of 100 mL oxalate ammonium solution (16 g/L) and 100 mL oxalic acid solution (4 g/L). The resulting mixture was stirred at 90 °C for 7 hours and then filtered through a Büchner funnel. The filtrate was retained for subsequent pectin extraction. The solid residue was dried in an oven at 30 °C for 8 hours before extraction.

Pretreatment by enzymatic catalysis was performed by a method adapted from that of Choudhari *et al*. [27]. We prepared 20 mL buffer solution (0.2 mol/L) at pH 4.5 from 0.18 g acetic acid and 0.164 g sodium acetate, with stirring for 3 minutes. We dissolved 5 mg of enzyme (5 mg of cellulase or 5 mg of a 50:50 mixture of pectinase and cellulase) in 1 mL of buffer solution with stirring for 2 min. The enzyme preparation was then added to 3 g DTW and incubated for 15 minutes at either 55 °C (for cellulase) or 65 °C for the cellulase/pectinase mixture. The solid material was filtered with a Büchner funnel, washed and dried for 8 hours at 40 °C.

2.3 Extraction by Cold Maceration

The material (with or without chemical pretreatment) was placed in 100 mL solvent (ethyl acetate, ethanol, a 1:1 combination of ethyl acetate and ethanol or a 1:1 mixture of ethanol and water) and stirred at room temperature for 24 hours. The solid material was collected by filtration and the solvent was evaporated off under a vacuum.

Material that had been subjected to enzymatic pretreatment was placed in 30 mL solvent (ethyl acetate or ethanol) and stirred at room temperature for 24 hours. The solid material was recovered by filtration and washed, and the solvent was evaporated off under a vacuum.

Extraction yields, expressed as a% (w/w) of dry weight (d.w.), were calculated as follows:

$$\text{Extraction yield (\%)} = 100 \times \frac{\text{weight of extract}}{\text{solid weight of tomato pomace}} \quad (1)$$

2.4 Extraction with SC-CO_2

Extraction was performed in a supercritical fluid unit in accordance with Directive 97/23/EC. This apparatus consisted of a 0.22 L extraction vessel, a 0.22 L separator, a pneumatic pump, and two thermostatic baths at 40 °C. For each experiment, 15 g ground tomato pomace (particles of 2–3 mm in diameter) was loaded into the extractor. The CO_2 flow rate was set to 80 g/min at a pressure of 200 bars. The extraction was stopped when the weight of the material collected from separator became stable.

2.5 Determination of the Bioactive Agent Composition of Extracts

2.5.1 Total Phenolic Content (TPC)

The total polyphenol content of extracts was determined with Folin-Ciocalteu reagent with a modified version of the protocol described by Papoutsis *et al*. [28]. We placed 1 mL dry extract (1 mg/mL ethanol) in a 6 mL tube, followed by 0.5 mL of 10% (w/v) Folin-Ciocalteu reagent. The tube was gently shaken for 5 minutes, and we then added 1.5 mL of 2% sodium carbonate solution. The mixture was stirred manually and incubated in the dark at room temperature for 30 minutes. The reaction was stopped by immersing the tubes in a cold bath for 20 minutes. A blank was prepared in a similar manner, but with the extract replaced with ethanol. Absorbance was measured at 760 nm with a UVD 1800 spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid was used as a standard for the preparation of a calibration curve, with concentrations between 0.01 and 0.20 g/L. The solutions of gallic acid at different concentrations were mixed with Folin-Ciocalteu reagent and subjected to the protocol described for aliquots of the

extracts. The results are expressed in mg gallic acid equivalent (GAE) per gram of extract (mg GAE/g extract).

2.5.2 Total Flavonoid Content (TFC)

The flavonoid content of extracts was determined according to a modified version of the aluminum chloride method described by Quettier-Deleu *et al*. [29]. We placed 1 mL of diluted extract (1 mg/mL in ethanol) or 1 mL of a standard solution of rutin in a flask, to which 1 mL of a 2% solution of aluminum chloride in methanol was added. The contents of the flask were mixed and incubated in the dark for 1 hour at room temperature. The absorbance of samples at 415 nm was measured with a UVD 1800 spectrophotometer (Shimadzu, Kyoto, Japan). We used 1 mL of solvent (ethanol) with 1 mL of 2% aluminum chloride in methanol as the blank for sample reading. A calibration curve was prepared with rutin as the standard, at concentrations of 0.005 to 0.090 g/L. Aliquots of rutin solution were mixed with aluminum chloride solution, and the protocol described above was followed. Flavonoid concentration is expressed in milligrams of rutin equivalent (RE) per gram of extract (mg RE/g extract).

2.5.3 Total Carotenoid Content (TCC)

A UV 1800 spectrophotometer (Shimadzu, Kyoto, Japan) was used to estimate total carotenoid content by measuring absorbance at 446 nm in a quartz cell with a path length of 1 cm. A wavelength of 471 nm was used for lycopene determinations, and a wavelength of 400 nm was used for β-carotene determinations. Dried extract (0.1 g) was dissolved in 50 mL ethanol and ultrasonicated for 1 minute. Two calibration curves were plotted from the absorbances measured at 400 and 471 nm for the solutions prepared with concentrations of 0.027 to 0.150 g/mL β-carotene and lycopene in ethanol. Moreover, total carotenoid content were determined by measuring absorbance at 446 nm and is expressed in mg of β-carotene equivalent per gram of extract.

2.6 Determination of the Biological Activity of Extracts

2.6.1 Determination of Antioxidant Activity with DPPH

The antioxidant activity of the extracts was assessed by determining their free radical scavenging activity against 2,2-diphenyl-1-picryldrazyl (DPPH), as described by Brand-Williams [30]. We dissolved 5 mg DPPH in 100 mL ethanol to obtain an absorbance of DPPH at 516 nm between 0.9 and 1.1. Extracts were diluted in ethanol to obtain concentrations of 500 μg/mL. We added a 150 μL aliquot of each extract and 150 μL of 50 μg/mL DPPH in ethanol to the wells of a 96-well microplate. The plate was kept in the dark for 40 minutes, with mixing by gentle rotation every 5 minutes. Absorbance was read at 516 nm with a spectrophotometer (Spectrostar, BMG Labtech, Ortenberg, Germany). Percent inhibition was calculated as follows:

$$\text{Inhibition } (\%) = 100 \times \left(1 - \frac{A}{A_0}\right) \quad (2)$$

where A is the absorption in the presence of the extract, and A_0 is the absorption of the solution obtained by mixing 150 μL of DPPH solution with 150 μL of ethanol.

2.6.2 Antidiabetic Activity

The ability of extracts to inhibit α-glucosidase activity was estimated in a modified version of the assay described by Oueslati *et al*. [31]. We used p-nitrophenyl-α-D-glucopyranoside (PNPG) as the substrate for this experiment. Various solutions of the extracts at different concentrations were prepared in dimethyl sulfoxide (DMSO). In brief, 50 μL of the extract tested (250 μg/mL in DMSO) was mixed with 100 μL of the enzyme solution (1 U/mL α-glucosidase in phosphate buffer (pH 6.9)) and 50 μL phosphate buffer (0.1 M, pH = 6.9). The mixture was incubated for 10 minutes at 25 °C, and 50 μL PNPG solution (5 mM in phosphate buffer, pH = 6.9) was added to the mixture (1% DMSO), which was then incubated for 5 minutes at 25 °C. Absorbance at 405 nm was measured in 96-well microplates with a Thermo Fisher Scientific Multiskan™ GO Microplate Spectrophotometer (Vantaa, Finland). Measurements were performed three times. Acarbose, an inhibitor of α-glucosidase, was used as a positive control. The percent inhibition (%) reflects the ability of the extract to inhibit α-glucosidase activity and was calculated as follows:

$$\alpha - \text{ glucosidase inhibition activity } (\%) = 100 \times \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \quad (3)$$

where $A_{control}$ is the absorbance (405 nm) of the control prepared with 100 μL potassium phosphate buffer (replacing α-glucosidase) and A_{sample} is the absorbance (405 nm) in the presence of the extract.

Inhibitory activity is expressed as the IC_{50} (μg/mL), corresponding to the concentration required to inhibit the enzymatic hydrolysis (by α-glucosidase) of the p-nitrophenyl-α-D-glucopyranoside substrate by 50%.

The ability of extracts to inhibit α-amylase activity was estimated by a modified version of the DNS method described by Saidi *et al*. [32]. Each extract was dissolved in a minimum amount of DMSO and diluted in sodium phosphate buffer (0.1 M, pH 6.9). The concentration of DMSO in the mixture did not exceed 1%. Diluted extract (50 μL of a 250 μg/mL dilution) was added to 50 μL of α-amylase solution (3 mg/5 mL of the same buffer). Samples were incubated for 15 minutes at 25 °C, and 100 μL starch solution (1% in buffer) was then added. The mixture was incubated at 25 °C for 3 minutes and was then mixed with 100 μL 3,5-dinitrosalicylic acid (DNS) solution. The reaction tube was placed in a boiling water bath for 10 minutes and was

then cooled to room temperature, before the addition of 1 mL sodium phosphate buffer. Absorbance was measured at 540 nm in triplicate in 96-well plates with a Thermo Fisher Scientific Multiskan^{TM} GO Microplate Spectrophotometer (Vantaa, Finland). Acarbose (1.3 mg/mL), an inhibitor of α-amylase, was used as a positive control. The percent inhibition (%) of α-amylase was calculated as follows:

$$\alpha - \text{ amylase inhibition activity } (\%) = 100 \times \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \quad (4)$$

where $A_{control}$ is the absorbance (540 nm) of the control prepared with 100 μL potassium phosphate buffer in place of α-amylase, A_{sample} is the absorbance (540 nm) in the presence of the extract. The concentration of the compound providing 50% inhibition (IC_{50} in μg/mL) was calculated by plotting percent inhibition against sample concentrations.

2.6.3 Anti-Inflammatory Activity

The anti-inflammatory activity of extracts was determined with 15-lipoxygenase (15-LOX), as described by Khelifi *et al.* [33], but with some modifications. In brief, 20 μL extract (250 μg/mL) was mixed with 150 μL sodium phosphate buffer (pH = 7.4), 20 μL 15-lipoxygenase and 60 μL linoleic acid (3.5 mM) as a substrate. For the blank, the substrate was replaced with 60 μL buffer. Then, the mixture was incubated at 25 °C for 10 minutes and absorbance at 234 nm was measured in 96-well microplates with a Thermo Fisher Scientific Multiskan^{TM} GO Microplate Spectrophotometer (Vantaa, Finland). Measurements were performed three times. Nordihydroguaiaretic acid (NDGA) at a concentration of 0.5 mg/mL was used as a positive control. The following equation was used to calculate the percent inhibition:

$$I\ (\%) = 100 \times \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \quad (5)$$

where $A_{control}$ is the absorbance (234 nm) of the reaction mixture when the extract is replaced with potassium phosphate buffer and A_{sample} is the absorbance (234 nm) in the presence of the extract.

2.6.4 Cell Viability Assay with HCT-116 Cells

This test is based on the reduction of MTT (a yellow water-soluble tetrazolium salt) by the mitochondrial dehydrogenase of intact cells to generate purple formazan. The anti-proliferation activity of the extracts against HCT-116 cells (a human colon cancer cell line) was estimated with a modified version of the protocol described by Dawra *et al.* [34]. The cells were purchased from ATCC, and cultured in RPMI 1640 medium at 37 °C in a humidified incubator (Thermo Fischer Scientific, Illkirch, France) under an atmosphere containing 5% CO_2. The HCT-116 cells were dispensed in a 96-well microplate, with 3×10^4 cells/well, in 100 μL of appropriate culture medium, and were incubated for 24 hours at 37 °C. The test compounds were suspended in DMSO and diluted to obtain solutions with a DMSO concentration below 1%. The microplate was incubated at 37 °C for 48 hours. The supernatant was then removed, 20 μL of MTT solution was added to each well and the plates were incubated for a further 40 minutes. The MTT reagent was removed and 80 μL DMSO was added to dissolve the formazan crystals. Absorbance was measured at 605 nm with a microplate reader (Multiskan^{TM} GO, F1-01620, Thermo Fisher Scientific, Vantaa, Finland). Measurements were performed three times. Tamoxifen at a concentration of 100 μM was used as a positive control. The percent inhibition of cellular activity was calculated as follows:

$$\text{Inhibition}(\%) = 100 \times \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \quad (6)$$

A_{blank} is the absorbance of the cell suspension without the extract.

2.6.5 Flow Cytometry Assay

For flow cytometry, the A549 cells were cultured in 96-well plates and infected with ZIKV^{GFP}, in the presence or absence of extracts, for 24 hours at a multiplicity of infection (MOI) of 1. The cells were then treated with trypsin and fixed by incubation with 3.7% PFA for 15 minutes. The cells were then subjected to a flow cytometry analysis in a Cytoflex machine (Beckman, Indianapolis, Indiana, USA). The results were analyzed with cytexpert software

2.7 High-Performance Liquid Chromatography Analysis (HPLC-DAD)

HPLC analyses were performed with a Thermo Fisher Scientific Spectra System P1000XR pump equipped with a Diode Array Detector (Waters, Massachusetts, 996). Separation was achieved on an RPC18 reverse-phase column (Phenomenex, Le Pecq, France) of 25 cm $\times$ 4.6 mm in size, with a particle size of 5 μm. Elution was performed at a flow rate of 1.2 mL/min, with a mobile phase consisting of acidified water at pH 2.6 (solvent A), and acidified water:acetonitrile (20:80 v/v) (solvent B). The samples were eluted with the following linear gradient: from 12% B to 30% B over a period of 35 minutes, from 30% B to 50% B over a period of 5 minutes, from 50% B to 88% B over a period of 5 minutes and, finally, from 88% B to 12% B over a period of 15 minutes. The extracts were prepared at a concentration of 1 mg/mL in acetonitrile and filtered with a Millex-HA (0.45 μm pores) syringe filter (Sigma Aldrich, Saint Louis, Missouri, USA). We injected 20 μL of each sample onto the column and detection was registered at 280 nm. The compounds were identified on the basis of their retention times (RT) and spectral characteristics relative to standards.

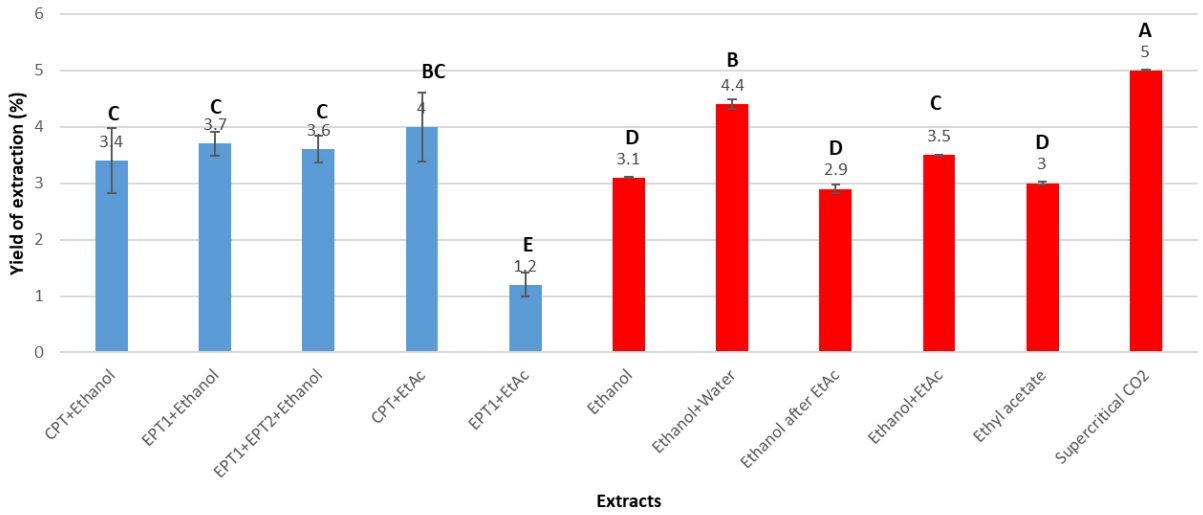

Fig. 2. Extraction yields for the various TP extracts (maceration at 20 °C for 24 hours and with SC-CO_2 at 40 °C and 200 bars). CPT, chemical pretreatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained after pretreatment, red histograms correspond to extracts obtained without pretreatment); each value is a mean of five replicates, columns with the same letters are not significantly different ($p > 0.05$).

2.8 Statistical Analysis

Data are expressed as the mean ± standard deviation. Statistical analysis was performed by one-way analysis of variance (ANOVA), with Duncan's multiple range test to compare means displaying significant variation ($p < 0.05$).

Statistical tests for antiviral activities were performed with Prism software (version 9.0; GraphPad software, La Jola, CA, USA).

3. Results and Discussion

3.1 Extraction Methods

Several fractions were isolated from DTW as described in Fig. 1. The ability of pretreatment to enrich the extract in metabolites was assessed. This step involved contact between the DTW and enzymes or an oxidative medium. But, this preliminary step could also potentially lead to a degradation or loss of some metabolites.

The chemical pretreatment was based on hydrolysis in an oxalic acid/sodium oxalate buffer. Grassino *et al.* [11] applied this pretreatment to TP before precipitating the pectins in the filtrate, by adding ethanol. Ecofriendly solvents (ethanol, ethyl acetate, and ethanol:water and ethanol:ethyl acetate mixtures) of various polarities were used to prepare several extracts by maceration. These biobased solvents were selected not only on the basis of their low toxicities, but also for their moderate boiling points (<100 °C), to limit the energy required for separation by distillation. Carotenoids have a higher affinity for lipophilic solvents, whereas polyphenols are better extracted in protic polar solvents. Ethanol and water can induce the swelling of cellulose, which may improve the release of metabolites. We chose to perform macerations at room temperature, to save energy and for ease of implementation. Extracts were also prepared with SC-CO_2, a method that usually efficiently extracts lipids and low-polarity molecules, such as carotenoids, whilst avoiding the need for a solvent separation step.

Extraction yields (Fig. 2) ranged from 1.2% to 5.0%. A yield of 5.0% was obtained with SC-CO_2 after 100 minutes of extraction at 80 g/min CO_2 and 200 bars. Increasing the pressure to 250 bars did not increase the yield. We therefore kept the pressure at 200 bars. Furthermore, finer grinding (particles <2–3 mm) did not improve extraction yield, probably due to the formation of channels described by Strati *et al.* [14]. Maceration in an ethanol and water mixture resulted in a yield of 4.4% yield. Chemical pretreatment increased the extraction yield obtained in ethyl acetate, by improving the release of lipophilic metabolites. The enzymatic pretreatment also tended to increase the yield obtained after maceration in ethanol, with the measured yields increasing from 3.1 to 3.7%. Successive macerations in ethyl acetate and then in ethanol resulted in the recovery of two fractions from the same sample of TP, corresponding to a total weight of 5.9 g per 100 g of TP. Finally, maceration in a mixture of ethyl acetate and ethanol resulted in a slightly higher yield (3.5%) than was obtained with either of these solvents used separately (3.0 and 3.1%).

3.2 Total Phenolic and Flavonoid Contents

The total flavonoid contents (TFC) and total phenolic content (TPC) were determined for the extracts (Figs. 3,4). Both these contents were highest for extracts in ethanol (99.5 mg RE/g extract and 99.8 mg GAE/g extract, respectively), or the ethanol:water mixture (93.2 mg RE/g and 93.0 mg GAE/g, respectively). The extract prepared with SC-CO_2 was also rich in phenolic compounds (87.6 mg

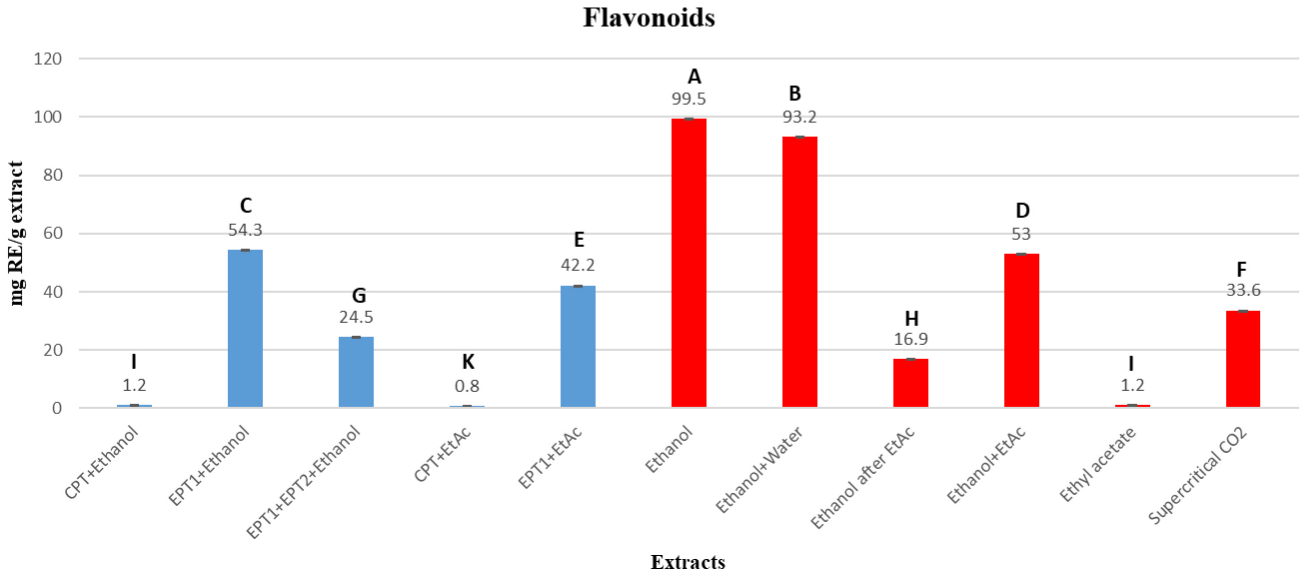

Fig. 3. Total flavonoid content in TP extracts (obtained by maceration at 20 °C for 24 hours and with SC-CO_2 at 40 °C with 80 g CO_2/min). Extracts were tested at 1 mg/mL. CPT, chemical pre-treatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained after pretreatment, red histograms correspond to extracts obtained without pretreatment; columns with the same letters are not significantly different ($p > 0.05$).

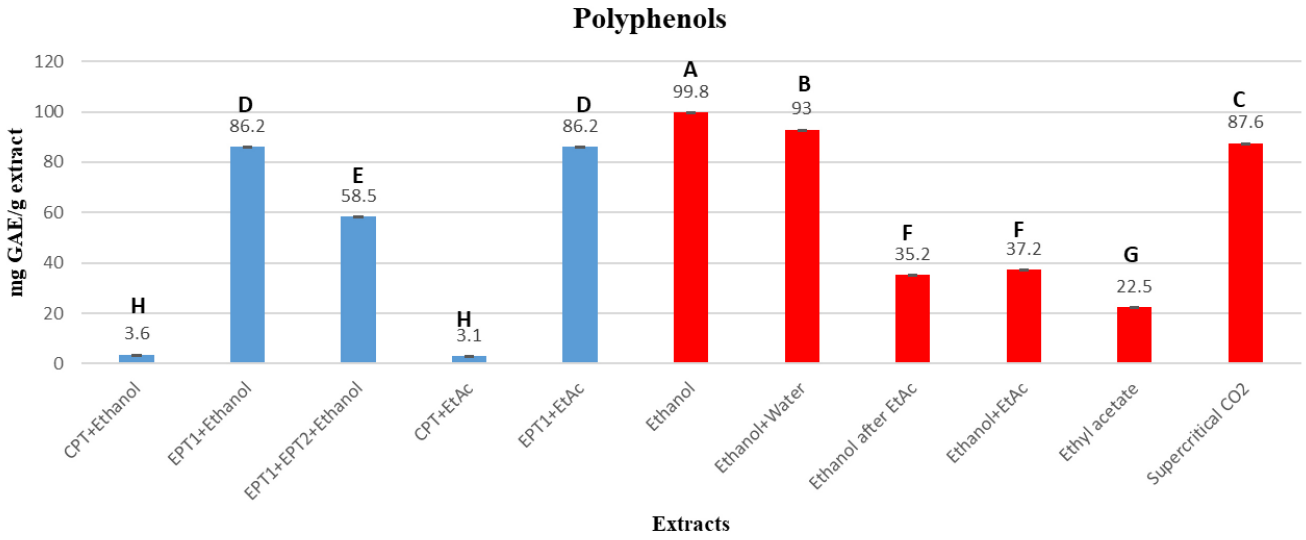

Fig. 4. Total polyphenol content in TP extracts (obtained by maceration at 20 °C for 24 hours and with SC-CO_2 at 40 °C and 200 bars). Extracts were tested at a concentration of 1 mg/mL. CPT, chemical pretreatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained after pretreatment. Red histograms correspond to extracts obtained without pretreatment, columns with the same letters are not significantly different ($p > 0.05$).

GAE/g) but had a lower flavonoid content than the extract obtained with ethanol (33.6 mg RE/g). For extracts obtained with ethyl acetate, enzymatic pretreatment increased the content of both polyphenols (up to 86.2 mg GAE/g) and flavonoids (42.2 mg RE/g) relative to the extract obtained with ethyl acetate in the absence of pretreatment. Nour *et al.* [12] found 1.2 mg GAE/g pomace and 0.4 mg RE/g pomace with an extraction in methanol with sonication. For the macerations performed in ethanol, the calculated values in mg/g TP are 3.09 mg GAE/g DW and 3.08 mg RE/g DW, showing that ethanol appears to be a suitable solvent to obtain these two categories of metabolites by maceration.

3.3 Total Carotenoid Content

The total carotenoid content determined by UV spectrophotometry is presented in Fig. 5. Extracts prepared by maceration in ethyl acetate after chemical pretreatment had the highest total carotenoid content, and the high-

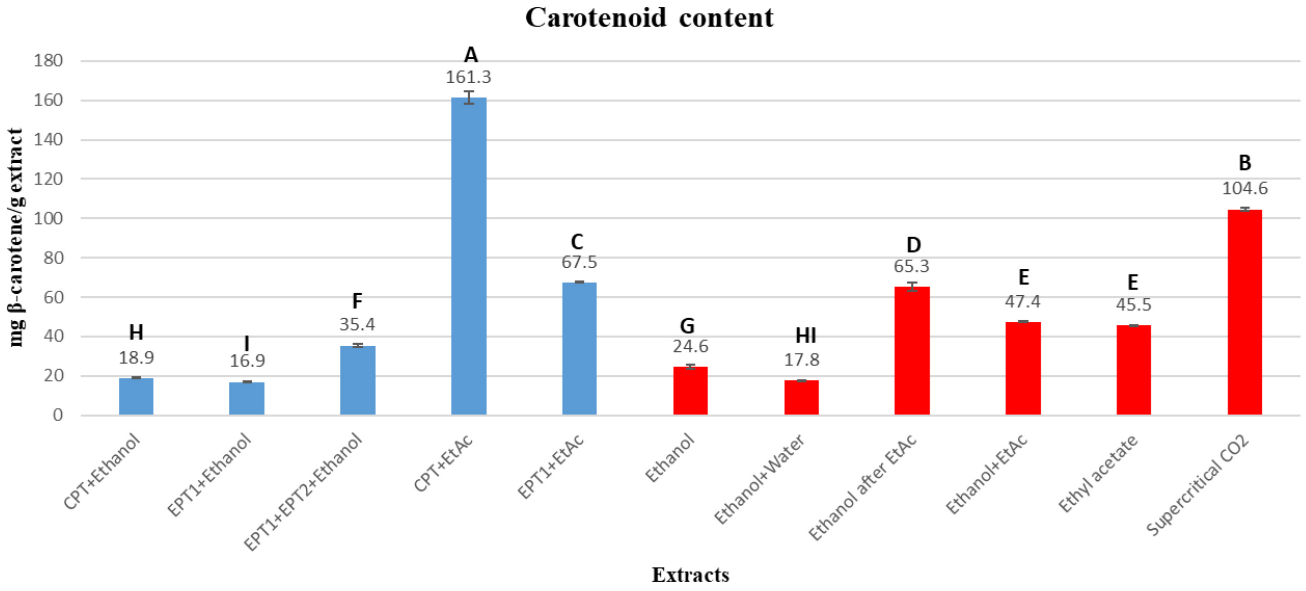

Fig. 5. Total carotenoid content of TP extracts (obtained by maceration at 20 °C for 24 hours or with SC-CO_2 at 40 °C and 200 bars). CPT, chemical pretreatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained with pretreatment. Red histograms correspond to extracts obtained without pretreatment, columns with the same letters are not significantly different ($p > 0.05$).

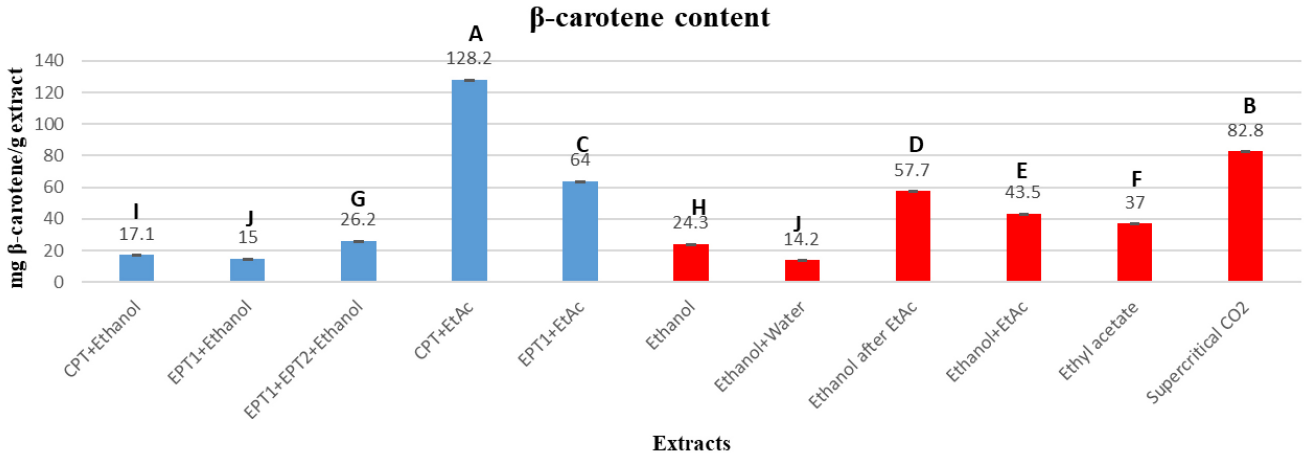

Fig. 6. Total β-carotene content of TP extracts (obtained by maceration at 20 °C for 24 hours or with SC-CO_2 at 40 °C and 200 bars). CPT, chemical pretreatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained with pretreatment; red histograms correspond to extracts obtained without pretreatment), columns with the same letters are not significantly different ($p > 0.05$).

est contents of lycopene (60.1 mg/g extract corresponding to 2.4 mg/g DTW) and β-carotene (128.2 mg/g extract) (Figs. 6,7).

The extraction yield of lycopene obtained in these conditions was greater than that (1.33 mg/g DTW) reported by Silva *et al.* [35] and Szabo *et al.* [36] for an extraction of TP with a mixture of ethyl acetate and ethyl lactate activated by ultrasound (63 °C, 100 mL/g). In addition, enzymatic pretreatment applied prior to ethyl acetate maceration was more effective to release lycopene (55.8 mg/g extract) compared to the case when it was coupled to ethanolic maceration (12.6 mg lycopene/g extract). The rates of lycopene (56 mg/g extract) and ß-caroten (82 mg/g extract) obtained in SC-CO_2 extract were greater than that obtained at higher pressures tested by Lenucci *et al.* [37]. Effective extractions can be thus achieved at moderate flows and pressures, justifying the selection of this process for the development of a tomato waste biorefinery [38].

3.4 Radical Scavenging Activity

The radical scavenging activity of each extract was expressed as a percentage inhibition in Fig. 8. The highest antioxidant activities (69.7% and 71.6% of inhibition) were detected for extracts obtained with ethanol:water and

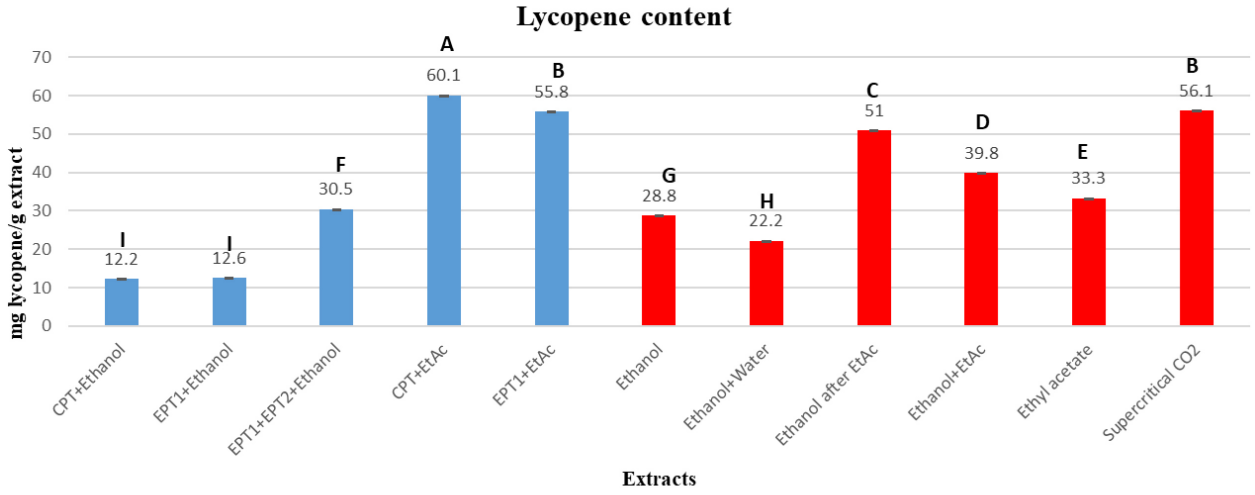

Fig. 7. Total lycopene content of TP extracts (obtained by maceration at 20 °C for 24 hours or with SC-CO_2 at 40 °C and 200 bars). CPT, chemical pretreatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained with pretreatment. Red histograms correspond to extracts obtained directly from the dried tomato waste (*i.e.*, without pretreatment), columns with at the same letters are not significantly different ($p > 0.05$).

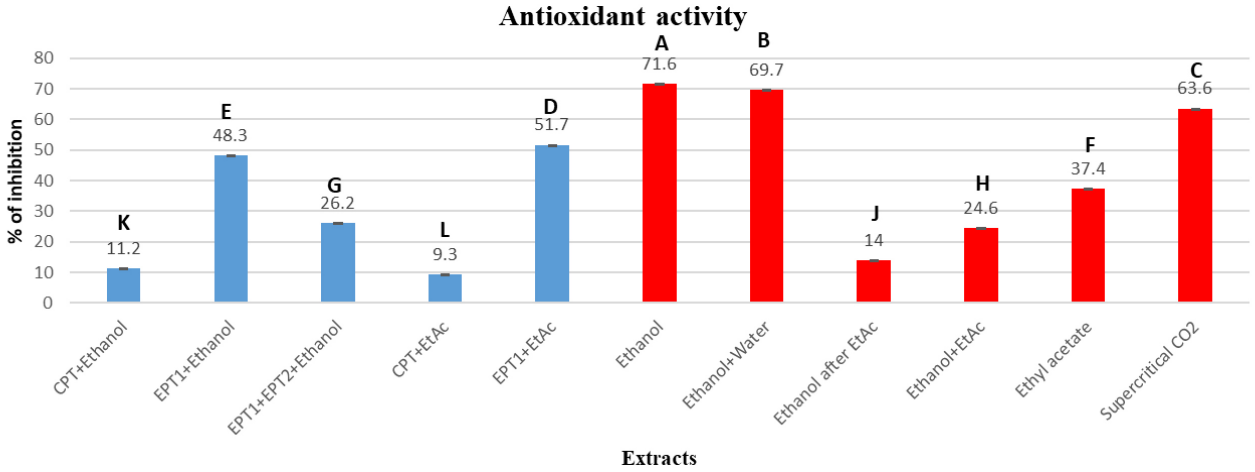

Fig. 8. DPPH radical scavenging activity of TP extracts (tested at 1 mg/mL) obtained by maceration at 20 °C for 24 hours or with SC-CO_2 at 40 °C and 200 bars. CPT, chemical pretreatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained with pretreatment. Red histograms correspond to extracts obtained without pretreatment, columns with the same letters are not significantly different ($p > 0.05$).

ethanol, samples which correspond to the highest contents in polyphenols and carotenoids. The extract prepared with SC-CO_2 also showed interesting antioxidant properties which can be attributed to its contents in polyphenols and lycopene. The contribution of methanolic fraction from fresh tomatoes, to the total antioxidant activity was clearly higher compared to that of lipophilic fractions obtained in hexane according to Lenucci *et al.* [39]. Promising antioxidant properties (around 300 mg TE/100 g of TP) were also found from ethanolic fraction of TP, isolated after the elimination of non-polar compounds in hexane [40]. Cetkovic *et al.* [41] suggested that the antioxidant activity of ethanolic extracts was due to effective scavenging of hydroxyl and superoxide anion radicals.

3.5 HPLC-DAD Analyses

The analyses described above showed that it was possible, in the extraction conditions used, to preserve heat-sensitive molecules and to obtain extracts with variable carotenoid, polyphenol and flavonoid contents. The enzymatic pretreatment enhanced the polyphenol and flavonoid contents of the ethyl acetate fraction, resulting in higher levels of antioxidant activity. Successive extractions (*i.e.*, ethanol after ethyl acetate) generated a final extract richer in carotenoids. SC-CO_2 extract was rich in lycopene, molecule which also contributed to its antioxidant activity.

HPLC-DAD analyses were used to determine the composition of the extracts more precisely (Table 1) and for instance to detect ascorbic acid as an antioxidant substance.

Table 1. Identification of molecules in extracts from tomato pomace by HPLC-DAD.

No.	RT (min)	Compound	With pretreatment (area, in AU)			Without pretreatment (area, in AU)					
			EPT1 + ethanol	EPT1 + EPT2 + ethanol	CPT + EtAc	Ethanol	Ethanol + water	Ethanol after EtAc	Ethanol + EtAc	EtAc	SC-CO_2
1	2.2	3-amino-4-hydroxybenzoic acid	2.6	9.5	-	2.8	-	12.3	18.7	1.9	-
2	2.81	ascorbic acid	-	15.6	0.2	13.3	26.0	14.6	45.8	6.2	-
3	4.1	gallic acid	-	-	0.1	-	‘	-	7.9		
4	7.66	hamamelitannin	-	-	-	-	-	0.6	4.4	-	-
5	9.88	chlorogenic acid	0.8	4.9	-	4.5	2.8	1.4	11.8	-	4.8
6	11.97	epicatechin (flavonol)	-	2.3	-	1.7	-	-	6.5	-	-
7	12.9	caffeic acid		1.2	-	-	1.1	-	-	-	-
8	15.3	2,4 dihydroxy cinnamic acid	-	3.1	-	1.6	-	1.1	4.6	4.6	-
9	18.07	6-hydroxycoumarine		0.5		0.5					3.5
10	19.15	3,4-dihydroxybenzoic acid methyl ester	-	-	-	-	-	0.7	-	-	-
11	19.88	7-hydroxycoumarin-3-carboxylic acid	1.2	-	-	-	-	-	-	-	-
12	20.94	ferulic acid	-	2.8	-	1.1	-	-	-	-	-
13	21.19	coumaric acid	-	-	-	-	-	0.6	3.4	2.1	-
14	22.74	rutin hydrate	-	-	1.7	-	-	5.1	-	-	-
15	23.56	sinapic acid	-	3.6	-	-	-	-	5.1	2.9	-
16	25.94	isoquercitrin (flavonoid glucoside)	-	4.8	-	0.8	1.1	-	12.3	-	-
17	27.41	trihydroxyethylrutin	-	-	-	-	-	2.4	-	-	14.2
18	39.89	salvianolic acid B	0.5	1.0	-	-	-	-	2.5	-	-
19	41.56	7,3- dihydroxyflavone	-	-	-	-	-	-	6.5	1.3	-
20	42.78	5,7-dihydroxy-4-propylcoumarin (tannin)	-	3.6	-	-	1.6	-	7.9	-	-
21	44.28	5,6,7-trihydroxyflavone (baicalein)	-	24.3	3.4	5.8	3.5	11.9	45.4	36.7	-
22	45.34	naringenin (flavanone)	9.7	-	-	–		6.0	-	-	-
23	46.18	kaempferol (flavonol)	-	-	24.2	-	-	-	-	-	-
24	46.98	cinnamyl-3,4-dihydroxy-α-cyanocinnamate (tannin)	-	-	20.0	-		-	-	-	13.9
25	47.54	3,7-dimethoxyflavone	-	10.8	-	6.6	5.0	7.5-	-	-	-
26	47.56	3,3′-dimethoxyflavone	8.5	-	-	-	-	-	-	7.9	-
27	48.5	5-hydroxyflavone	-	7.7	-	4.2	3.7	3.4	7.3	-	-
28	49.54	b-carotene	3.0	-	77.7	-	-	-	-	-	82.1
29	50.12	lycopene	-	4.2	124.5	-	-	-	7.7	10.7	66.8
30	50.47	lutein	-	0.4	-	0.3	-	-	-	-	4.0

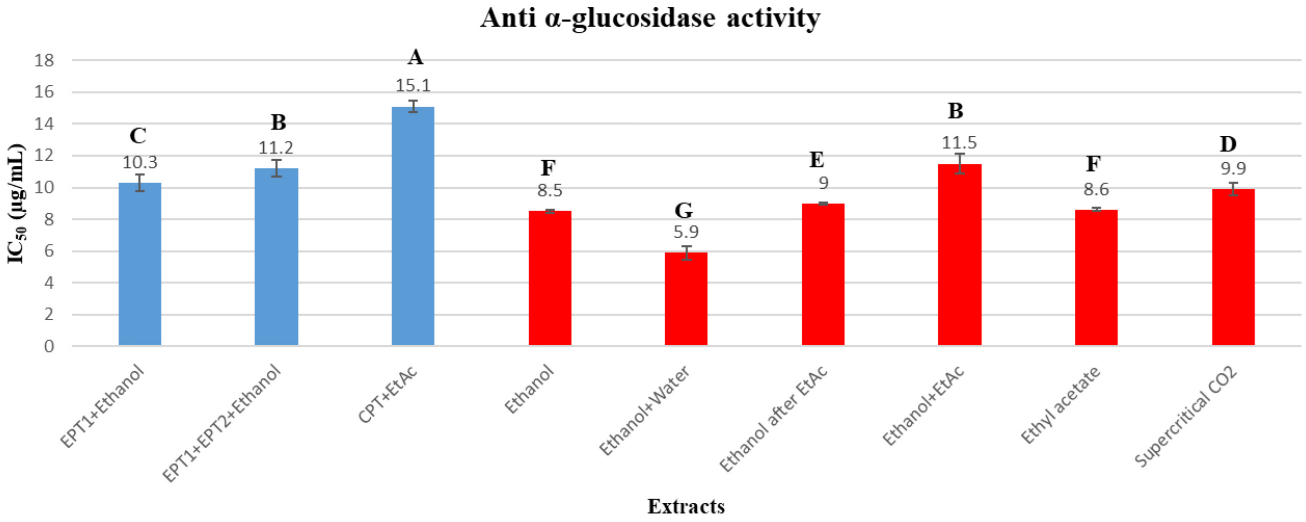

Fig. 9. Anti-α-glucosidase activity of TP extracts (tested at 250 μg/mL) obtained by maceration at 20 °C for 24 hours or with SC-CO_2 at 40 °C and 200 bars. CPT, chemical pretreatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained with pretreatment; Red histograms correspond to extracts obtained without pretreatment, columns with at the same letters are not significantly different ($p > 0.05$).

HPLC on the ethyl acetate extract obtained after chemical pretreatment revealed the presence of rutin hydrate, kaempferol, tannins and derivatives of *p*-hydroxy benzoic acid, together with high levels of carotenoids. Enzymatic pretreatment (with a mixture of two enzymes) enriched the ethanolic fraction in tannins, lycopene, isoquercitrine and specific phenol acids (sinapic acid, ferulic acid). Extraction in an ethanol ethyl acetate mixture resulted in higher levels of tannins and phenolic acids in the resulting extract than were obtained with ethanol alone. As for the ethanol-water mixture, overall composition was similar to that for the ethanol extract, with similar levels of antioxidant activity, but a lower carotenoid content. In the ethanol fraction obtained after maceration in ethyl acetate were detected rutin hydrate and trihydroxyethylrutin, as well as naringinin. Finally, the extract obtained with SC-CO_2 contained polyphenols (including phenolic acid), carotenoids (lycopene in particular) and trihydroxyethylrutin (a flavonoid) and tannin (a cinnamic acid ester).

3.6 Biological Activities

The biological activities of the extracts with the greatest bioactive molecule contents were assessed. We analyzed nine extracts by HPLC (Table 1).

3.6.1 Antidiabetic Activity

We used the α-glucosidase and α-amylase enzymes to assess antidiabetic activity. Acarbose, an α-glucosidase inhibitor prescribed for the treatment of diabetes, was used as a positive control. It has an IC_{50} of 48.8 μg/mL for these two enzymes. The percent inhibition of α-glucosidase and α-amylase achieved with the various extracts is presented in Figs. 9,10. These extracts clearly outperformed the positive control (with IC_{50} = 15.1 and 8.7 μg/mL for anti α-glucosidase and α-amylase activities, respectively), probably due to synergy between the various molecules present in the extracts.

The extract obtained with the ethanol:water (50:50) mixture yielded the lowest IC_{50} values (5.9 and 4.9 μg/mL). This extract contains flavones (molecules 21, 25 and 26) and phenolic acids (molecules 1, 5 and 7), which are known to have antidiabetic activity. The contribution of 5,7-dihydroxy-4-propyl coumarin (molecule 20) to several biological activities has been investigated with African mustard extracts [42]. In their review, Khalid Al-Ishaq *et al.* [43] described the antidiabetic activity of flavonoids, including flavones, together with their mechanism of action. Gheonea *et al.* [17] also reported an inhibitory effect (79.9%) against α-amylase for lycopene extracted from tomato skin at a concentration 8.5 mg/mL.

3.6.2 Evaluation of Anti-Inflammatory Activity

Nordihydroguaiaretic acid (NDGA) was used as a positive control in the 15-lipoxygenase (15-LOX) inhibition assays, with an IC_{50} of 4 μg/mL. The IC_{50} values of the extracts ranged from 9.3 to 12.3 μg/mL (Fig. 11), indicating clear activity against 15-lipoxygenase whatever the solvent used for extraction. The extract obtained with ethyl acetate after chemical pretreatment had one of the lowest IC_{50} values recorded (*i.e.*, 9.3). Kaempferol (molecule 23), cinnamyl-3,4-dihydroxy-α-cyanocinnamate (molecule 24) and rutin hydrate (molecule 14) are probably involved in this bioactivity, as all these molecules have already been reported to have anti-inflammatory properties. Devi *et al.* [44] described the mechanisms of action of kaempferol, and Pergola *et al.* [45] showed that molecule 24 is a potent in-

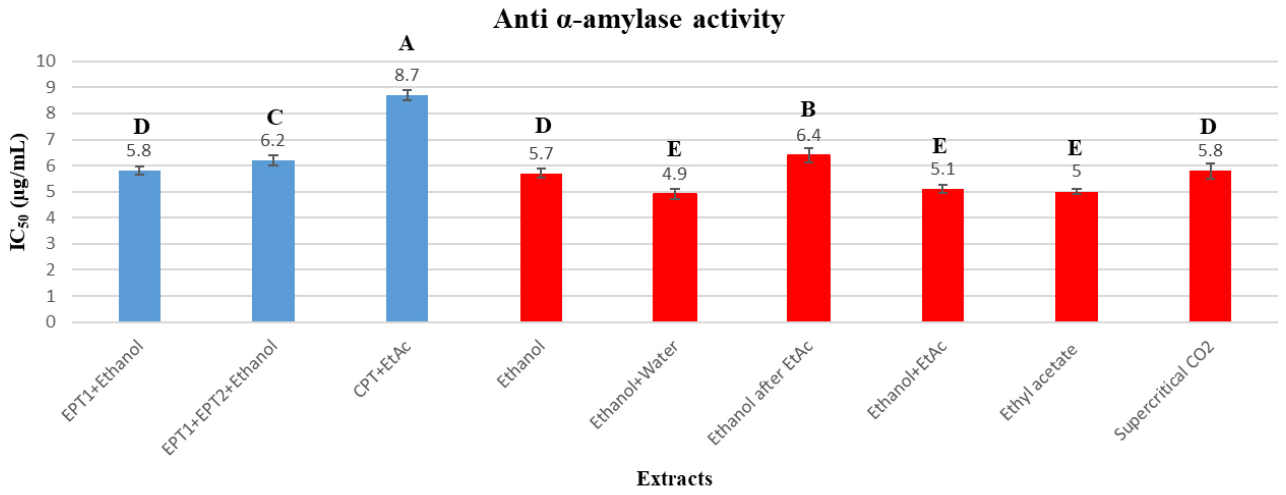

Fig. 10. Anti-α-amylase activity of TP extracts (tested at 250 μg/mL) obtained by maceration at 20 °C for 24 hours or with SC-CO_2 at 40 °C and 200 bars. CPT, chemical pretreatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained with pretreatment. Red histograms correspond to extracts obtained without pretreatment), columns with the same letters are not significantly different ($p > 0.05$).

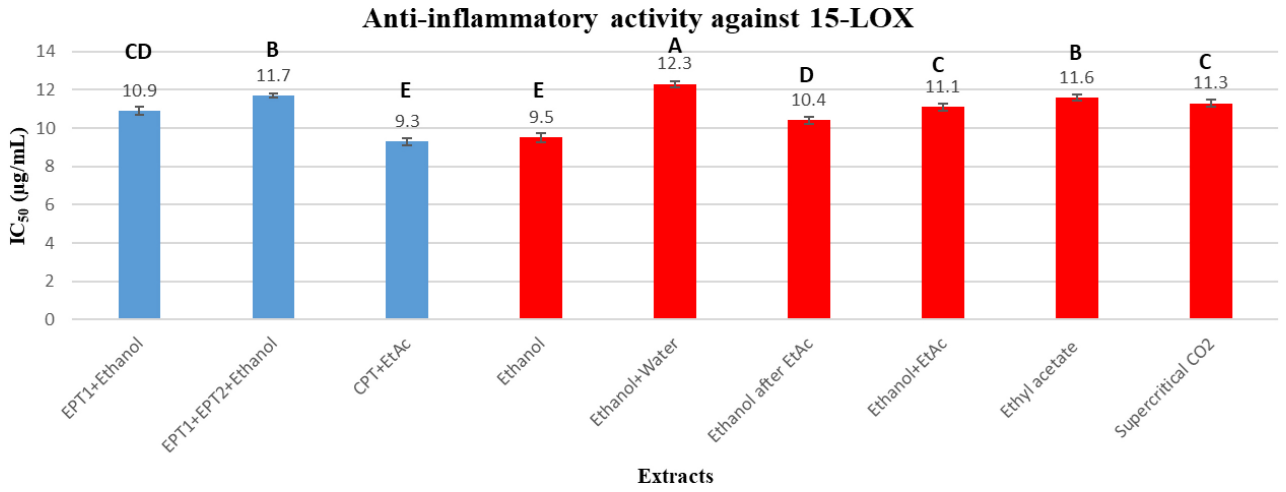

Fig. 11. Anti-inflammatory activity of TP extracts (tested at 250 μg/mL) against the 15-lipoxygenase enzyme, after maceration at 20 °C for 24 hours or with SC-CO_2 at 40 °C and 200 bars. CPT, chemical pretreatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained with pretreatment. Red histograms correspond to extracts obtained without pretreatment, columns with the same letters are not significantly different ($p > 0.05$).

hibitor of 5-LO that is effective *in vivo*.

Based on recent data from *in vitro* studies, Maleki *et al*. [46] highlighted the possibility that different subclasses of flavonoids (including rutin hydrate) might modulate different stages of inflammation, thereby playing key roles in diseases such as diabetes, asthma, cardiovascular diseases and cancer. Finally, the phenolic acids present in the ethanol-based extracts have also been described as potent inhibitors of 15-lipoxygenase [47,48].

3.6.3 Cytotoxicity against Tumor Cell Lines

The cytotoxicity of extracts (50 μg/mL) was evaluated against a variant HCT 116 tumor cell line (Fig. 12). Tamoxifene (100 μM), used as a positive control, gave 93% growth inhibition. The SC-CO_2 extract had a clear anticancer effect, yielding 64.5% inhibition. Four other extracts in ethanol had a moderate anticancer effect, with inhibition rates between 31% and 37%. These four extracts had high total polyphenol contents (Fig. 4), with high levels of chlorogenic acid (molecule 5) and several flavones in common. Their activities may be linked to several molecules previously reported to have anticancer properties. Trihydroxethylrutin (molecule 17), used in a pure state, has been shown to be an effective inhibitor of the growth of HCT-116 cells [33]. Chlorogenic acid extracted from decaffeinated coffee beans was found to be cytotoxic

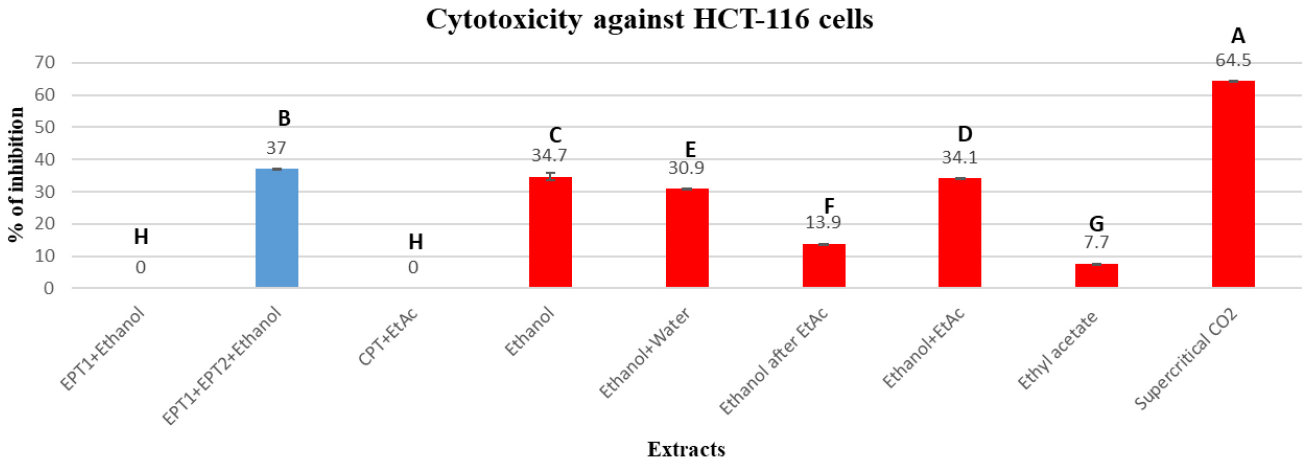

Fig. 12. Cytotoxic activity of TP extracts (tested at 50 μg/mL) against HCT 116 cancer cell lines, after maceration at 20 °C for 24 hours or with SC-CO_2 at 40 °C and 200 bars. CPT, chemical pretreatment; EPT1, enzymatic pretreatment with cellulase; EPT2, enzymatic pretreatment with pectinase; EtAc, ethyl acetate. Blue histograms correspond to extracts obtained with pretreatment. Red histograms correspond to extracts obtained without pretreatment, columns with the same letters are not significantly different ($p > 0.05$).

in the MTT test on HCT 116 cancer cells, with an IC_{50} of 0.84 mg/mL [49]. Lycopene (molecule 29) increased the inhibition of HCT116 cell growth by 58.3% when used at a concentration of 100 μg/mL [50]. Moreover, 5,6,7-trihydroxyflavone (*i.e.*, baicalein, molecule 21) extracted from the fruits of *Oroxylum indicum*, widely used in traditional Chinese herbal medicine for its anti-inflammatory properties, inhibited the growth of HL 60 cells by 50% at concentrations of 25 to 30 mM [51]. Flavones from tomato skin have also been reported to have inhibitory activity against SCC 9 human oral cancer cells [52,53].

3.6.4 Evaluation of Antiviral Activity against ZIKV *in Vitro*

The potential antiviral activities of the six extracts prepared without TP pretreatment were investigated against Zika virus (ZIKV), a mosquito-borne enveloped arbovirus from the *Flavivirus* genus that constitutes a major health burden, due to the diverse human cells it can infect [54]. For this assay, human A549 cells were infected with ZIKV carrying a reporter gene encoding green fluorescent protein (GFP) [55] for 24 hours in the presence or absence of various nontoxic concentrations of the extracts. The concentrations inhibiting infection by 50% were determined. The results of antiviral activity assays are presented in Fig. 13.

Cellular mitochondrial activities were preserved below 20 μg/mL (Fig. 13A). Above this concentration, cell viability was conserved for extracts 1 (ethanol) and 4 (ethanol and water), with values of 80–90% at 200 μg/mL relative to vehicle-treated control cells. In antiviral activity assays, extracts 1, 3 and 6 (prepared with ethanol, ethanol:ethyl acetate and SC-CO_2, respectively), inhibited ZIKV infection in a dose-dependent manner at concentrations above 10 μg/mL (Fig. 13B). At a concentration of 200 μg/mL these three extracts inhibit ZIKV infection in human cells up to 75%. Time-of-drug-addition assays (Fig. 13C) showed that the most active extracts (1, 3 and 6) impeded ZIKV entry into cells (Fig. 13D). Gaafar *et al.* [56] showed that an ethanol extract of tomato pomace (400 μg/μL) inhibited avian influenza A H5N1 virus by 63%. The epicatechin (molecule 6) detected in extracts 1 and 3 was described in the review by Behl *et al.* [57] as having antiviral activity against Zika virus. Baicalin (a trihydroxyflavone, molecule 20), which was also detected in ethanol extracts, has been reported to downregulate ZIKV replication [57,58]. Isoquercitrin (quercetin-3-O-glucoside, molecule 16), which was detected in extracts 1, 2 and 3, has been identified as a potent inhibitor of ZIKV in various cell types of human origin, as this molecule prevent the initiation of host-cell infection [59]. Some flavonoids have been reported to have anti-Zika virus activities, and several possible mechanisms of action have been suggested [54,60]. Finally, hamametannin (molecule 4 in extracts 2 and 3) has been described as a potential SARS-CoV-2 inhibitor, based on molecular docking data [61]. These classes of molecules may account for the observed antiviral activities of some TP extracts against ZIKV. They probably act in synergy, as the ethanolic fraction obtained after maceration in ethyl acetate, which would have concentrated the extract in polar molecules only (extract 5), had lower bioactivities.

4. Conclusions

Macerations of TP in the selected green solvents and extractions with SC-CO_2 conditions led to the isolation of fractions containing cocktails of preserved molecules. HPLC analysis identified 30 molecules in the various extracts, including hydroxy/methoxyflavones, phenolic acids, carotenoids and tannins as the most abundant groups of molecules. Our analyses confirmed that the choice of solvent and pretreatment determined the selectivity of the ex-

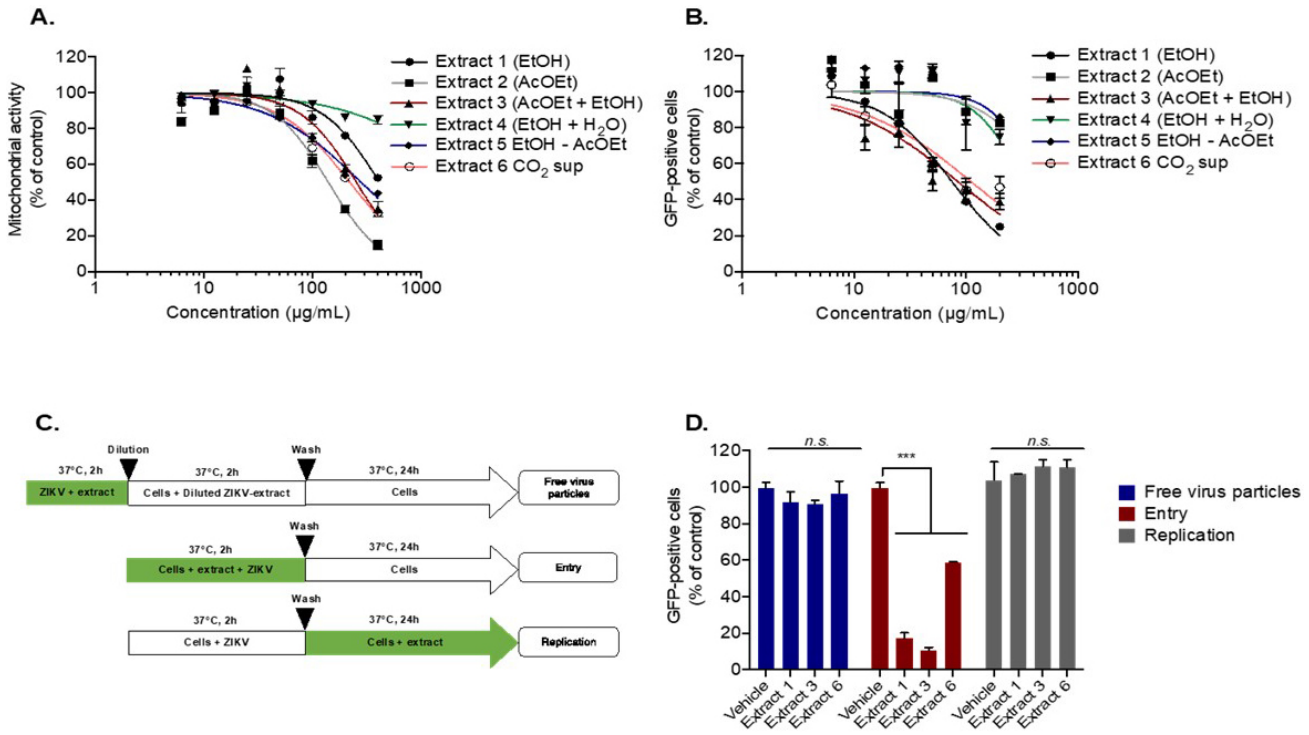

Fig. 13. Extracts display antiviral activity against ZIKV. (A) A549 cells were incubated with two-fold serial dilutions (400 to 6.25 μg/mL) of plant extracts for 24 hours. Cellular mitochondrial activity was evaluated in an MTT assay. (B) A549 cells were infected with ZIKV^{GFP} at an MOI of 1 in the presence of different concentrations of extracts. Flow cytometry analysis of GFP fluorescence was performed 24 hours post-infection. (C) Schematic representation of time-of-drug-addition assays used to characterize the antiviral activity of extracts 1, 3 and 6 (100 μg/mL). (D) Flow cytometry analysis of GFP expression in A549 cells infected with ZIKV^{GFP} for 24 hours at an MOI of 1 under the various experimental conditions shown in (C). The data shown are the means $\pm$ SD of four independent experiments performed in triplicate, and are expressed relative to vehicle-treated/infected cells. Statistical analysis consisted of one-way ANOVA followed by Dunnett's test for multiple comparisons with $p < 0.05$ considered significant.

traction process. Total polyphenol and flavonoid contents showed a significant contribution to the antioxidant properties of the fractions, with inhibition rates above 48%. The utility of pretreatment depends on the extraction solvent used and the properties targeted. Pretreatment is justified in the following cases. For extractions in ethyl acetate, the chemical pretreatment of TP enhanced its anti-inflammatory activity, with an IC_{50} of 9.3 μg/mL, highlighting the role of carotenoids in regulating the inflammatory process. For the same extract, enzymatic pretreatment was adapted to increase antioxidant activity from 37 to 51% inhibition. For ethanol extract, enzymatic pretreatment tended to enhance cytotoxicity activity against HCT 116 cells (37% rather than 34.7% inhibition). However, cytotoxicity was highest for the SC-CO_2 extract, for which 65% inhibition was recorded. In terms of antidiabetic potential, the ethanol:water extract inhibited both α-amylase and α-glucosidase activities with IC_{50} values of 4.9 and 5.9 μg/mL, respectively. At non-cytotoxic concentrations, the ethanol fraction of TP extract showed the highest antiviral effect against ZIKV with an IC_{50} of 75 μg/mL. This fraction acts on the early stages of the viral cycle by blocking viral entry into the host cell. Furthermore, conditions promoting more selective extraction (extraction in ethanol after the removal of lipophilic molecules in ethyl acetate) resulted in only moderate biological properties. This finding suggests that there may be synergy between some polar and non-polar molecules. Finally, the associations of metabolites obtained from tomato pomace under mild, ecofriendly conditions have potential benefits for human health. These fractions could be used without further fractioning, for formulations of pharmaceuticals, nutraceuticals or cosmetic formulations. Based on these findings, we hope to determine the role of the matrix (lipids or polysaccharides) in molecule preservation. Moreover, pretreatments may be of interest for extractions from fresh tomato pomace. We are currently designing eco-materials for upgrading the residual material from the TP.

Author Contributions

PDC, OM and AH conceived and designed this study; AJ and PDC performed the experiments, interpreted the results and wrote the article; JB supervised the therapeutic assays and HPLC analyses; CEK and JGH supervised the

antiviral assays; PE, PDC and OM revised the article and analyzed the data. All the authors read and approved the final version.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. OM is serving as one of the Editorial Board members of this journal. We declare that OM had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to MI.

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