Green pathway for utilisation of orange peel dust and *in silico* evaluation of pharmacological potential

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Abstract

This study is focused on the investigation of the new ways of valorization and maximal utilisation of the fruit dust obtained from the orange peel during industrial processing in the filter tea production. Therefore, the possibility of the integration of two attractive "green" extraction methods, CO_2-assisted extraction and ultrasound-assisted extraction, were investigated. In the first step to produce herbal extracts, supercritical carbon dioxide (Sc-CO_2) extraction of the orange peel dust (OPD) was applied in the pressure range of 10–30 MPa. The second step was the ultrasound-assisted extraction of the OPD remaining after the Sc-CO_2 extraction, with varying sonication amplitudes (20-100%). Gas chromatography combined with mass spectrometry was used for the chemical characterization of Sc-CO_2

extracts. Among the valuable volatile compounds, the most abundant are linoleic (31.11-41.47%) and hexadecanoic (15.22-20.58%) acid, osthol (4.53-5.42%), and 7-methoxy-8-(2-oxo-3-methylbutyl) coumarin (12.65-15.45%). Extracts obtained by UAE were analyzed by HPLC, where the dominant compounds were identified as hesperidin (848-900.24 [µg/ml]), narirutin (16.70-18.16 [µg/ml]), naringin (771.45 -830.39 [µg/ml]) and rutin (47.53-104.40 [µg/ml]). Finally, the *in silico* testing was performed to identify pharmacologically the most valuable bioactive compounds in the extracts, and to enable screening of the possible pharmacological effect and applications of the obtained.

Keywords: Orange peel; Herbal dust; Supercritical extraction; Ultrasound-assisted extraction; Polyphenols; *In silico* evaluation

1. Introduction

Citrus fruits are the most produced and consumed fruits in the world, both in fresh form and as a juice (Murador et al., 2019). According to the data of the Food and Agriculture Organization of the United Nations (FAO, 2021), it is estimated that 143,755.6 thousand tons of citrus were produced in 2019, of which 76,292.6 thousand tons were oranges. Because of a large world production and processing, large quantities of various citrus by-products and wastes are formed. This represents a huge problem in the waste disposal means, as well as a large economic burden for the citrus processing sectors. In the case of citruses, according to Mahato et al. (2018), the edible parts of citrus are pulp and juice, while segment walls, peel, pith residues and seeds are not edible and represent citrus waste (CW). The amount of CW ranges between 50-60% of processed fruit and depends on several factors such as the juice extraction system, citrus varieties, and the amount of water for processing (Taghizadeh-Alisaraei et al., 2017). Sharma et al. (2019) defined the composition of CW as follows: 40-55% of peel, 30-35% of internal tissues and less than 10% of seeds. The orange peel, as one of the dominant CWs, contains a large amount of high value-added compounds such as polyphenols, carotenoids, dietary fibre, sugars, essential oils, ascorbic acid, and significant amounts of certain trace elements (zinc, magnesium, etc.) (Osarumwense et al., 2013; Putnik et al., 2017).

Essential oil (EO) and polyphenolics of the orange peel are the most important health beneficial constituents of this raw material. The unique orange peel EO aroma is formed due to the presence of terpene hydrocarbons, ketones, aldehydes, esters, and alcohols (Qiao et al., 2008). Limonene is mentioned as the main component of this EO, however, other components such as linalool, octanal, citronellal, geranial and α-terpineol are present too, but in the lower concentrations (Gonçalves et al., 2018). The application of this kind of EO, as well as of individual volatiles of the EO, is significant in the production of pharmaceutical products,

food flavour additives, natural antimicrobials (Bakkali et al., 2008), in production of cleaning products, and in the perfume industry (Sharma et al., 2017). Flavonoids and phenolic acids are the most important polyphenolics of orange peel (Feng, 2022). Hesperidin can be isolated as the most common flavonoid in every part of citrus, as well as in the peel (Huang & Ho, 2010). Hesperidin has a number of different properties, such as a positive effect on the vascular or cardiovascular system (Morand et al., 2011), protective effect in case of radiation exposure (Hosseinimehr et al., 2009; Kalpana et al., 2009), anticancer activity (Aggarwal et al., 2020), antimicrobial activity (Corciova et al., 2015), anti-inflammatory and antioxidant activity (Tejada et al., 2018). In addition to hesperidin, naringin is another important flavonoid present in citrus fruits. Several studies have shown that naringin supplementation is useful in the treatment of obesity, hypertension, diabetes, and metabolic syndrome (Alam et al., 2014).

Bioactive compounds from waste and by-products, such as orange peel, can be extracted using traditional extraction techniques (Haya et al., 2019). However, due to the insufficient efficiency and cost-effectiveness of these methods, long extraction time, thermal degradation of thermolabile active compounds, as well as frequent use of toxic, organic solvents (hexane, petroleum ether, etc.) (Putnik et al., 2017), there is an increasing tendency for the application of modern green extraction techniques in order to overcome such limitations (Vidović et al., 2021a). The choice of the most adequate technique, as well as the set-up of working conditions, significantly affects the chemical composition of the extracts obtained and their biological activity. According to the literature data, supercritical carbon dioxide extraction (Sc-CO_2) is an excellent technique for the isolation of citrus peel EO, while for the isolation of peel polar components, ultrasound-assisted extraction (UAE) can be selected (Jokić et al., 2020). Extraction technology based on the use of CO_2 in the supercritical state is widely recognized as safe and has been used as a green approach for productive extraction and recovery of valuable compounds from various raw materials (Vidović et al., 2021). CO_2 has

many advantages over organic solvents, while its selectivity, as well as density and other physico-chemical properties, can be adjusted by the change of process temperature and pressure (or both) (Aiello et al., 2020). As extracts obtained by Sc-CO_2 are free of applied extraction solvent, the production of the extract without need for additional purification can be highlighted as another important aspect of this technique. UAE can be performed using an ultrasound bath or ultrasound probe, which are based on a piezoelectric transducer as a source of ultrasound power. UAE, in addition to being relatively cheap, has many advantages over conventional methods, some of which are less time and energy, low temperature extraction and maintaining the quality of the extract (Kumar et al., 2021). According to the studies, UAE gives higher contents of total phenols and flavonoids compared to conventional solvent extractions due to the cavitation and destruction of the cell walls, which occur during the propagation of ultrasound waves through the solvent. Consequently, there is an increase in the mass transfer and release of compounds from the material (Živković et al., 2022). For both extraction methods the important fact is that they are already recognized by the industry and are scalable on the industrial level.

Considering all, the study has targeted the orange peel dust (OPD) as the waste material of interest which was generated in the filter tea factory. Namely, during industrial processing of dried orange peel a large amount of powder (even up to 35%) accumulates with a particle diameter <0.315 mm, which is less than the pore size of filter paper; therefore, this kind of material is impossible to be used in the further production (Vidović et al., 2021). This residual fraction represents a raw material still containing a wide range of bioactive compounds which can be isolated and potentially value-added products can be obtained. As novel studies are showing, the integration of different extraction processes represents an attractive approach to the production of different valuable products from the same raw material, where both non-polar and polar fractions can be isolated (Osorio-Tobón, 2020). Besides, according to the

results of Dias et al. study (2019) the integrated process (Sc-CO_2 and UAE) can increase the UAE efficiency. Therefore, this approach has been applied in the case of OPD processing and utilisation. To the best of knowledge, this is the first report focused on the application of integrated, green-based extraction technologies (Sc-CO_2 + UAE) for the recovery of high-value compounds from this kind of material.

2. Material and Methods

2.1. Plant material and chemicals

The orange peel (*Citrus sinensis* L.) dust (OPD) was obtained from the local filter tea factory Fructus d.o.o. (Bačka Palanka, Serbia). It represents the material in the form of powder with particle size <0.315 μm. The moisture content of the raw material was 3.36 ± 0.63%.

For Sc-CO_2, commercial CO_2 (purity 99.9%, v/v) was used (Messer, Novi Sad, Serbia). All other chemicals used during the experiment were analytical purity reagents. For HPLC, mobile phases (methanol and acetic acid) were obtained from J.T. Baker (Phillipsburg, NY, USA) where methanol was HPLC grade, while acetic acid was an analytical grade. The ultrapure water (conductivity ≤ 0.054 μS/cm) used for the preparation of the mobile phase was obtained from a Milli-Q water purification system Simplicity 185 by Millipore (Bedford, MA, USA). The standard of rutin (purity 97%) was purchased from Acros Organics (Geel, Belgium), the standard of hesperidin (purity 89.5%) was obtained from Dr Ehrenstorfer GmBH (Augsburg, Germany), while narirutin (purity 98%) and naringin (purity 95%), were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Sc-CO_2 extraction

The high-pressure extraction was carried out using a high-pressure extraction system (HPEP, NOVA-Swiss, Effretikon, Switzerland) with Sc-CO_2 as the extraction solvent. The Sc-CO_2

extraction of OPD was conducted at pressures 100, 200 and 300 bar at temperature 40 °C using 30 g of the plant material. The extraction kinetics were monitored in the time intervals of 0.5, 1, 1.5, 2, 3 and 4 h. During extraction, the pressure and temperature in the separator unit were 15 bar and 23 °C, while the flow rate was set at 1.94 kg/h. Using this extraction procedure 3 different supercritical OPD extracts were prepared.

2.3. Ultrasound-assisted extraction

UAE was provided for 3 different materials left after extraction by Sc-CO_2. These are: material left after extraction by Sc-CO_2 at 100 bar, marked as SFE-100, material left after extraction by Sc-CO_2 at 200 bar, marked as SFE-200, and material left after extraction by Sc-CO_2 at 300 bar, marked as SFE-300. Each material was extracted by 50% ethanol/water solution (v/v) in a solid liquid ratio of 1:20 (w/v) using a sonotrode (UP400St ultrasonic processor, Hielscher, Germany) with varying amplitude (20, 60 and 100%) and at the temperature up to 50 °C. Thus, from each material left after Sc-CO_2 extraction three different liquid OPD extracts were prepared. The extraction time was limited by reaching the set temperature. Energy consumption, as well as the increase and change of the process temperature itself were recorded. Beside of this, using the same procedure extraction of untreated raw material (OPD which was not previously extracted by Sc-CO_2) was performed in order to evaluate efficiency of the process integration-coupling of two green methods (Sc-CO_2 extraction followed by UAE). After extraction, obtained extracts were stored in the refrigerator until the analysis.

2.4. Determination of total phenols and total flavonoids in OPD liquid extracts obtained by UAE

The content of total phenols (TPC) in extracts was determined by the Folin-Ciocalteu method (Singleton et al., 1999). At a wavelength of 750 nm, the absorbance was measured using a UV/VIS single-beam spectrophotometer (6300 Spectrophotometer, Jenvai, UK). The results

158 are expressed as gallic acid equivalent (GAE) per gram of dry weight (mg GAE/g dw). All
159 experiments were performed in triplicate, and the results were expressed as mean values.

160 The content of total flavonoids (TFC) was determined by colorimetric analysis of aluminium
161 chloride (Harborne, 1989). The results obtained are expressed as mg of catechin equivalent
162 (CE) per gram of dry weight (mg CE/g dw). All experiments were performed in triplicate, and
163 the results were expressed as mean values.

164 **2.5. GC-MS analysis of supercritical OPD extracts**

165 For the analysis of supercritical OPD extracts obtained in the first process step- SC-CO_2, a gas
166 chromatograph and mass spectrometer instrument model 7890A (Agilent Technologies, Palo
167 Alto, CA, USA) with 5975C mass detector (Agilent Technologies, Palo Alto, CA, USA) was
168 used. The capillary column was HP-5 MS (30 m, 0.25 mm, 0.25 μm). As the carrier gas
169 helium was used with a flow rate of 1 mL/min. The conditions for the analysis were: the
170 injector temperature of 250 °C (split ratio 1:50), and the temperature regime was isothermal at
171 70 °C for 2 min and then, increased from 70 °C to 200 °C at 3 °C/min. The parameters for the
172 MS detector were: ionisation energy of 70 eV, ion source temperature 230 °C, and scanning
173 range of 45-450 *m*/*z*. The extracts obtained with Sc-CO_2 (10 mg) were diluted with hexane
174 and 1 μL of the solution was inserted into the GC injector. Identification of the compounds
175 was performed by the comparison of their retention indices (RI), determined relative to the
176 retention times of *n*-alkanes (C_9-C_{25}), and qualitative identifications of present compounds
177 were performed using Wiley 9 (Wiley, New York, NY, USA) and NIST 17 (D-Gaithersburg)
178 databases. Percentage of the compound's composition was calculated from the GC peak areas
179 using the normalisation method without correction factors. The average components values
180 were calculated from duplicate GC-MS analyses of all extracts (Šafranko, 2021).

2.6. HPLC analysis of liquid OPD extracts obtained by UAE

Determination of polyphenols was performed by HPLC (high performance liquid chromatography) method, on Cosmosil 5C18-MS-II column (Nacalai Tesque, Inc., Kyoto, Japan), 250 mm long with an internal diameter of 4.6 mm, filled with 5 μm particles. The analysis was performed on a semi-preparative HPLC device (Agilent, 1260 Infinity II series). The HPLC system used for the analysis consisted of a quaternary pump (G7111A), a column chamber (G7116A), a photo-diode array detector (G7115A), an autosampler (G7157A), and a fraction collector (G1364E). The system was operated using a computer program Prep LC Online. The separation of analysed compounds was performed by gradient elution for 50 min, where 1% CH_3COOH (in Milli-Q water) was used as the phase A and methanol as the phase B, with 80:20 ratio of A:B. The flow rate was 1.0 mL min^{-1}, the injection volume was 20 μL, the UV detection wavelength was 283 nm, and the analysis was performed at 25 °C. The gradient conditions were: 0-5 min 80% of A, 5-15 min 80-40% of A, 15-35 min holding 20% of A, 35-40 min 20-40% of A, 40-50 min 40-80% of A. The standard stock solution for hesperidin was prepared in dimethyl sulfoxide (DMSO) and diluted with methanol. The calibration for hesperidin was obtained at seven concentrations (1.0-1500 mg/L). The linearity of the calibration curve was confirmed by $R^2 = 0.99920$ for hesperidin. The retention time for hesperidin was 18.838 min (Šafranko, 2021).

2.7. *In silico* studies

The target prediction for a selected set of small molecules was carried out using pharmacophore approach implemented in Pharmmapper, a reverse docking server (Liu et al., 2010). For each molecule, three-dimensional structures stored in a.mol2 format were submitted to generate a list of 300 potential proteins from the database of all targets containing 7302 entries (v2010). All other parameters were kept at their default values.

The docking of selected components identified in the citrus extracts against liver carboxylesterase 1 (PDB ID: 2DR0) was conducted using AutoDock Vina (ver. 1.1.2) (Trott & Olson, 2010) within the VegaZZ scripting environment (ver 3.2.2.21). The library of the natural products was built by converting SMILES strings into mol2 files using OpenBabel (ver. 2.4.1) (Pedretti et al., 2021). The protein target was prepared by removing water molecules and adding hydrogens atoms. The co-crystalized ligand in the binding site was removed from structures prior to docking and its centre of mass was used to position a box with the sizes of 24 Å x 24 Å x 24 Å. Generally, the docking settings were kept to its default values except exhaustiveness that was set to 50, and the top five favourable binding poses were saved for each molecule.

Molecular properties of selected components were predicted using DataWarrior software (Sander et al., 2015) and SwissADME web server (Daina et al., 2017).

3. Results and Discussion

3.1. Sc-CO_2 extraction of OPD

By integrating two advanced green extraction techniques, Sc-CO_2 extraction and UAE, the more efficient reuse of citrus by-products and wastes could be achieved, and a higher number of valuable compounds and products could be obtained. Side to this, according to Jokić et al. (2020) the advantage of using Sc-CO_2 extraction is not only related to better oil yield, but also to the reduction of artefacts created by conventional methods. The application of Sc-CO_2 extraction enables isolation of aromatic and volatile compounds from citrus waste, as well as fatty acids, certain pigments, and waxes, while in exhausted material left after Sc-CO_2 extraction, polar constituents, such as bioflavonoids, stayed unused. This material therefore could be further processed for the isolation of polar compounds, using techniques such as UAE, making the whole process of citrus peel utilisation much more efficient.

Obtaining non-polar compounds of different complexity can be achieved by varying the operating pressures during Sc-CO_2 extraction (Jokić et al., 2020). Pressure is the most dominant process parameter in Sc-CO_2 extraction, and its variation is making a large difference in general extraction yield and extract quality. Therefore, in order to find out the best process set-up for the extraction of non-polar compounds from the targeted material OPD, the efficiency of Sc-CO_2 extraction in dependence to working pressure (100, 200 and 300 bar) was analysed, while the process temperature and the CO_2 flow were kept as constant.

According to the results, Sc-CO_2 extraction of OPD provided, for a total extraction time of 4 h, very similar yields in the range of 0.61-0.64%. The highest yield Sc-CO_2 extraction was achieved at 300 bar. Barrales et al. (2018), in the study on supercritical extraction of orange peel, obtained a total extraction yield of 0.48 ± 0.02% at a pressure of 350 bar and a temperature of 40 ºC, which is less compared to our study. On the other hand, Benelli et al. (2010) at the temperature of 40 ºC and a pressure of 300 bar achieved the yield of 1.37 ± 0.09% in the total extraction time of 5 h. The difference between the extraction yields is impacted by the raw material drying process, quality and characteristics (particle size and shape), the orange variety, and this may explain variation in the total extraction yield in the literature data.

Supercritical OPD extracts obtained at different process pressures were analysed by GC-MS. The obtained volatile profile of extracts is shown in Table 1.

Table 1. Volatile profile of supercritical OPD extracts.

No.	Compound	RI	Area percentage [%]		
			100 bar	200 bar	300 bar
1.	Hexanoic acid	973	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00
2.	Limonene	1033	0.07 ± 0.00	0.04 ± 0.01	0.05 ± 0.01
3.	2-Acetylpyrrole	1062	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
4.	Octan-1-ol	1073	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.01

5.	*cis*-Linalool oxide	1076	0.02 ± 0.00	0.01 ± 0.01	0.03 ± 0.01
6.	Linalool	1100	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
7.	Menthone	1158	0.01 ± 0.00	0.01 ± 0.01	-
8.	Benzoic acid	1163	0.12 ± 0.02	0.18 ± 0.02	0.15 ± 0.01
9.	Octanoic acid	1173	0.02 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
10.	Menthol	1175	0.08 ± 0.01	0.06 ± 0.02	0.06 ± 0.01
11.	(*Z*)-Hex-3-enyl butanoate	1190	0.07 ± 0.00	0.06 ± 0.01	0.08 ± 0.01
12.	α-Terpineol	1191	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
13.	Geraniol	1257	0.05 ± 0.00	0.04 ± 0.01	0.05 ± 0.00
14.	Linalyl acetate	1260	0.03 ± 0.00	0.03 ± 0.02	0.02 ± 0.00
15.	Nonanoic acid	1270	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00
16.	2,6-Dimethylocta-1,7-diene-3,6-diol* (Terpendiol II)		0.10 ± 0.01	0.10 ± 0.02	0.12 ± 0.02
17.	Anethole	1286	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
18.	Perilla alcohol	1297	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
19.	Carvacrol	1301	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
20.	*p*-Mentha-1,8-diene-1,2-diol	1341	0.25 ± 0.03	0.21 ± 0.01	-
21.	Decanoic acid	1368	0.06 ± 0.01	0.07 ± 0.00	0.08 ± 0.01
22.	Vanillin	1395	0.02 ± 0.00	0.02 ± 0.01	0.03 ± 0.00
23.	*trans*-Caryophyllene	1419	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
24.	Undecanoic acid	1466	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.00
25.	Ar-curcumene	1484	-	-	0.20 ± 0.12
26.	α-Zingiberene	1495	-	-	0.33 ± 0.23
27.	β-Bisabolene	1509	-	-	0.17 ± 0.12
28.	β-Sesquiphellandrene	1525	-	-	0.20 ± 0.12
29.	Dihydroactinidiolide	1526	0.06 ± 0.01	0.03 ± 0.01	-
30.	Dodecanoic acid	1566	0.51 ± 0.13	0.56 ± 0.03	0.86 ± 0.01
31.	Spathulenol	1576	0.08 ± 0.01	0.06 ± 0.01	0.08 ± 0.01
32.	Caryophyllene oxide	1582	0.15 ± 0.02	0.12 ± 0.02	0.13 ± 0.03
33.	Veridiflorol	1589	0.05 ± 0.02	0.04 ± 0.01	0.03 ± 0.00
34.	Hexadecane	1600	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
35.	α-Cadinol	1654	0.05 ± 0.00	0.04 ± 0.00	-
36.	Heptadecane	1700	0.04 ± 0.02	0.04 ± 0.00	0.06 ± 0.01
37.	Tetradecanoic acid	1763	0.29 ± 0.05	0.32 ± 0.03	0.45 ± 0.04
38.	Nootkatone	1800	0.46 ± 0.03	0.36 ± 0.02	0.41 ± 0.10
39.	Hexahydrofarnesyl acetone	1846	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.03
40.	Hexadecan-1-ol	1880	0.24 ± 0.22	0.39 ± 0.08	0.57 ± 0.15
41.	Methyl hexadecanoate	1928	0.25 ± 0.02	0.28 ± 0.02	0.35 ± 0.14
42.	(*E*)-Hexadec-9-enoic acid	1942	0.08 ± 0.00	0.08 ± 0.02	0.13 ± 0.12
43.	Hexadecanoic acid	1970	20.58 ± 1.35	15.22 ± 1.16	15.27 ± 0.81
44.	Bergaptene	2048	1.75 ± 0.09	1.85 ± 0.35	2.51 ± 0.25
45.	Oleyl alcohol	2060	1.22 ± 0.08	1.09 ± 0.34	1.84 ± 0.48

46.	Octadecan-1-ol	2086	0.52 ± 0.46	0.53 ± 0.53	1.30 ± 1.30
47.	Methyl linoleate	2098	0.82 ± 0.44	0.89 ± 0.03	1.70 ± 0.70
48.	Oleic acid	2104	1.13 ± 0.74	2.05 ± 0.14	3.74 ± 0.63
49.	Methyl octadecanoate (Methyl stearate)	2130	1.07 ± 0.85	2.24 ± 0.39	1.60 ± 0.24
50.	Osthole	2135	5.42 ± 0.61	4.53 ± 0.14	5.11 ± 0.48
51.	Linoleic acid	2143	41.47 ± 1.83	38.80 ± 5.53	31.11 ± 3.18
52.	Ethyl stearate	2201	0.77 ± 0.20	1.07 ± 0.20	1.78 ± 0.01
53.	7-Methoxy-8-(2-formyl-2-methylpropyl)coumarin*	2206	1.00 ± 0.22	1.27 ± 0.27	2.31 ± 0.05
54.	7-Methoxy-8-(2-oxo-3-methylbutyl)coumarin*	2234	14.82 ± 2.13	12.65 ± 0.24	15.45 ± 2.99
55.	Tricosane	2300	0.34 ± 0.11	0.52 ± 0.20	1.02 ± 0.16
56.	Tetradecane	2400	0.35 ± 0.24	0.62 ± 0.26	0.23 ± 0.07

* Tentatively identified based only on MS data.

The supercritical OPD extracts obtained contain a wide range of compounds including mainly fatty acids and their esters, terpenes, coumarins and their derivatives. Out of a total of 56 phytochemicals detected, 11 are fatty acids, of which linoleic (31.11-41.47%) and hexadecanoic (15.22-20.58%) were the most common in all extracts. Apart from being an important source of energy, each of these acids possess other activities. Linoleic acid is an essential acid, and its consumption is of great importance for the human body. It can be converted to arachidonic acid, which further serves as a precursor in the synthesis of a group of hormones called prostaglandins, which have significant function in muscle contractions and are also important due to their anti-inflammatory properties (Özcan et al., 2013). Arachidonic and linoleic acid are omega-6 fatty acids, which have been shown to have protective properties against cancer (Rajasekaran, 2017). Various clinical tests have proven the local application of linoleic acid, as well as its polyunsaturated derivatives to reduce the effects of transepidermal water loss, and also to achieve a soothing effect on the skin (Ghafoor et al., 2017); this data could be of importance in the potential application of supercritical OPD extracts in skin cosmetic preparation. Palmitic (hexadecanoic) acid as saturated fatty acid present in the extracts obtained has been also shown to possess a wide

range of pharmacological activities, such as anti-inflammatory, antiviral, analgesic and regulatory activity of lipid metabolism (Mayneris-Perxachs et al., 2014; Librán-Pérez et al., 2019).

A study conducted by Jokić et al. (2020) showed the presence of fatty acids such as linoleic and hexadecanoic acid in Sc-CO_2 extracts of orange peel. The abundance of fatty acids in their study, at 300 bar and 40 ºC was 4.8% for hexadecanoic and 9.2% for linoleic acid. Also, the presence of fatty acids in the petrol ether extract of the orange peel after derivatization in their methyl esters (Islam et al., 2012), as well as in the hexane extract of orange peel without derivatization (Esquivel-Ferriño et al., 2014) has been reported.

Coumarins were identified as the second most represented group of compounds in the supercritical OPD extracts, and these were: osthol (4.53-5.42%), bergapten (1.75-2.51%), 7-methoxy-8-(2-formyl-2-methylpropyl)-coumarin (1.00-2.31%) and 7-methoxy-8-(2-oxo-3-methylbutyl) coumarin (12.65-15.45%). Due to its various functions, this group of compounds is very attractive. Namely, coumarins have a wide range of different biological and pharmacological effects, such as anti-inflammatory, antiviral, antibacterial, antioxidant, cyclooxygenase and lipoxygenase inhibition, antithrombotic, xanthine oxidase inhibition, anti-Alzheimer's disease (AD), anticonvulsant, antidiabetic, as well as antitumor effects (Annunziata et al., 2020). Thanks to their fungicidal and antioxidant effects, they are used as additives in the cosmetics and food industry (Mark et al., 2019), and thanks to their aromatic fragrance, their use in perfumes production is also significant (Matos et al., 2015). The coumarin derivative identified in the highest amount was 7-methoxy-8-(2-oxo-3-methylbutyl)coumarin (isomeranzin), which is found in the literature as one of the constituents of citrus peel (Gyawali et al., 2012). However, the literature data on the biological activity of the most dominant coumarin in the obtained supercritical OPD extracts is rather limited, unlike data on others coumarins present. Osthol (7-methoxy-8-(3-methyl-2-

291 butenyl)coumarin), second dominant coumarin in the extracts obtained, is a natural coumarin
292 that exhibits anti-inflammatory, anticancer, neuroprotective, immunomodulatory properties,
293 as well as hepatitis suppressants (Zhang et al., 2015). Several studies have shown that this
294 compound enhances antitumor immune responses (Liu et al., 2015; Zhang et al., 2015) and
295 reduces the development of hepatocellular carcinoma (Zhang et al., 2012). During Sc-CO_2
296 extraction of bitter orange peel using ethanol as a co-solvent, Trabelsi et al. (2016) found the
297 presence of osthol as the main compound (47%). Also, according to the research of Jerković
298 et al. (2015), the presence of osthol in Sc-CO_2 extracts of orange peel from the Dubrovnik
299 region was detected (up to 1.1%). Coumarin bergapten or 5-methoxypsoralen, identified in
300 supercritical OPD extracts is the lower concentration up to 2,51%, is a furocoumarin
301 derivative and is mainly found in bergamot EO, but can also be isolated from other citrus EOs
302 as well as grapefruit juice (Sicari et al., 2018). According to Jerković et al. (2015) in the
303 extracts of the peel of different types of oranges from the Dubrovnik region obtained by Sc-
304 CO_2 extraction at 40 ºC and 10 MPa, the presence of bergapten (up to 1.4%) was found too.

305 **3.2. Ultrasound-assisted extraction of OPD**

306 In the second part of this study, materials left after Sc-CO_2 extraction (SFE-100, SFE-200 and
307 SFE-300) were subjected to the UAE in order to isolate the polar constituents. The control
308 was performed using raw OPD (the material which was not subjected to the previous Sc-CO_2
309 step). By varying the extraction amplitude (20, 60 and 100%) from each processed material
310 three different OPD liquid extracts were obtained. Thus, totally 12 extracts were prepared and
311 further chemically analysed.

312 UAE is based on the mechanism of acoustic cavitation, where due to the implosion of
313 cavitation bubbles on the surface of plant material, damage or erosion occurs, which allows
314 easier contact with the solvent and the increased extraction yield (Petigny et al., 2013). It has

been proven that the UAE process is influenced by various parameters, of which special emphasis is placed on the solvent, ultrasonic power, time, and temperature of the extraction.

The solvent stands out as a dominant parameter generally in the extraction processes, which, regardless of the extraction technique, has a very significant impact (Tomšik et al., 2016) on the process efficiency and product quality. Due to its high affinity for the phenols, ethanol solution is considered the first-choice solvent for the extraction of phenolic compounds from fruit and vegetable matrices and wastes (Ramić et al., 2015). As study conducted by Lapornik et al. (2005) showed, the extraction efficiency is significantly reduced if pure ethanol is used as the solvent because phenols (especially flavonoids with a sugar component) are hydrophilic due to the number of hydroxyl groups, and thus show greater solubility in ethanol solutions. According to Shehata et al. (2021), investigation of the effect of ethanol/water ratio (30 to 80%) on the polyphenol extraction from orange peel shows the total phenolic content increases with the ethanol concentration up to 50%, emphasising that this concentration is the optimal for maximum extraction of polyphenols from the orange peel. Besides, the accessibility, origin from a renewable source, as well as categorisation as a GRAS solvent (Rodrigues et al., 2015) are some of the additional reasons why ethanol solution is a suitable solvent to be applied in the "green" process of OPD utilisation.

Based on all the above, 50% ethanol water solution was chosen as the solvent in this study, while the influence of temperature and time was investigated. Since the UAE probe was used, in addition to the above parameters, the analysis of power and energy consumption was also considered.

Based on Figure 1A, the highest TPC was achieved in the liquid extract obtained from SFE-100 material processed by UAE at an amplitude of 100% (24.43 ± 0.01 mg GAE/g dw). An increase in TPC content in the liquid extracts obtained from SFE-100 can be clearly seen with

increasing amplitude, as well as a decrease in the value in the liquid extracts obtained from other materials, SFE-200 and SFE-300. TPC in the liquid extracts obtained from the untreated material (OPD not exposed to Sc-CO_2) increases with the increasing amplitude, with the highest values reached at the highest amplitude, 22.94 ± 0.05 (mg GAE/g dw). If we compare the quality of liquid extracts obtained from SFE-100, SFE-200 and SFE-300, with extracts obtained from raw OPD untreated material, it can be noticed that higher concentration of TPC, approximately up to around 6%, can be achieved if OPD was processed in the previous step by Sc-CO_2 at 100 bar. Namely, the application of Sc-CO_2 extraction enabled the isolation of low-polarity constituents, and the obtained degreased material without traces of toxic organic solvents was used for further "cleaner and safer" processing, and at the same time, the increased destruction of the material walls enabled the "easier" extraction of phenolic constituents found in deeper layers . This is consistent with a study conducted by Rodríguez-Rojo et al. (2012) where a positive effect of treatment with supercritical carbon dioxide on the further isolation of phenolic constituents was observed.

The application of Sc-CO_2 prior to UAE was not beneficial in the case of flavonoid extraction, as at all parameters applied the UAE of raw untreated OPD gave the better results. According to the obtained results, in the case of untreated raw OPD material, there is a slight increase in the liquid extracts TFC with increasing amplitude, reaching the maximum of 6.44 ± 0.01 (mg CE/g dw) at the highest amplitude applied, what is the case if raw OPD material is processed. Aside from this, it has been noticed that if the pretreated material is applied in this process, an increase of amplitude in UAE will be beneficial, meaning the content of flavonoids in the liquid OPD extracts produced will be increased in all cases (Figure 1B).

There are several studies in the literature which were focused on the application of UAE for the isolation of polyphenolic constituents from the orange peel. Razola-Diaz et al. (2021) determined TPC for orange peel using a sonotrode and the values obtained ranged between

8.7 and 29.7 (mg GAE/g dw). The highest value was achieved under ethanol/water (50:50, v/v), for 25 min extraction, using 100% of amplitude and 100% pulse, while the lowest value corresponds to application of 100% ethanol as extraction solvent, 25 min extraction, 20% amplitude and 50% pulse. As the similar extraction procedure was applied, the difference in TPC in the study of Razola-Diaz et al. (2021) and presented study most dominantly arise from the difference in the quality of the starting raw material. According to Sandhu et al. (2021) TPC of orange peel extract was 258.67 ± 1.14 (mg GAE/100 g), while for pulp it was 735.54 ± 1.83 (mg GAE/100 g) which is significantly less compared to the results of present study.

As already mentioned, temperature and time are important parameters of the UAE process. As chemical changes in the extract can occur at elevated temperatures, as well as the degradation of the target compounds, the process is set-up in such a way to allow the increase of the process temperature up to maximal 50 ºC. Therefore, the extraction time was dependable on the temperature, while the temperature was dependable on the applied amplitudes. All three were monitored. Figure 2 shows the time dependence of the temperature in the case of SFE-100 material extraction, as a representative of the UAE of materials previously processed by Sc-CO_2, while Figure 3 is showing the time change in the case of raw OPD extraction. As it was expected, the decrease in the extraction time with increasing amplitude causes that at higher amplitudes it takes a shorter time to reach a defined maximum process temperature. Therefore, at amplitude of 20%, the extraction times were 20.5, 21 and 22 minutes for SFE-100, SFE-200 and SFE-300, respectively, while at amplitudes of 60% and 100%, the time for the tested samples was uniform, 5 and 3 minutes, respectively. In the case of the raw OPD extraction the maximum temperature is reached somewhat faster, by 18.5, 4 and 2.5 minutes at amplitudes of 20, 60 and 100%, respectively.

During the UAE, the energy consumption was also monitored (Figure 4). The increase in the energy consumption over the time was observed, which would mean that changed process

parameters affect the final energy consumption. This needs to be considered too in order to select the most efficient process in terms of extract quality and energy consumption at the same time. This is especially important if the process results in the similar content of targeted bioactive compounds, such as in the case of UAE of TPC or TFC. In those cases, it is reasonable to select the process which saves energy.

The lowest energy consumption (6.566 W/h) was observed in the processing of SFE-100 material at an amplitude of 60% in 5 min extraction, while the highest (10.115 W/h) was observed in processing of the SFE-300 at an amplitude of 20% in 22 minutes.

Table 2. UAE performance review.

Type of extracted material	Amplitude [%]	Time [min]	Energy [W/h]	TPC [mg GAE/g dw]	TFC [mg CE/g dw]
SFE-100	20	20.5	**0.23 - 9.699**	22.67 ± 0.04	5.82 ± 0.01
	60	5	0.75 - 6.566	24.41 ± 0.02	5.99 ± 0.01
	100	3	1.24 - 7.066	**24.43 ± 0.01**	6.2 ± 0.02
SFE-200	20	21	**0.23 - 9.980**	23.77 ± 0.04	5.83 ± 0.01
	60	5	0.72 - 6.818	23.25 ± 0.03	5.9 ± 0.01
	100	3	1.23 - 7.080	22.63 ± 0.02	6.17 ± 0.00
SFE-300	20	22	**0.23 - 10.115**	23.84 ± 0.01	5.81 ± 0.01
	60	5	0.73 - 7.022	22.97 ± 0.07	5.83 ± 0.01
	100	3	1.2 - 6.851	22.42 ± 0.06	6.09 ± 0.01
Raw OPD	20	18.5	**0.31 - 9.348**	21.31 ± 0.06	6.41 ± 0.01
	60	4	0.74 - 5.686	22.91 ± 0.05	6.42 ± 0.00
	100	2.5	1.26 - 6.1	22.94 ± 0.05	6.44 ± 0.01

From Table 2, if we want to draw a general conclusion based on all the results, the processes of material SFE-100 at an amplitude of 60% could be considered as optimal, as the TPC and TFC reached almost maximal value, while energy consumption is the lowest compared to all other cases of processing of material pretrated by supercritical process. Basides, the time is significantly saved as it is approximately 4 times lower than extraction time at amplitude 20%, while it is just 2 minutes higher than extraction at amplitude 100%. Also, it is noticeable

404 that in all samples, during extractions with the longest duration, i.e. at an amplitude of 20%,
405 the highest energy consumption occurs.

406 **3.3. HPLC chromatographic profiling of liquid OPD extracts**

407 HPLC analysis liquid OPD extracts obtained in UAE confirmed the presence of bioactive
408 compounds, namely hesperidin, naringin, narirutin and rutin. According to the literature data
409 the two most dominant flavonoids, in fresh and dried orange by-products, were hesperidin and
410 narirutin (Razola-Díaz et al., 2021). Londoño-Londoño et al. (2010) studied the application of
411 UAE in the extraction of polyphenols from the citrus peel. According to their study, orange
412 peel was the most complex source of these bioactive compounds as it contained hesperidin,
413 neohesperidin, diosmin, nobiletin and tangerine and. In another study, M'hiri et al. (2017)
414 identified 10 phenolic compounds in ethanolic extracts of Maltese orange peel, namely
415 flavanones (eriocitrin, narirutin, naringin, hesperidin, neohesperidin, didimine) and
416 polymethoxylated flavones (sinensetin, hexamethoxyflavone, tangeretin, nobiletin). The
417 extracts were obtained by various methods, and one of the ones used was UAE with the use of
418 a sonicator. Toledo-Guillen et al. (2010) identified hesperidin, narirutin, sinensetin, nobiletin,
419 tetramethylscutellarein and tangeretin in orange peel extracts.

420 Table 3. Components detected by HPLC in OPD extracts.

Samples	Amplitude [%]	Hesperidin [µg/ml]	Narirutin [µg/ml]	Naringin [µg/ml]	Rutin [µg/ml]
SFE-100	100	860.08 ± 6.31	17.08 ± 0.26	804.04 ± 9.28	75.10 ± 27.56
	60	865 ± .35	17.23 ± 0.08	809.05 ± 5.10	48.37 ± 0.03
	20	848 ± 6.50	17.10 ± 0.13	794.13 ± 2.83	48.31 ± 0.55
SFE-200	100	863.6 ± 2.55	17.31 ± 0.19	801.77 ± 0.38	48.70 ± 0.42
	60	857.13 ± 1.73	17.20 ± 0.19	795.89 ± 3.99	47.92 ± 0.04
	20	884.64 ± 21.59	16.70 ± 0.51	771.45 ± 28.66	47.53 ± 0.67
SFE-300	100	849.13 ± 12.23	16.81 ± 0.06	785.56 ± 12.64	104.40 ± 0.18
	60	878.30 ± 33.60	17.34 ± 0.35	814.18 ± 32.64	48.10 ± 0.11

	20	852.57 ± 13.82	16.92 ± 0.10	781.03 ± 4.29	47.58 ± 0.57
Initial OPD	100	900.24 ± 0.21	18.16 ± 0.12	830.39 ± 0.62	100.44 ± 0.58
	60	865.62 ± 0.60	17.61 ± 0.43	797.22 ± 0.42	50.66 ± 0.93
	20	891.13 ± 0.89	17.85 ± 0.31	824.09 ± 0.64	100.06 ± 0.24

As already mentioned, the most common flavonoid in the orange peel is hesperidin. In this research, this statement was confirmed even though the study investigated the orange peel dust which is actually the orange peel waste product. Based on Table 3, the content of hesperidin is the highest compared to the other identified components. The second dominant flavonoid in liquid OPD extracts was naringin, present in just some lower concentration in comparison to hesperidin. According to the literature using an ultrasound sonifier, Khan et al. (2010) isolated hesperidin and identified it as the main compound of orange by-products present in the amount of 205.2 mg/100 g fresh weight. Giannuzzo et al. (2003) and Taktak et al. (2018) conducted studies on extraction methods, which showed the effect of processing variables on naringin release. Comparing the yields obtained after UAE and solvent extraction (SE) for 60 min extraction, Wang et al. (2008) found out that the amounts of naringin and hesperidin obtained in UAE (70.3 and 205.2 mg/100 g fresh weight, respectively) were significantly higher than those obtained by SE (50.9 and 144,7 mg/100 g fresh weight, respectively). Authors of the same study stated that no evidence was found that flavanone degradation occurred under sonication, and based on the yield, it was confirmed that the UAE was significantly more efficient. Same has been noticed in the study of Khan et al. (2010) where results of the Valencia orange peel variety extraction were presented. This is in line with the fact that the UAE has the potential to extract natural compounds with higher yields than conventional techniques. The concentration of hesperidin, in the liquid OPD extracts obtained by UAE from materials pretreated by Sc-CO_2, ranges from 848 ± 6.50 to 884.64 ± 21.59 (μg/ml). If the process of OPD utilisation is set without supercritical pretreatment, then

liquid OPD extracts with some higher concentration of hesperidin can be obtained. But, in this case the concentration of hesperidin is higher up to 1,8%, and what needs to be kept in mind is that the non-polar fraction is lost as it is not prior isolated by a supercritical process. Glycosylated flavonoid naringin, known for its antioxidant activities (Nakao et al., 2011) ranged in the liquid OPD extracts obtained from previously pretreated materials, between 771.45 ± 28.66 and 814.18 ± 32.64 (μg/ml), with the highest concentration measured in the extract obtained from SFE-300 sample by application of 60% amplitude. Similar as in the case of hesperidin, its concentration can be increased in the liquid extracts if the untreated OPD is applied at the amplitude of 100%, but again, this means that non-polar fraction will be lost as isolation in the supercritical phase will not be applied.

Compounds detected in the lower concentration in the liquid OPD extracts are narirutin and rutin. The content of narirutin ranged between 16.70 ± 0.51 and 17.34 ± 0.35 (μg/ml) for extracts obtained from samples previously processed by Sc-CO_2. The concentrations of narirutin in these extracts were quite similar regardless to the material or amplitude applied. Slightly higher results were determined in the liquid extract obtained from the previously untreated OPD material, but again quite similar regardless of the amplitude applied. Rutin is a glycoside of the flavonoid quercetin, which is characterised by a strong antioxidant effect. It is found in many plant fruits, as well as in fruit peels (especially in citrus fruits: oranges, grapefruit, lemon and lime) (Omoba et al., 2015). In the study of Omoba et al. (2015) routine concentrations ranged between 18.6 and 17.93 mg/g for mature and immature peels, respectively. In the present study, rutin was identified in the liquid OPD extracts (obtained from material processed by Sc-CO_2) at a concentration of 47.53 ± 0.67 to 104.40 ± 0.18 (μg/ml). The highest concentration was determined in SFE-300 extract using UAE at the amplitude of 100%.

3.4. Predicted pharmacological potential of extracts

As established previously, the extracted compounds have a wide range of important activities, with the mechanism of action not fully established for the most of the compounds. In this study, we focussed on the two most abundant non-fatty acid molecules found in the extracts obtained using Sc-CO_2 extraction, isomerazin and osthole. These were chosen for further *in silico* studies that include protein target prediction and evaluation of potential interaction with putative targets. Linoleic and hexadecenoic acids, most abundant fatty-acids, were considered as the candidates for synergistic action on a selected target. Besides, all four constituents detected in the liquid OPD extracts obtained by UAE (hesperidin, naringin, narirutin and rutin) were evaluated too.

The Pharmmapper results indicated that the top scoring targets for isomeranzin and osthole do not have specified indications (Table 4), leading the choice of liver carboxylesterase 1 (CES1) as a potential target for coumarin analogues. The indications for this protein listed by Pharmmapper include a range of diseases arteriosclerosis, hypercholesterolemia, hyperlipidaemia, cardiovascular and cerebrovascular diseases, various cancers and multiple myeloma, Alzheimer's disease, dementia and osteoporosis. Interestingly, three out of four main components identified in the liquid OPD extracts obtained by UAE have a potential to interact with the same protein, albeit with lower certainty as indicated by lower z-scores. Previous studies have shown that citrus peel can have beneficial preventative effects in atherosclerosis (Feng et al., 2020) and cancer, suggesting that interactions of its components with the CES1 could be further explored.

Table 4. Potential top scoring targets of selected components of OPD extracts predicted using Pharmmapper. Detailed information is included for liver carboxylesterase 1, a putative target of isomerazin, with defined indications.

	Top scoring target		Liver carboxylesterase 1		
Compound	**Name**	**Indication**	**Rank**	**Normalised fit**	**Z-score**
Isomerazin	Stromelysin-1	None	2	0.99	1.26
Osthole	ATP synthase C chain	None	12	0.98	1.13
Linoleic acid	Steryl-sulfatase	Cognitive disorder; Cancer	40	0.87	1.19
Hexadecanoic acid	NitFhit	None	179	0.82	0.70
Hesperidin	Xylose isomerase	Scurvy	-	-	-
Naringin	Arylesterase	None	111	0.92	0.01
Narirutin	Xylose isomerase	Scurvy	113	0.93	-0.08
Rutin	Lysozyme	Myasthenic syndrome	198	0.87	-0.46

Table 5. Molecular properties (logP and solubility) and drug likeness of selected compounds predicted using DataWarrior software. The results of the molecular docking of these small molecules into the binding pocket of liver carboxylesterase 1 are shown as binding affinities of top binding poses calculated using Vina software.

Compound	**clogP**	**clogS**	**Druglikeness**	**Binding affinity (kcal/mol)**
Isomerazin	2.26	-3.2	-5.64	-8.1
Osthole	3.44	-3.3	-3.29	-7.7
Linoleic acid	6.47	-4.3	-25.56	-6.2
Hexadecanoic acid	6.06	-4.2	-25.22	-5.5
Hesperidin	-0.81	-2.7	2.04	-9.5
Naringin	-0.74	-2.7	0.64	-9.7
Narirutin	-0.74	-2.7	1.89	-10.0
Rutin	-1.26	-2.4	1.93	-8.5

Molecular docking simulations have shown that all molecules can be accommodated into the CES1 active site with different binding affinities (Table 5). Isomerazin and osthole bind with similar binding scores, and they form favourable interactions in two different parts of the binding site that are promiscuous. However, both molecules interact with a residue of catalytic triad residues (S1221–E354–H468) that is located at the base of the active gorge.

These interactions have a potential to prevent generation of the Ser221 oxygen nucleophile and disrupt the action of the enzyme (Briand et al., 2019).

Similar types of interaction can be formed by other docked molecules (Figures 5C, 5D and 6), except the naringin which interacts with another residue of the catalytic triad, H1468. Although the four compounds from liquid OPD extract obtained by UAE have better binding affinities, they have lower predicted gastrointestinal absorption and may have lower inhibitory potential than isomerazin and osthole due to potentially lower blood concentrations. However, they can contribute additively to the action of the extract against CES1. Overall, these small molecules from the extracts can act as non-specific inhibitors of CES1 and therefore could module its function resulting in multiple beneficial effects. However, it should be also considered that these molecules can also affect the function of other proteins and that warrants further investigation.

4. Conclusions

Based on the results of the study, it can be concluded that the OPD is a rich source of bioactive compounds and therefore should be further utilised for more valuable products-extracts production. Fatty acids (linoleic, hexadecane and oleic), 7-methoxy-8-(2-oxo-3-methylbutyl) coumarin and osthole were present as dominant compounds in Sc-CO_2 extracts, while in UAE extracts hesperidin, naringin, narirutin and rutin were the most abundant. In the utilisation pathway it is meaningful to combine two green extraction techniques for isolation of first non-polar constituents (Sc-CO_2 extraction), and then, in the second phase, to isolate the polar one using UAE and ethanol solution as the extraction solvent. Although the general results of the UAE are showing that liquid OPD extracts obtained by UAE alone, without previous supercritical extraction, contained slightly higher concentration of the dominant polar bioactive compounds, this difference is not of the significant level to favour the

application of UAE without previous supercritical extraction step. Depending on the selection of targeted-priority compounds, the optimal conditions could be viewed from two different perspectives. However, if both polar and non-polar compounds are equally important, and if the goal of final producer is production of two different extracts-fitopreparation from the orange peel herbal dust, Sc-CO_2 extraction at 200 bar and then ultrasound-assisted extraction at an amplitude of 60% could be considered as the optimal conditions. In this way, a sufficient amount of non-polar compounds would be isolated, and on the other hand, second extract with the high concentration of both, TPC and TFC, as well as the dominant polar compounds will be produced, with significant energy savings.

According to the *in silico* evaluation the obtained extracts, due to the bioactive constituents present, are showing a wide range of important activities. Results of the investigation are indicating the potential application toward atherosclerosis prevention.

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References

Aggarwal, V., Tuli, H. S., Thakral, F., Singhal, P., Aggarwal, D., Srivastava, S., Pandey, A., Sak, K., Varol, M., Khan, Md. A., & Sethi, G. (2020). Molecular mechanisms of action of hesperidin in cancer: Recent trends and advancements. Experimental Biology and Medicine, 245(5), 486–497. https://doi.org/10.1177/1535370220903671

Aiello, A., Pizzolongo, F., Scognamiglio, G., Romano, A., Masi, P., & Romano, R. (2020). Effects of supercritical and liquid carbon dioxide extraction on hemp (*Cannabis sativa* L.) seed oil. International Journal of Food Science & Technology, 55(6), 2472–2480. https://doi.org/10.1111/ijfs.14498

Alam, M. A., Subhan, N., Rahman, M. M., Uddin, S. J., Reza, H. M., & Sarker, S. D. (2014). Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Advances in Nutrition, 5(4), 404–417. https://doi.org/10.3945/an.113.005603

Annunziata, F., Pinna, C., Dallavalle, S., Tamborini, L., & Pinto, A. (2020). An overview of coumarin as a versatile and readily accessible scaffold with broad-ranging biological activities. International Journal of Molecular Sciences, 21(13), 4618. https://doi.org/10.3390/ijms21134618

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. Food and Chemical Toxicology, 46(2), 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Barrales, F. M., Silveira, P., Barbosa, P. de P. M., Ruviaro, A. R., Paulino, B. N., Pastore, G. M., Macedo, G. A., & Martinez, J. (2018). Recovery of phenolic compounds from citrus by-

products using pressurized liquids—An application to orange peel. Food and Bioproducts Processing, 112, 9–21. https://doi.org/10.1016/j.fbp.2018.08.006

Benelli, P., Riehl, C. A. S., Smânia, A., Smânia, E. F. A., & Ferreira, S. R. S. (2010). Bioactive extracts of orange (*Citrus sinensis* L. Osbeck) pomace obtained by SFE and low pressure techniques: Mathematical modeling and extract composition. The Journal of Supercritical Fluids, 55(1), 132–141. https://doi.org/10.1016/j.supflu.2010.08.015

Briand, E., Thomsen, R., Linnet, K., Rasmussen, H. B., Brunak, S., & Taboureau, O. (2019). Combined ensemble docking and machine learning in identification of therapeutic agents with potential inhibitory effect on human CES1. Molecules, 24(15), 2747. https://doi.org/10.3390/molecules24152747

Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Perino, S., Fabiano-Tixier, A.-S., & Abert-Vian, M. (2017). Review of green food processing techniques. Preservation, transformation, and extraction. Innovative Food Science & Emerging Technologies, 41, 357–377. https://doi.org/10.1016/j.ifset.2017.04.016

Corciova, A., Ciobanu, C., Poiata, A., Mircea, C., Nicolescu, A., Drobota, M., Varganici, C.-D., Pinteala, T., & Marangoci, N. (2015). Antibacterial and antioxidant properties of hesperidin: β-cyclodextrin complexes obtained by different techniques. Journal of Inclusion Phenomena and Macrocyclic Chemistry, 81(1–2), 71–84. https://doi.org/10.1007/s10847-014-0434-2

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7(1), 42717. https://doi.org/10.1038/srep42717

592 Dias, J. L., Mazzutti, S., de Souza, J. A. L., Ferreira, S. R. S., Soares, L. A. L., Stragevitch, L.,
593 & Danielski, L. (2019). Extraction of umbu (*Spondias tuberosa*) seed oil using CO2,
594 ultrasound and conventional methods: Evaluations of composition profiles and antioxidant
595 activities. The Journal of Supercritical Fluids, 145, 10–18.
596 https://doi.org/10.1016/j.supflu.2018.11.011

597 Esquivel-Ferriño, P. C., Clemente-Soto, A. F., Ramírez-Cabriales, M. Y., Garza-González, E.,
598 Álvarez, L., & Camacho-Corona, M. D. R. (2014). Volatile constituents identified in hexane
599 extract of Citrus sinensis peel and anti-mycobacterial tuberculosis activity of some of its
600 constituents. Journal of the Mexican Chemical Society, 58(4), 431-434.

601 FAO. 2021. Citrus Fruit Statistical Compendium 2020. Rome.

602 Feng, C.-H. (2022). Optimizing procedures of ultrasound-assisted extraction of waste orange
603 peels by response surface methodology. Molecules, 27(7), 2268.
604 https://doi.org/10.3390/molecules27072268

605 Feng, X., Zhang, L., Xu, S., & Shen, A. (2020). ATP-citrate lyase (ACLY) in lipid
606 metabolism and atherosclerosis: An updated review. Progress in Lipid Research, 77, 101006.
607 https://doi.org/10.1016/j.plipres.2019.101006

608 Ghafoor, K., Özcan, M. M., AL-Juhaımı, F., Babıker, E. E., Sarker, Z. I., Ahmed, I. A. M., &
609 Ahmed, M. A. (2017). Nutritional composition, extraction, and utilization of wheat germ oil:
610 A review. European Journal of Lipid Science and Technology, 119(7), 1600160.
611 https://doi.org/10.1002/ejlt.201600160

612 Giannuzzo, A. N., Boggetti, H. J., Nazareno, M. A., & Mishima, H. T. (2003). Supercritical
613 fluid extraction of naringin from the peel of *Citrus paradisi*. Phytochemical Analysis, 14(4),
614 221–223. https://doi.org/10.1002/pca.706

615 Gonçalves, D., Costa, P., Rodrigues, C. E. C., & Rodrigues, A. E. (2018). Effect of *Citrus*
616 *sinensis* essential oil deterpenation on the aroma profile of the phases obtained by solvent
617 extraction. The Journal of Chemical Thermodynamics, 116, 166–175.
618 https://doi.org/10.1016/j.jct.2017.09.011

619 Gyawali, R., Jeon, D. H., Moon, J., Kim, H., Song, Y. W., Hyun, H. B., Jeong, D., & Cho, S.
620 K. (2012). Chemical composition and antiproliferative activity of supercritical extract of
621 *Citrus grandis* (L.) Osbeck fruits from Korea. Journal of Essential Oil Bearing Plants, 15(6),
622 915–925. https://doi.org/10.1080/0972060X.2012.10662594

623 Harborne, J. B. (1989). Methods in plant biochemistry. Volume 1. Plant phenolics. Academic
624 Press Ltd.

625 Haya, S., Bentahar, F., & Trari, M. (2019). Optimization of polyphenols extraction from
626 orange peel. Journal of Food Measurement and Characterization, 13(1), 614–621.
627 https://doi.org/10.1007/s11694-018-9974-2

628 Hosseinimehr, S. J., Mahmoudzadeh, A., Ahmadi, A., Mohamadifar, S., & Akhlaghpoor, S.
629 (2009). Radioprotective effects of hesperidin against genotoxicity induced by γ-irradiation in
630 human lymphocytes. Mutagenesis, 24(3), 233–235. https://doi.org/10.1093/mutage/gep001

631 Huang, Y.-S., & Ho, S.-C. (2010). Polymethoxy flavones are responsible for the anti-
632 inflammatory activity of citrus fruit peel. Food Chemistry, 119(3), 868–873.
633 https://doi.org/10.1016/j.foodchem.2009.09.092

634 Islam, M. R., Muslim, T., & Rahman, M. A. (2012). Investigation on orange peel:
635 Derivatization of isolated cellulosic material and analysis of the fatty acids composition.
636 Dhaka University Journal of Science, 60(1), 77–78. https://doi.org/10.3329/dujs.v60i1.10341

637 Jerković, I., Družić, J., Marijanović, Z., Gugić, M., Jokić, S., & Roje, M. (2015). GC-FID/MS
638 Profiling of Supercritical CO 2 Extracts of Peels from *Citrus aurantium*, *C. sinensis* cv.
639 Washington navel, *C. sinensis* cv. Tarocco and *C. sinensis* cv. Doppio Sanguigno from
640 Dubrovnik Area (Croatia). Natural Product Communications, 10(7), 1934578X1501000.
641 https://doi.org/10.1177/1934578X1501000745

642 Jokić, S., Molnar, M., Cikoš, A. M., Jakovljević, M., Šafranko, S., & Jerković, I. (2020).
643 Separation of selected bioactive compounds from orange peel using the sequence of
644 supercritical CO_2 extraction and ultrasound solvent extraction: Optimization of limonene and
645 hesperidin content. Separation Science and Technology, 55(15), 2799–2811.
646 https://doi.org/10.1080/01496395.2019.1647245

647 Kalpana, K. B., Devipriya, N., Srinivasan, M., & Menon, V. P. (2009). Investigation of the
648 radioprotective efficacy of hesperidin against gamma-radiation induced cellular damage in
649 cultured human peripheral blood lymphocytes. Mutation Research/Genetic Toxicology and
650 Environmental Mutagenesis, 676(1–2), 54–61.
651 https://doi.org/10.1016/j.mrgentox.2009.03.005

652 Khan, M. K., Abert-Vian, M., Fabiano-Tixier, A.-S., Dangles, O., & Chemat, F. (2010).
653 Ultrasound-assisted extraction of polyphenols (Flavanone glycosides) from orange (*Citrus*
654 *sinensis* L.) peel. Food Chemistry, 119(2), 851–858.
655 https://doi.org/10.1016/j.foodchem.2009.08.046

656 Kumar, K., Srivastav, S., & Sharanagat, V. S. (2021). Ultrasound assisted extraction (UAE) of
657 bioactive compounds from fruit and vegetable processing by-products: A review. Ultrasonics
658 Sonochemistry, 70, 105325. https://doi.org/10.1016/j.ultsonch.2020.105325

659 Lapornik, B., Prošek, M., & Golc Wondra, A. (2005). Comparison of extracts prepared from
660 plant by-products using different solvents and extraction time. Journal of Food Engineering,
661 71(2), 214–222. https://doi.org/10.1016/j.jfoodeng.2004.10.036

662 Librán-Pérez, M., Pereiro, P., Figueras, A., & Novoa, B. (2019). Antiviral activity of palmitic
663 acid via autophagic flux inhibition in zebrafish (*Danio rerio*). Fish & Shellfish Immunology,
664 95, 595–605. https://doi.org/10.1016/j.fsi.2019.10.055

665 Liu, X., Ouyang, S., Yu, B., Liu, Y., Huang, K., Gong, J., Zheng, S., Li, Z., Li, H., & Jiang,
666 H. (2010). PharmMapper server: A web server for potential drug target identification using
667 pharmacophore mapping approach. Nucleic Acids Research, 38(suppl_2), W609–W614.
668 https://doi.org/10.1093/nar/gkq300

669 Liu, Y.-W., Chiu, Y.-T., Fu, S.-L., & Huang, Y.-T. (2015). Osthole ameliorates hepatic
670 fibrosis and inhibits hepatic stellate cell activation. Journal of Biomedical Science, 22(1), 63.
671 https://doi.org/10.1186/s12929-015-0168-5

672 Londoño-Londoño, J., Lima, V. R. de, Lara, O., Gil, A., Pasa, T. B. C., Arango, G. J., &
673 Pineda, J. R. R. (2010). Clean recovery of antioxidant flavonoids from Citrus peel:
674 Optimizing an aqueous ultrasound-assisted extraction method. Food Chemistry, 119(1), 81–
675 87. https://doi.org/10.1016/j.foodchem.2009.05.075

676 M'hiri, N., Irina, I., Cédric, P., Ghoul, M., & Boudhrioua, N. (2017). Antioxidants of
677 Maltease orange peel: Comparative investigation of the efficiency of four extraction
678 methods. Journal of Applied Pharmaceutical Science, 7(11), 126-135.
679 https://doi.org/10.7324/JAPS.2017.71119

680 Mahato, N., Sharma, K., Sinha, M., & Cho, M. H. (2018). Citrus waste derived nutra-
681 /pharmaceuticals for health benefits: Current trends and future perspectives. Journal of
682 Functional Foods, 40, 307–316. https://doi.org/10.1016/j.jff.2017.11.015

683 Mark, R., Lyu, X., Lee, J. J. L., Parra-Saldívar, R., & Chen, W. N. (2019). Sustainable
684 production of natural phenolics for functional food applications. Journal of Functional Foods,
685 57, 233–254. https://doi.org/10.1016/j.jff.2019.04.008

686 Matos, M. J., Santana, L., Uriarte, E., Abreu, O. A., Molina, E., & Yordi, E. G. (2015).
687 Coumarins—An important class of phytochemicals. In A. V. Rao & L. G. Rao (Eds.),
688 Phytochemicals—Isolation, Characterisation and Role in Human Health. InTech.
689 https://doi.org/10.5772/59982

690 Mayneris-Perxachs, J., Guerendiain, M., Castellote, A. I., Estruch, R., Covas, M. I., Fitó, M.,
691 Salas-Salvadó, J., Martínez-González, M. A., Aros, F., Lamuela-Raventós, R. M., & López-
692 Sabater, M. C. (2014). Plasma fatty acid composition, estimated desaturase activities, and
693 their relation with the metabolic syndrome in a population at high risk of cardiovascular
694 disease. Clinical Nutrition, 33(1), 90–97. https://doi.org/10.1016/j.clnu.2013.03.001

695 Morand, C., Dubray, C., Milenkovic, D., Lioger, D., Martin, J. F., Scalbert, A., & Mazur, A.
696 (2011). Hesperidin contributes to the vascular protective effects of orange juice: A
697 randomized crossover study in healthy volunteers. The American Journal of Clinical
698 Nutrition, 93(1), 73–80. https://doi.org/10.3945/ajcn.110.004945

699 Murador, D. C., Salafia, F., Zoccali, M., Martins, P. L. G., Ferreira, A. G., Dugo, P.,
700 Mondello, L., de Rosso, V. V., & Giuffrida, D. (2019). Green extraction approaches for
701 carotenoids and esters: Characterization of native composition from orange peel.
702 Antioxidants, 8(12), 613. https://doi.org/10.3390/antiox8120613

Nakao, K., Murata, K., Itoh, K., Hanamoto, Y., Masuda, M., Moriyama, K., Shintani, T., & Matsuda, H. (2011). Anti-hyperuricemia effects of extracts of immature *Citrus unshiu* fruit. Journal of Traditional Medicines, 28(1), 10-15. https://doi.org/10.11339/jtm.28.10

Omoba, O., Obafaye, R., Salawu, S., Boligon, A., & Athayde, M. (2015). HPLC-DAD phenolic characterization and antioxidant activities of ripe and unripe sweet orange peels. Antioxidants, 4(3), 498–512. https://doi.org/10.3390/antiox4030498

Osarumwense, P. O., Okunrobo, L. O., & Uwumarongie-Ilori, E. G. (2013). Phytochemical screening, proximate and elemental analysis of *Citrus sinensis* peels (L.) Osbeck. Journal of Applied Sciences and Environmental Management, 17(1), 47-50.

Osorio-Tobón, J. F. (2020). Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. Journal of Food Science and Technology, 57(12), 4299–4315. https://doi.org/10.1007/s13197-020-04433-2

Özcan, M. M., Rosa, A., Dessi, M. A., Marongıu, B., Pıras, A., & AL Juhaimi, F. (2013). Quality of wheat germ oil obtained by cold pressing and supercritical carbon dioxide extraction. Czech Journal of Food Sciences, 31(No. 3), 236–240. https://doi.org/10.17221/172/2012-CJFS

Pedretti, A., Mazzolari, A., Gervasoni, S., Fumagalli, L., & Vistoli, G. (2021). The VEGA suite of programs: An versatile platform for cheminformatics and drug design projects. Bioinformatics, 37(8), 1174–1175. https://doi.org/10.1093/bioinformatics/btaa774

Petigny, L., Périno-Issartier, S., Wajsman, J., & Chemat, F. (2013). Batch and continuous ultrasound assisted extraction of boldo leaves(*Peumus boldus* Mol.). International Journal of Molecular Sciences, 14(3), 5750–5764. https://doi.org/10.3390/ijms14035750

Putnik, P., Bursać Kovačević, D., Režek Jambrak, A., Barba, F., Cravotto, G., Binello, A., Lorenzo, J., & Shpigelman, A. (2017). Innovative “green” and novel strategies for the extraction of bioactive added value compounds from citrus wastes—A review. Molecules, 22(5), 680. https://doi.org/10.3390/molecules22050680

Qiao, Y., Xie, B., Zhang, Y., Zhang, Y., Fan, G., Yao, X., & Pan, S. (2008). Characterization of aroma active compounds in fruit juice and peel oil of Jinchen sweet orange fruit (*Citrus sinensis* (L.) Osbeck) by GC-MS and GC-O. Molecules, 13(6), 1333–1344. https://doi.org/10.3390/molecules13061333

Rajasekaran, A. (2017). Nutraceuticals. In Comprehensive Medicinal Chemistry III (pp. 107–134). Elsevier. https://doi.org/10.1016/B978-0-12-409547-2.12287-5

Ramić, M., Vidović, S., Zeković, Z., Vladić, J., Cvejin, A., & Pavlić, B. (2015). Modeling and optimization of ultrasound-assisted extraction of polyphenolic compounds from *Aronia melanocarpa* by-products from filter-tea factory. Ultrasonics Sonochemistry, 23, 360–368. https://doi.org/10.1016/j.ultsonch.2014.10.002

Razola-Díaz, M. del C., Guerra-Hernández, E. J., Rodríguez-Pérez, C., Gómez-Caravaca, A. M., García-Villanova, B., & Verardo, V. (2021). Optimization of ultrasound-assisted extraction via sonotrode of phenolic compounds from orange by-products. Foods, 10(5), 1120. https://doi.org/10.3390/foods10051120

Rodrigues, S., Fernandes, F. A. N., de Brito, E. S., Sousa, A. D., & Narain, N. (2015). Ultrasound extraction of phenolics and anthocyanins from jabuticaba peel. Industrial Crops and Products, 69, 400–407. https://doi.org/10.1016/j.indcrop.2015.02.059

Rodríguez-Rojo, S., Visentin, A., Maestri, D., & Cocero, M. J. (2012). Assisted extraction of rosemary antioxidants with green solvents. Journal of Food Engineering, 109(1), 98–103. https://doi.org/10.1016/j.jfoodeng.2011.09.029

Šafranko, S., Ćorković, I., Jerković, I., Jakovljević, M., Aladić, K., Šubarić, D., & Jokić, S. (2021). Green extraction techniques for obtaining bioactive compounds from mandarin peel (*Citrus unshiu* var. *Kuno*): Phytochemical analysis and process optimization. Foods, 10(5), 1043. https://doi.org/10.3390/foods10051043

Sander, T., Freyss, J., von Korff, M., & Rufener, C. (2015). Datawarrior: An open-source program for chemistry aware data visualization and analysis. Journal of Chemical Information and Modeling, 55(2), 460–473. https://doi.org/10.1021/ci500588j

Sandhu, H. K., Sinha, P., Emanuel, N., Kumar, N., Sami, R., Khojah, E., & Al-Mushhin, A. A. M. (2021). Effect of ultrasound-assisted pretreatment on extraction efficiency of essential oil and bioactive compounds from citrus waste by-products. Separations, 8(12), 244. https://doi.org/10.3390/separations8120244

Sharma, K., Mahato, N., & Lee, Y. R. (2019). Extraction, characterization and biological activity of citrus flavonoids. Reviews in Chemical Engineering, 35(2), 265–284. https://doi.org/10.1515/revce-2017-0027

Sharma, K., Mahato, N., Cho, M. H., & Lee, Y. R. (2017). Converting citrus wastes into value-added products: Economic and environmently friendly approaches. Nutrition, 34, 29–46. https://doi.org/10.1016/j.nut.2016.09.006

Shehata, M. G., Abd El Aziz, N. M., Youssef, M. M., & El-Sohaimy, S. A. (2021). Optimization conditions of ultrasound-assisted extraction of phenolic compounds from orange

768 peels using response surface methodology. Journal of Food Processing and Preservation,
769 45(10). https://doi.org/10.1111/jfpp.15870

770 Sicari, V. (2018). Optimization of the supercritical carbon dioxide separation of bergapten
771 from bergamot essential oil. Journal of AOAC International, 101(1), 293–297.
772 https://doi.org/10.5740/jaoacint.17-0190

773 Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total
774 phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent.
775 In Methods in Enzymology (Vol. 299, pp. 152–178). Elsevier. https://doi.org/10.1016/S0076-
776 6879(99)99017-1

777 Taghizadeh-Alisaraei, A., Hosseini, S. H., Ghobadian, B., & Motevali, A. (2017). Biofuel
778 production from citrus wastes: A feasibility study in Iran. Renewable and Sustainable Energy
779 Reviews, 69, 1100–1112. https://doi.org/10.1016/j.rser.2016.09.102

780 Taktak, F., Ciğeroğlu, Z., Öğen, Y., & Kirbaşlar, Ş. İ. (2018). Resin-loaded cationic hydrogel:
781 A new sorbent for recovering of grapefruit polyphenols. Chemical Engineering
782 Communications, 205(10), 1442–1456. https://doi.org/10.1080/00986445.2018.1457028

783 Tejada, S., Pinya, S., Martorell, M., Capó, X., Tur, J. A., Pons, A., & Sureda, A. (2019).
784 Potential anti-inflammatory effects of hesperidin from the genus citrus. Current Medicinal
785 Chemistry, 25(37), 4929–4945. https://doi.org/10.2174/0929867324666170718104412

786 Toledo-Guillén, A. R., Higuera-Ciapara, I., García-Navarrete, G., & de la Fuente, J. C.
787 (2010). Extraction of bioactive flavonoid compounds from orange (*Citrus sinensis*) peel using
788 supercritical CO_2. Journal of Biotechnology, 150, 313–314.
789 https://doi.org/10.1016/j.jbiotec.2010.09.292

790 Tomšik, A., Pavlić, B., Vladić, J., Ramić, M., Brindza, J., & Vidović, S. (2016). Optimization
791 of ultrasound-assisted extraction of bioactive compounds from wild garlic (*Allium ursinum*
792 L.). Ultrasonics Sonochemistry, 29, 502–511. https://doi.org/10.1016/j.ultsonch.2015.11.005

793 Trabelsi, D., Aydi, A., Zibetti, A. W., Della Porta, G., Scognamiglio, M., Cricchio, V., Langa,
794 E., Abderrabba, M., & Mainar, A. M. (2016). Supercritical extraction from *Citrus aurantium*
795 amara peels using CO_2 with ethanol as co-solvent. The Journal of Supercritical Fluids, 117,
796 33–39. https://doi.org/10.1016/j.supflu.2016.07.003

797 Trott, O., & Olson, A. J. (2009). AutoDock Vina: Improving the speed and accuracy of
798 docking with a new scoring function, efficient optimization, and multithreading. Journal of
799 Computational Chemistry, NA-NA. https://doi.org/10.1002/jcc.21334

800 Vidović, S., Vasić, A., Vladić, J., Jokić, S., Aladić, K., Gavarić, A., & Nastić, N. (2021).
801 Carbon dioxide supercritical fluid extracts from yarrow and rose hip herbal dust as valuable
802 source of aromatic and lipophilic compounds. Sustainable Chemistry and Pharmacy, 22,
803 100494. https://doi.org/10.1016/j.scp.2021.100494

804 Vidović, S., Vladić, J., Nastić, N., & Jokić, S. (2021a). Subcritical and supercritical extraction
805 in food by-product and food waste valorization. In Innovative Food Processing Technologies
806 (pp. 705–721). Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.23014-X

807 Wang, Y.C., Chuang, Y.C., & Hsu, H.W. (2008). The flavonoid, carotenoid and pectin
808 content in peels of citrus cultivated in Taiwan. Food Chemistry, 106(1), 277–284.
809 https://doi.org/10.1016/j.foodchem.2007.05.086

810 Zhang, L., Jiang, G., Yao, F., He, Y., Liang, G., Zhang, Y., Hu, B., Wu, Y., Li, Y., & Liu, H.
811 (2012). Growth inhibition and apoptosis induced by osthole, a natural coumarin, in

812 hepatocellular carcinoma. PLoS ONE, 7(5), e37865.
813 https://doi.org/10.1371/journal.pone.0037865

814 Zhang, L., Jiang, G., Yao, F., Liang, G., Wang, F., Xu, H., Wu, Y., Yu, X., & Liu, H. (2015).
815 Osthole promotes anti-tumor immune responses in tumor-bearing mice with hepatocellular
816 carcinoma. Immunopharmacology and Immunotoxicology, 37(3), 301–307.
817 https://doi.org/10.3109/08923973.2015.1035391

818 Zhang, Z.-R., Leung, W. N., Cheung, H. Y., & Chan, C. W. (2015). Osthole: A review on its
819 bioactivities, pharmacological properties, and potential as alternative medicine. Evidence-
820 Based Complementary and Alternative Medicine, 2015, 1–10.
821 https://doi.org/10.1155/2015/919616

822 Živković, J., Vladić, J., Naffati, A., Nastić, N., Šavikin, K., Tomić, M., & Vidović, S. (2022).
823 Comparative chemical profiling of underexploited *Arctostaphylos uva-ursi* L. Herbal dust
824 extracts obtained by conventional, ultrasound-assisted and subcritical water extractions.
825 Waste and Biomass Valorization, 13(10), 4147–4155. https://doi.org/10.1007/s12649-022-
826 01770-5

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834 **Figure captions**

835 Figure 1. Influence of UAE amplitudes on A) the TPC and B) TFC of raw OPD, and SFE-
836 100, SFE-200, and SFE-300 samples.

837 Figure 2. Dependence of the process time on the process temperature in the case of SFE-100
838 material extraction.

839 Figure 3. Dependence of the process time on the process temperature in the case of raw OPD
840 extraction.

841 Figure 4. Dependence of energy consumption presented for the case of on the UAE of SFE-
842 300 material.

843 Figure 5. Top binding poses of components from supercritical OPD extracts docked into liver
844 carboxylesterase 1 binding site, A) isomerazin, B) osthole, C) linoleic acid and D)
845 hexadecanoic acid. The amino acid residues of the protein are shown in a thin line
846 representation, while the small molecules are shown as thick cyan bond in 3D plots, while the
847 amino acid residues are shown as circles coloured according the type of the interaction
848 involved with small molecules (thick stick representation (light green – van der Waals
849 interaction, dark green – conventional hydrogen bond, pink – interaction involving alkyl
850 groups, purple – pi-sigma interaction, dark pink – amide -pi stacked interaction).

851 Figure 6. Top binding poses of components from liquid OPD extracts obtained by UAE
852 docked into liver carboxylesterase 1 binding site, A) hesperidin, B) naringin, C) narirutin and
853 D) rutin. The amino acid residues of the protein are shown in a thin line representation, while
854 the small molecules are shown as thick cyan bond in 3D plots, while the amino acid residues
855 are shown as circles coloured according the type of the interaction involved with small
856 molecules (thick stick representation (light green – van der Waals interaction, dark green –

857 conventional hydrogen bond, pink – interaction involving alkyl groups, purple – pi-sigma

858 interaction, dark pink – amide -pi stacked interaction).