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Oxalis erythrorhiza Gillies ex Hooker et Arnott (Oxalidaceae): Chemical Analysis, Biological In Vitro and In Vivo Properties and Behavioral Effects

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Abstract: In this work, a decoction (DOe) and a methanolic global extract (MGEOe), obtained with the aerial parts of *Oxalis erythrorhiza* Gillies ex Hooker et Arnott (Oxalidaceae), were evaluated. The high-resolution liquid chromatography in conjunction with electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-ESI-QTOF-MS) analysis showed forty compounds in MGEOe and twenty-nine in DOe, including flavones, C-glycosyl flavones, isoflavones, fatty acids, terpenes, phenolic acids, and sterols. The antioxidant properties were evaluated by DPPH, TEAC, FRAP, and ILP assays. Both DOe and MGEOe showed stronger antioxidant activities. The anti-inflammatory effects were evaluated by COX inhibition method, where DOe demonstrated a significant inhibitory effect. The cytotoxic effects were evaluated in the tumoral HCT-116 and non-tumoral HBL-100 cell lines, revealing a selective action from DOe and MGOe on cancer cells. DOe was evaluated in an animal model of insulin resistance, which is characterized by alterations in glucose and lipid metabolism, as well as cognitive impairments, including anxiety-like behavior and memory deficits. Male SD rats received sucrose (10% *w/v*, SUC), a half dilution of DOe (5% *w/v*) with sucrose (HDOeS) or DOe with sucrose (DOeS) from PND21 to PND61. Then, anxiety-like behavior and spatial memory were assessed using the open field (OF), elevated plus maze (EPM) and the novel object location (NOL) tests, respectively. Serum parameters basal glycemia, total cholesterol (TC) and tryglicerides were measured using commercial kits. The lipid peroxidation was determined in homogenates of cerebral cortex, hippocampus and hypothalamus by TBAR assay. Only HDOeS exhibited lower anxiety-like behavior in OF and improved performance in NOL compared to SUC. Furthermore, DOeS showed reduced serum parameters, while HDOeS presented lower TC levels than SUC. No differences were observed on TBAR assay. The beneficial properties of these preparations could be attributed to the identified metabolites. These findings highlighted *O. erythrorhiza* as a potential source of compounds to improve human health; however, further research is required to elucidate its mechanisms of action.

Keywords: UHPLC-ESI-QTOF-MS analysis; antioxidant; anti-inflammatory; cytotoxicity; open field; elevated plus maze; novel object location

1. Introduction

Plants not only fulfill essential ecological roles but also serve as an abundant source of inspiration for innovations in green nanotechnology, biomedicine, and architecture. In particular, phytomedicine research holds significant value in the biomedical field, offering the potential to uncover novel therapeutic agents derived from natural sources [1]. For centuries, plants have been central to traditional medicine, providing bioactive compounds with diverse pharmacological properties, including anti-inflammatory, antioxidant, and anticancer effects. Phytomedicine bridges the gap between traditional knowledge and modern biomedical applications, enabling the discovery and development of safer, more cost-effective treatments. Furthermore, studying the mechanisms of action of plant-derived compounds can inspire innovative approaches to addressing complex health challenges, such as chronic diseases and drug resistance. Advancing research in this field is therefore essential for expanding the range of biomedical tools and enhancing global healthcare outcomes.

In the central Andean regions of the province of San Juan of Argentina, there is a significant number of plants used in popular medicine to treat several health disorders. The Andean cushion herb *Oxalis erythrorhiza* Gillies ex Hooker et Arnott (Oxalidaceae), commonly known as “boldo de la cordillera”, is employed in the treatment of hepatic and heart complaints even in the absence of scientific support [2]. This plant is a perennial shrub that forms very dense cushions, often exceeding 30 cm in diameter. Its rhizomes and woody stems are dark at the base, while its petioles, peduncles, and branches are reddish. These branches are covered by the stipules of the leaves, which consist of three narrow, obcordate, and hairy leaflets measuring 1 to 2 mm. The flowers are yellow and solitary, with five petals ranging from 6 to 8 mm, barely protruding above the foliage. The fruit is a small globose capsule, and its seeds are ovoid-asymmetric, measuring 1.2 mm in length, sharp, brown, and marked with interrupted transverse striations. This plant thrives in high mountain habitats, growing at altitudes between 1800 and 4000 m.a.s.l. on the eastern slopes of the Andes [3]. In the literature, there are few reports on the chemistry and biological activity of *O. erythrorhiza*. The hexane and dichloromethane (DCM) extracts of *O. erythrorhiza* showed activity against dermatophytes and bacteria [4]. So far, the literature only reports the isolation of embelin and alkyl phenols from DCM extracts [5]. Other reports state that extracts of Oxalidaceae family members produce antioxidant, cytotoxic and anti-inflammatory effects [6–8], improve parameters like basal glycemia (BG), total cholesterol (TC) and triglycerides (TG) [9–11] and produce beneficial effects on anxiety behavior and memory [12,13]. However, these latter biological properties of extracts and decoction are poorly explored in *O. erythrorhiza*. On the other hand, although various drugs are used to treat different health impairments, many produce side effects or lack therapeutic action in certain patients, and represent high costs for health systems. That is why the search for improved and low-cost medicinal treatments remains in force. Taking into account this aspect and the fact that the extracts and decoction properties of *O. erythrorhiza* are poorly explored, the aim of this work was to update the chemical profile, the antioxidant, anti-inflammatory and cytotoxic properties in vitro, along with the hypoglycemic, hypolipidemic and anxiolytic properties in vivo. This is the first study to investigate a decoction similar to that traditionally used by the local population, with the aim of supporting the medicinal application of this Andean species while also exploring its potential biological activities.

2. Materials and Methods

2.1. Chemicals

Ultra-pure water, with total organic carbon (TOC) levels below 5 µg/L, was sourced using an Arium 126 61316-RO purification system coupled with an Arium 611 UV unit (Sartorius, Goettingen, Germany). High-purity methanol (HPLC grade) and mass spectrometry-grade formic acid were supplied by J. T. Baker (Phillipsburg, NJ, USA), while HPLC-grade chloroform was provided by Merck (Santiago, Chile). Various drugs and HPLC standards, including gallic acid, quercetin, catechin, orientin, isoorientin, isovitexin, and rutin, among others, were acquired from Sigma-Aldrich Chem. Co. (St Louis, MO, USA) or Extrasynthèse (Genay, France).

2.2. Plant Material, Decoction (DOe) and Methanolic Extract (MGEOe)

The aerial parts of *O. erythrorhiza* were collected from the Bauchaceta Mountains in San Juan Province, Argentina, during the blossoming period (December–February 2022) at an altitude between 4000 and 4500 m.a.s.l. The entire plants were dried at room temperature (25 °C) and stored in conditions devoid of light and heat. A voucher specimen had been previously deposited in the herbarium of the Escuela de Química y Farmacia, Universidad de Chile (SQF), Santiago, Chile, under the reference number SQF 21009.

The DOe was prepared at 10% *w/v* with dried and milled leaves of the plant and water purified with PSA equipment. After 30 min of boiling, the decoctions were filtered and then stored at −20 °C until the animal treatments. Representative samples of each decoction (100 mL) were lyophilized with LA-B3 RIFICOR equipment (Buenos Aires, Argentina; yield: 1.13% *w/w*). The samples were stored in a freezer at −40 °C until the UHPLC-ESI-QTOF-MS analysis, phenolic and flavonoid quantification, and in vitro and in vivo assays.

The MGEOe was obtained by reflux extraction from the air-dried, ground aerial parts of the plant (1 kg) using methanol (3000 mL) in three separate 30 min intervals at 60 °C. The resulting mixtures were concentrated under reduced pressure using a Yamato rotary evaporator (RE-300-AW2, Santa Clara, CA, USA). The final MGEOe product (200 g, yielding 20% *w/w* relative to the initial dry material) was stored at −20 °C until it was analyzed via UHPLC-PDA-QTOF-MS, as well as for the quantification of phenols and flavonoids, and in vitro assays.

2.3. Ultra-High-Resolution Liquid Chromatography Analysis (UHPLC-ESI-QTOF-MS)

The separation and identification of secondary metabolites in DOe and MGEOe were performed following previous studies [14,15], utilizing a UHPLC-ESI-QTOF-MS system. This system included a UHPLC Ultimate 3000 RS with Chromeleon 6.8 software (Dionex GmbH, Idstein, Germany) and a Bruker maXis ESI-QTOF-MS. Mass spectrometry data were processed using Bruker Data Analysis 4.0 software (Bruker Daltonik GmbH, Bremen, Germany) and ACD Lab Spectrum Processor (New York, NY, USA), with sodium formate as the internal standard.

2.4. The Total Phenol and Flavonoid Content

The total phenol content and the flavonoid content were expressed as milligrams of gallic acid equivalents (GAE) or milligrams of quercetin equivalents (QE) per gram of DOe and MGEOe (mg GAE/g DOe or MGEOe), respectively [16]. The values were obtained in quadruplicate using a Multiskan FC Microplate Photometer (Thermo Scientific, Waltham, MA, USA). The results are expressed as mean ± SD.

2.5. In Vitro Studies

2.5.1. Antioxidant Activity

Radical Scavenging Capacity Assay of 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

The free radical scavenging activity of DOe and MGEOe was assessed using the DPPH assay. The concentration required to achieve 50% radical scavenging (EC₅₀) was

determined from a graph plotting inhibition percentage at 517 nm against concentrations of DOe and MGEOe. Catechin (Sigma-Aldrich, $\geq 98\%$, St. Louis, MO, USA) served as the reference compound (EC₅₀ 4.1 $\mu\text{g/mL}$). Each test was conducted in quadruplicate [16].

Ferric-Reducing Antioxidant Power Assay (FRAP)

Results from the FRAP assay were calculated using linear regression based on the FRAP–Trolox calibration curve and are expressed as milligrams of Trolox equivalents per gram of DOe or MGEOe (mg Trolox/g DOe or MGEOe). All tests were conducted in quadruplicate [16].

Trolox Equivalent Antioxidant Activity Assay (TEAC)

The TEAC assay results were obtained through linear regression from a calibration curve created using Trolox concentrations ranging from 0 to 1 mM and are reported as milligrams of Trolox equivalents per gram of DOe and MGEOe (mg Trolox/g DOe or MGEOe). Each test was performed in quadruplicate [16].

Inhibition of Lipid Peroxidation (ILP) in Erythrocytes

The ILP assay in erythrocytes was performed following the previously described methodology [16], employing two concentrations of DOe and MGEOe (100 and 250 $\mu\text{g/mL}$). Catechin was employed as positive control (70% of ILP at 100 $\mu\text{g/mL}$). The obtained results in quadruplicate were expressed as percentages of ILP.

2.5.2. Anti-Inflammatory Effects: Cyclooxygenase (COX) Inhibition Method

The inhibitory activity of DOe (18; 36 and 72 $\mu\text{g/mL}$) on COX-1 (ovine) and COX-2 (human) was determined using a commercial colorimetric kit 701050 (COX Colorimetric Inhibitor Screening Assay Kit, Cayman Chemical Co., Ann Arbor, MI, USA) following the manufacturer's instructions, which involved measuring prostaglandin (PG) levels by ELISA. Diclofenac sodium (1–2 $\mu\text{g/mL}$) and etoricoxib COX-2 specific inhibitor (2.5–5.0 $\mu\text{g/mL}$) were used as a positive control. Once the buffer, hemin and enzymes were added to the background wells, 100% initial activity wells (Abs COX) and inhibitor wells, they were incubated for 5 min at 25 °C. Then, the colorimetric substrate was added to all the wells and arachidonic acid was quickly added. Finally, the solution was incubated for 2 min at 25 °C and measured for absorbance at 590 nm using a Multiskan FC Microplate Photometer (Thermo Scientific, Waltham, MA, USA). The results are expressed as mean $\pm$ SD. The obtained results were expressed as percentage of inhibition [17,18] of COX-1 and COX-2 enzymes, defined as:

$$\% \text{ of Inhibition} = ((A - B) / A) \times 100 \quad (1)$$

$$A = (\text{Abs } 100\% \text{ initial Act} - \text{Abs background}); B = (\text{Abs inhibitor} - \text{Abs background})$$

2.5.3. Antitumoral and Cytotoxic Assays

Cell Culture Procedure

Two different human cell lines were used: HCT-116, representative of colorectal cancer, and HBL-100, considered as non-tumoral epithelial cells. Both cell lines were obtained from the American Type Culture collection (ATCC, Manassas, VA, USA). The cells were cultured as a monolayer in a 37 °C humidified atmosphere enriched with 5% of CO₂ in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Sigma-Aldrich Chem. Co. St Louis, MO, USA; pH 7.6), supplemented with 10% (*v/v*) fetal bovine serum (FBS, Internegocios, Mercedes, Buenos Aires Province, Argentina), 3.7 g/L NaHCO₃ (Sigma-Aldrich, USA) and antibiotics (100 IU/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin, Gibco, Waltham, MA, USA).

Methyl Thiazolyl Tetrazolium (MTT) Assay

Cell proliferation was assessed by the MTT colorimetric assay as described previously [16]. Briefly, 3.5×10^4 cells/100 μL were seeded in a 96-well microplate and let to attach overnight. Cells were treated with DOe (125, 250, 500, 1000 and 2000 $\mu\text{g/mL}$) and/or MGEOe (62.5, 125, 250, 500 and 1000 $\mu\text{g/mL}$) for 48 h. The stock solutions (100 mg/mL) of

Doe and MGEOe were prepared in water or dimethyl sulfoxide (DMSO), respectively. The highest concentration of MGEOe was determined by DMSO toxicity. Solvent presence in concentrations higher than 1000 µg/mL resulted in intrinsic cytotoxicity. Thereafter, the medium was replaced with MTT solution (0.5 mg/mL) in DMEM lacking phenol red, FBS, and antibiotics. After 4 h incubation in the dark, the solution was removed, and 100 µL of DMSO per well was added to dissolve the formazan crystals. The optical density was measured at 570 nm with a Thermo Scientific Multiscan Elisa reader. The median effect dose (Dm), obtained with CompuSyn 1.0 software [19], was used as indicative of extract potency. All assays were performed three times in triplicate.

2.6. Experimental Procedure In Vivo

Adult male Sprague-Dawley rats (PND 21; $n = 60$) were maintained under standard laboratory conditions within a temperature- and humidity-controlled vivarium, adhering to a 12 h light/dark cycle with ad libitum access to food and water. All procedures related to animal care and use were conducted in accordance with the European Community Council Directive (86/609/EEC) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the ethical committee of IBYME (resolution number 021/2021) in CABA, Argentina. The animals were fed with a normal diet consisting of 3.3 kcal/g, with the following composition: protein (18.2%), carbohydrates (56.9%), lipids (3.9%), vitamins and minerals (3%), fiber (5%), and moisture (13%) (sourced from Gepsa feeds, Grupo Pilar SA, Buenos Aires Province, Argentina). The animal model was developed as described previously [20] with modifications. Briefly, from PND 21, the animals received water (CON, $n = 10$), sucrose 10% *w/v* (S, $n = 10$, commercial sugar, Ledesma, Ingenio Ledesma S.A.A.I, Buenos Aires, Argentina), a half dilution of Doe (5% *w/v*) alone (HDOe, $n = 10$) or with sucrose 10% *w/v* (HDOeS, $n = 10$), or Doe alone ($n = 10$) or with sucrose 10% *w/v* (DoeS, $n = 10$) for 40 days. The groups CON, HDOe and Doe were developed to evaluate the treatment effects in the normal condition. Their comparisons and comparisons between SUC and CON are in the Supplementary Materials. The body weight (BW) of the animals was recorded at the beginning (PND21) and at the end (PND61) of the treatments. The BW gain was calculated by subtracting the BW at PND21 from the BW at PND61. Beverage consumption was recorded every other day between 09:00 and 10:00 a.m. for 40 days of treatment, inside each group. At PND 61, all beverages were replaced by water. After one day, the animals were evaluated with a glucose tolerance test (GTT). The behavioral tests were performed between PND 63 and 69. After this period, the animals, fasted for 6 h, were rendered unconscious using CO₂ and then decapitated. Blood was collected from the trunk to assess lipid parameters. The hypothalamus, hippocampus and cerebral cortex were dissected, frozen and stored at $-80\text{ }^{\circ}\text{C}$ [21,22] to determine the degree of lipid peroxidation by thiobarbituric acid reactive substances (TBAR) assay. The determination of BW gain, the beverage volume, the GTT, the serum parameters and TBAR were conducted on 7 animals from each group from two independent assays ($n = 3$ or 4/assay).

2.6.1. Determination of GTT and Serum Parameters

The BG was determined in a drop of blood, obtained from the tail vein of the 6 h fasted animal, using a test strip and the “One Touch Ultra” glucometer (Johnson & Johnson® Plaza, New Brunswick, NJ, USA). The GTT and the area under curve (AUC) were obtained as described previously [20]. The TC and TG levels were determined in the serum obtained from the trunk blood, as described previously [22,23].

2.6.2. TBAR Assay Method

The homogenates of hypothalamus, hippocampus and cerebral cortex were obtained following the protocol described previously [21,22]. The protein concentration was determined with the spectrophotometric method described by [24]. The extent of lipid peroxidation in these homogenates was evaluated by measuring the production of TBAR,

which indicates the presence of malondialdehyde (MDA) in the tissue, according to the previously described protocol [20].

2.6.3. Behavioral Tests Procedure

All the tests were performed as described previously [20]. All devices were illuminated by a 42 W light positioned 100 cm above, directed towards the ceiling, ensuring uniform light intensity across all areas. The animals were placed in the testing room and allowed to acclimate for at least 15 min before testing. To eliminate olfactory cues, the devices were thoroughly cleaned with a 70% ethanol solution after each trial. All tests were recorded on video and analyzed using ANY-maze Video Tracking System version 7.44 (Stoelting Co., Wood Dale, IL, USA) by two observers who were blind to the treatments administered to the animals.

Open Field and Elevated Plus Maze Tests

The open field (OF) was digitally partitioned into zones: central, middle, and peripheral, while the elevated plus maze (EPM) was divided into central, open, and closed arms. The number of entries, distance traveled, and time spent in each zone were analyzed. The events of rearing [25] and grooming [26] were also analyzed. Additionally, the head dip events (when the animals protrude the head over the edge of an open arm) were determined in the elevated plus maze as a measure of risk assessment [27–30].

Novel Object Location Test

The novel object location test (NOL) consists of two trials. In the first trial (T1), the animal is placed in a device (a wooden box with an open top and dark interior) with two identical objects positioned equidistantly for 5 min. After a 2 h intertrial interval, the second trial (T2) is conducted, where the animal is placed back in the device with one object relocated to a new position (N), while the other remains in its original, familiar location (F). The animal explores the objects for 5 min. The time spent exploring each object is recorded and it is important to note that the animal should explore each object for 20 s to be considered for the analysis. The results are presented as the exploration ratio (ER), calculated as the time spent exploring object N divided by the total time spent exploring both objects $[tN/(tN + tF)]$. An ER of 0.50 or lower indicates either an equal preference for objects N and F or a preference for F, suggesting potential memory impairment. To ensure that the animals traveled the same distance within each trial, the number of virtual lines crossed was also analyzed.

2.6.4. Statistical Analysis

The results were analyzed with GraphPad Prism (GraphPad Software Inc., v.9, San Diego, CA, USA). The comparisons between two groups were conducted using Student t test, whereas the comparisons between three groups were assessed using one-way ANOVA followed by Duncan or Newman–Keuls post hoc tests. All results are expressed as mean $\pm$ standard deviation (SD). All differences were considered significant when $p < 0.05$.

3. Results

3.1. Metabolite Profiling: UHPLC-ESI-QTOF-MS Analysis of Doe and MGEOe

The exhaustive UHPLC-ESI-QTOF-MS analysis in negative mode revealed the presence of thirty-eight peaks in MGEOe, corresponding to forty compounds, and twenty-seven peaks corresponding to twenty-nine compounds in Doe. These included C-glycosyl flavones, flavones, aromatics, terpenes, sterols, furans, and several fatty acids; all of them are tentatively identified for the first time in *O. erythrorhiza*. The identification strategy implemented involved spiking experiments with available standards. Additionally, a comprehensive access strategy to several databases, including MONA mass spectrometry and Metaboscape, was employed, along with the use of specific software such as Data Analysis 4.0 (Bruker Daltonik GmbH, Bremen, Germany) and ACD Lab Spectrum Processor (Toronto,

Canada). Figure 1 and Table 1 display the high-resolution UHPLC–PDA–QTOF analysis results for metabolite identification in Doe and MGEOe. Additionally, Figure 2 shows the chemical structures of some of the main compounds identified in Doe and MGEOe.

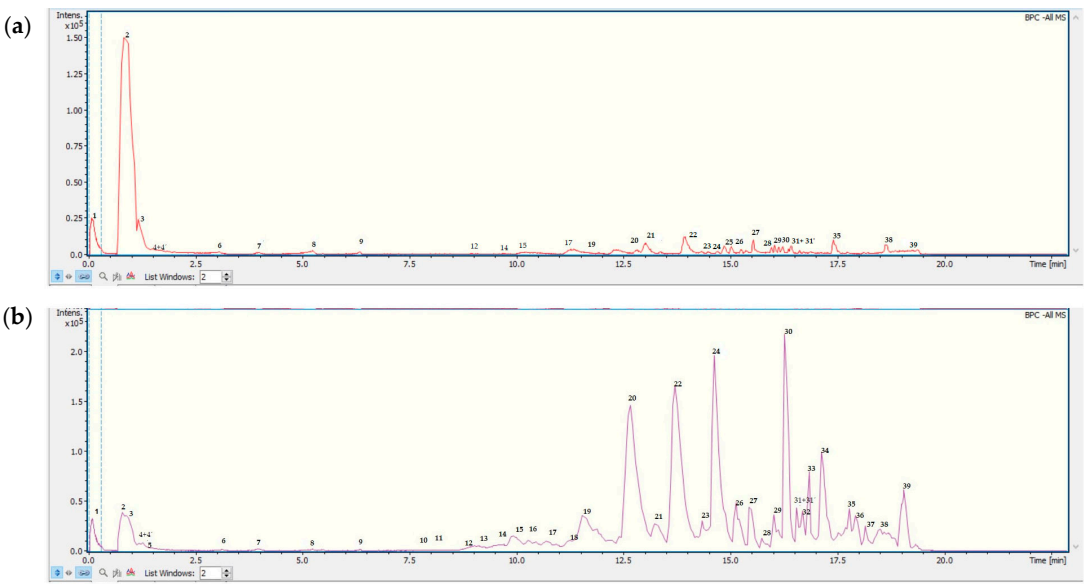


Figure 1. UHPLC Q-TOF-TIC chromatogram of DOe (a) and MGEOe (b). The peak numbers correspond to internal standard Na formiate (1); monohexosyldiglyceride (2); quinic acid (3); vicenin II (apigenin 6,8-di C-B-D-glucopiranoside) (4); chlorogenic acid (4’); luteolin-6-C-glucoside (isoorientin; (5); bis-3-(4’,5,7-trimethoxy-flavanone)-5’-(2’,4,4’-trimethoxy-chalcone) (6); rutin (7); isovitexin (8); meliartenin (9); 4’,5,7-trihydroxy-3,6-dimethoxyflavone (10); 8-hydroxy-5-methoxyflavanone (11); 17-hydroxylinolenic acid (12); diffractaic acid (13); 9-HPODE octadecenedioic acid (14); 9-hydroxy-10,12-octadecadienoic acid (15); 13-HPODE octadecenedioic acid (16); (9Z,12R)-12-hydroxyoctadec-9-enoic acid (17); ricinoleic acid (18); 5-(octadecyloxy) isophthalic acid (19); ricinoleic acid isomer (20); lesquerolic acid (21); pinolenic acid (22); 9-octadecenoic acid (Z)-,2-hydroxyethyl ester (23); roccellaric acid (24); alpha-boswellic acid (25); eicosapentaenoic acid (26); pristimerin (27); dodecanoic acid,2-ethylhexanoic acid, propane-1,2,3-triol (28); palmitic acid (29); FAHFA 18:1/2:0 hydroxy fatty acid ester: icos-10-enedioic acid (30); 22-oxodocosanoic acid (31); PI 34:21-phosphatidyl-1D-myo-inositol (31’); isopropyl linoleate (32); PI(16:0/13-HODE) hydroxyoctadecadienoic acid (33); muricatenol (34); 28-O-acetylbutulin-3-yl-β-D-glucopyranoside (35); 3β-O-acetyl-28-O-lup-20(29)-ene (36); 1-stearyl-2-cholesterylcarbonoyl-3-trityl glycerol (37); aipolic acid(38); daucosterol (39).

Table 1. UHPLC-PDA-Q-TOF identification of metabolites from DOe and MGEOe.

Peak	Tentative Identification	[M-H]-	Retention Time (min)	Measured Mass (m/z)	Molecular Formula	Accuracy (ppm)	Metabolite Type	MS Ions (m/z)
1	Naformiate (internal standard)	NaC ₂ H ₂ O ₄	0.12	112.9829	112.9856	3.10	A	
2	Monohexosyldiglyceride	C ₁₂ H ₁₈ O ₁₂	0.78	353.0786	354.0859	17.08	Fatty acid	
3	Quinic acid	C ₇ H ₁₂ O ₆	0.84	191.0563	192.0636	2.26	Acid	93.0345
4	Vicenin II (apigenin 6,8-di C-B-D-glucopiranoside)	C ₂₇ H ₃₀ O ₁₅	1.20	593.2185	594.2199	2.45	C-glycosil flavone	353.1079
4'	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	1.20	353.0886	354.0959	2.45	Phenolic acid	191.0513
5	Luteolin-6-C-glucoside (Isoorientin)	C ₂₁ H ₂₀ O ₁₁	1.50	447.0953	448.1025	3.93	C-glycosil flavone	429.1389 357.1038 327.0886 298.0861
6	Bis-3-(4',5,7-trimethoxy-flavanone)-5'-(2',4,4'-trimethoxy-chalcone)	C ₃₆ H ₃₄ O ₉	3.13	609.2154	610.2130	3.89	Flavone	447.1456 341.1492 112.9882

Table 1. Cont.

Peak	Tentative Identification	[M-H] ⁻	Retention Time (min)	Measured Mass (m/z)	Molecular Formula	Accuracy (ppm)	Metabolite Type	MS Ions (m/z)
7	Rutin	C ₂₇ H ₃₀ O ₁₆	4.42	609.1477	610.1550	4.67	Flavone	301.0333 300.0258
8	Isovitexin	C ₂₁ H ₂₀ O ₁₀	4.48	431.1006	432.1078	5.07	Isoflavone	311.0563 283.0608
9	Meliartenin	C ₂₈ H ₃₆ O ₁₀	6.25	531.2313	532.2236	3.05	Furane	486.2168 385.3007
10	4',5,7-trihydroxy-3,6-dimethoxyflavone	C ₁₇ H ₁₄ O ₇	7.54	329.0677	330.0750	3.31	Flavone	311.1599 255.0120
11	8-hydroxy-5-methoxyflavanone	C ₁₆ H ₁₄ O ₄	7.93	269.0817	270.0894	4.06	Flavone	254.0580
12	17-hydroxylinolenic acid	C ₁₈ H ₃₀ O ₃	8.45	293.2134	294.2207	4.00	Aromatic	439.0996 311.1636
13	Diffraetaic acid	C ₂₀ H ₂₂ O ₇	9.62	373.1304	374.1376	4.47	Aromatic	282.0879 135.0810
14	9-HPODE octadecenedioic acid	C ₁₈ H ₃₂ O ₄	9.80	311.223	312.2305	0.80	Fatty acid	269.2072 257.2058
15	9-hydroxy-10,12-octadecadienoic acid	C ₁₈ H ₃₂ O ₃	10.50	295.2283	296.2351	1.62	Fatty acid	269.2077
16	13-HPODE octadecenedioic acid	C ₁₈ H ₃₂ O ₄	10.56	311.2235	312.2308	3.49	Fatty acid	257.2058
17	(9Z,12R)-12-hydroxyoctadec-9-enoic acid	C ₁₈ H ₃₄ O ₃	11.52	311.2235	312.2308	5.76	Fatty acid	257.2057
18	Ricinoleic acid	C ₁₈ H ₃₄ O ₃	11.88	297.2437	298.2513	0.44	Fatty acid	253.5113
19	5-(octadecyloxy) isophthalic acid	C ₂₆ H ₄₁ O ₅	11.57	433.2866	434.2960	−21.04	Phenolic acid	
20	Ricinoleic acid isomer	C ₁₈ H ₃₄ O ₃	12.54	297.2427	298.2513	5.04	Fatty acid	283.0608 311.0561
21	Lesquerolic acid	C ₂₀ H ₃₈ O ₃	13.22	325.2745	326.2748	8.33	Fatty acid	299.2586 227.2255
22	Pinolenic acid	C ₁₈ H ₃₀ O ₂	14.23	277.2184	278.2257	4.04	Fatty acid	283.2152
23	9-octadecenoic acid (Z)-, 2-hydroxyethyl ester	C ₂₀ H ₃₈ O ₃	14.31	325.2767	326.2748	5.74	Fatty acid	307.2263 299.2586 293.2044
24	Roccellaric acid	C ₁₉ H ₃₄ O ₄	14.62	325.2396	326.2464	2.70	Fatty acid	281.2495
25	Alpha-boswellic acid	C ₃₀ H ₄₈ O ₃	14.85	455.3540	456.3613	1.37	Fatty acid	227.2255
26	Eicosapentaenoic acid	C ₂₀ H ₃₀ O ₂	15.02	301.2184	302.2257	1.79	Fatty acid	251.2660
27	Pristimerin	C ₃₀ H ₄₀ O ₄	15.02	463.2860	464.2933	1.44	Terpene	449.2673 433.2385
28	Dodecanoic acid, 2-ethylhexanoic acid, propane-1,2,3-triol	C ₂₃ H ₄₈ O ₇	15.58	435.3275	436.3327	−11.95	Fatty acid	255.2610
29	Palmitic acid	C ₁₆ H ₃₂ O ₂	16.13	255.2338	256.2411	3.29	Fatty acid	112.9879
30	FAHFA 18:1/2:0 hydroxy fatty acid ester: icos-10-enedioic acid	C ₂₀ H ₃₆ O ₄	16.28	339.2553	340.2625	3.48	Fatty acid	281.2811
31	22-oxodocosanoic acid	C ₂₂ H ₄₁ O ₃	16.39	353.3116	354.3061	15.40	Fatty acid	339.2952
31'	PI 34:21-phosphatidyl-1D-myo-inositol	C ₄₃ H ₇₉ O ₁₃ P	16.39	833.5264	834.5337	9.47	Fatty acid	717.4680 533.4085 403.2977 323.2977
32	Isopropyl linoleate	C ₂₁ H ₃₉ O ₂	16.45	323.2969	324.2956	4.12	Fatty acid	275.0326
33	PI(16:0/13-HODE) hydroxyoctadecadienoic acid	C ₄₃ H ₇₉ O ₁₄ P	17.02	849.5204	850.5276	8.12	Fatty acid	353.3115

Table 1. Cont.

Peak	Tentative Identification	[M-H] ⁻	Retention Time (min)	Measured Mass (m/z)	Molecular Formula	Accuracy (ppm)	Metabolite Type	MS Ions (m/z)
34	Muricatenol	C ₃₇ H ₆₈ O ₆	17.43	607.4909	607.4943	−5.49	Fatty acid	599.4280 574.4127 409.3437
35	28-O-acetylbetuln-3-yl-β-D-glucopyranoside	C ₃₈ H ₆₂ O ₈	17.52	645.4363	646.4372	−1.33	Fatty acid	607.4901 573.4062
36	3β-O-acetyl-28-O-lup-20(29)-ene	C ₃₈ H ₆₂ O ₈	17.74	645.4363	645.4367	−1.31	Sterol	409.3437
37	1-stearyl-2-cholesterylcarbonoyl-3-trityl glycerol	C ₄₀ H ₅₉ O ₃	18.56	586.4309	587.4391	−14.02	Fatty acid	281.2111
38	Aipolic acid	C ₃₂ H ₅₂ O ₅	18.51	515.3647	515.3589	11.09	Terpene	361.3160
39	Daucosterol	C ₃₅ H ₆₀ O ₆	19.12	575.4261	576.4317	−9.66	Sterol	477.3778

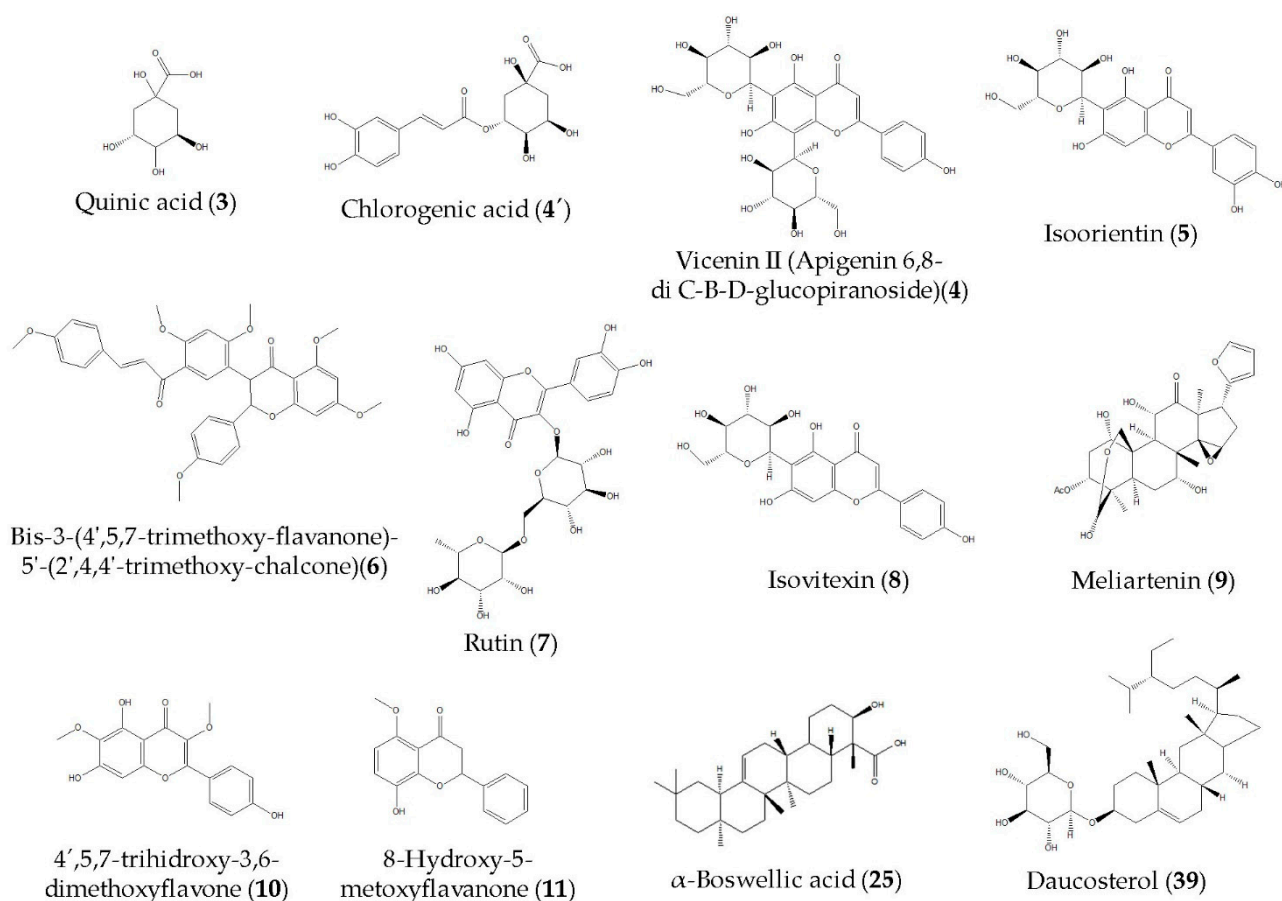


Figure 2. Molecular structures of main compounds identified in DOe and MGEOe.

3.2. In Vitro Studies

3.2.1. Total Phenolic and Flavonoid Contents, Antioxidant Activity and Inhibition Enzyme COX of DOe and MGEOe

Both extracts exhibited high levels of total phenolics, measuring 69 mg GAE/g for DOe and 65 mg GAE/g for MGEOe. Also, the flavonoid contents were 43 mg QE/g DOe and 31 mg QE/g MGEOe (Table 2). Compared to catechin, quercetin and gallic acid (value range EC₅₀: 1–10 µg/mL), the antioxidant activity in the DPPH assay was moderate for both extracts (DOe EC₅₀: 70 µg/mL and MGEOe EC₅₀: 62 µg/mL). DOe and MGEOe exhibited a significant ILP in erythrocytes, reaching 81–83%, respectively, at 250 µg DOe or

MGEOe/mL (Table 2), compared to positive control catechin, which showed 70% of ILP at 100 µg/mL. Regarding enzyme inhibition of COX-1 and 2, DOe showed a good percentage of inhibition of COX-2 (86.97% at 72 µg/mL).

Table 2. Total phenols, flavonoids, antioxidant activity and inhibition COX-2 assays of DOe and MGEOe.

Spectrophotometric Quantification of Compounds of Interest		
Total phenols (mg GAE/g extracts)	DOe	69.24 ± 5.78
	MGEOe	65.45 ± 0.06
Flavonoids (mg QE/g extracts)	DOe	43.31 ± 4.13
	MGEOe	31.90 ± 0.05
Antioxidant and Inhibition COX-2 Assays		
DPPH (EC ₅₀ in µg extracts/mL)	DOe	70.11 ± 0.07
	MGEOe	62.07 ± 1.74
FRAP (mg TE/g of extracts)	DOe	4.95 ± 0.08
	MGEOe	3.25 ± 0.06
TEAC (mg TE/g of extracts)	DOe	0.67 ± 0.05
	MGEOe	1.24 ± 0.05
Percentage ILP (at 250 µg extract/mL)	DOe	83.81 ± 3.59
	MGEOe	90.46 ± 0.03
Percentage inhibition COX-2 (at 72 µg/mL)	DOe	86.97 ± 0.05

3.2.2. Cytotoxicity Study of DOe and MGEOe

DOe produced a slight cytotoxic effect on HCT-116, but not on HBL-100 (Figure 3A,B). The dose–response effect against proliferation was present ($R^2 = 0.88$), but the maximum dose was not enough to reach the Dm. The estimated Dm value was 1708.99 ± 302.72 µg/mL. In contrast, MGEOe induces marked cytotoxic effects in both cell lines. The dose–response effects against proliferation were present in both cell lines ($R^2 = 0.99$). The Dm estimated was 354.15 ± 6.13 µg/mL for HCT-116 (Figure 3C) and 571.36 ± 8.11 µg/mL for HBL-100 (Figure 3D). The comparison between both Dm values revealed significant differences, revealing specific cytotoxic effects on HCT-116 ($p = 0.002$). The comparison between the extracts revealed that MGEOe showed higher potency than DOe on HCT-116 ($p = 0.0015$).

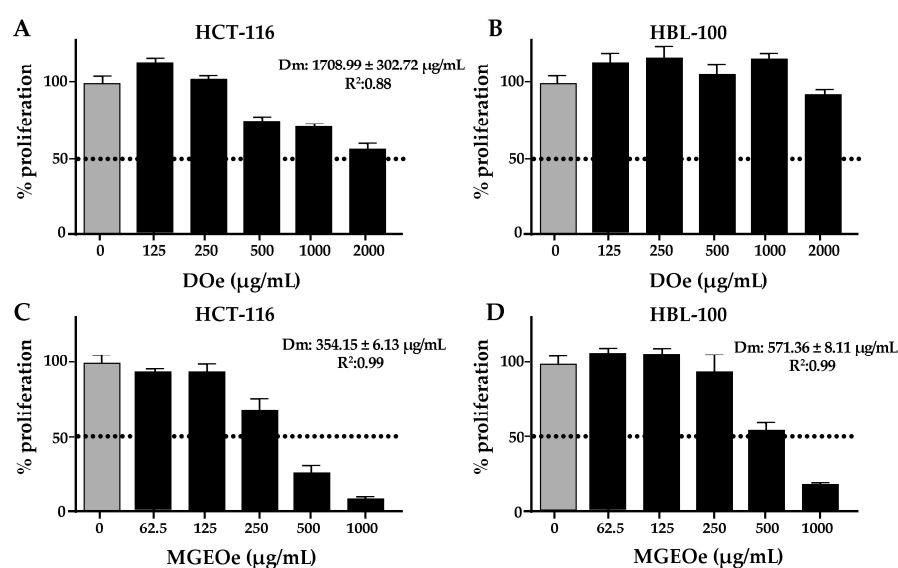


Figure 3. HCT-116 and HBL-100 cell proliferation after 48 h treatment with DOe (A,B) or MGEOe (C,D) and their respective Dm values.

3.3. In Vivo Study

3.3.1. BW Gain and Average Beverage Volume

The analysis of BW gain did not show any significant differences between HDOeS, DOeS and SUC (Figure 4a). Regarding to the average beverage volume, HDOeS and DOeS drank less beverage than SUC (16.35% and 29.21%, respectively; $p < 0.0001$). Also, DOeS drank less beverage than HDOeS (15.39%; $p < 0.0001$ Figure 4b).

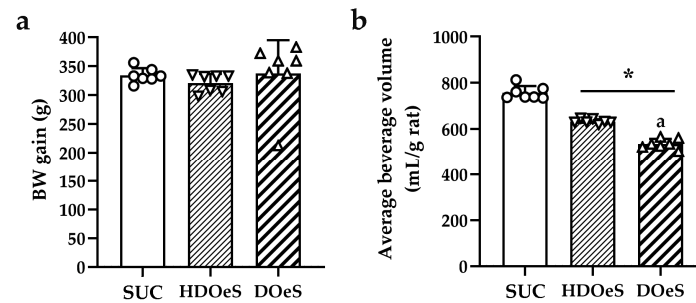


Figure 4. BW gain (a) and average beverage volume (b) of SUC (empty circles), HDOeS (empty inverted triangles) and DOeS (empty triangles). Results are reported as mean $\pm$ SD from two independent assays ($n = 7$ animals per group). Significant differences were assessed using one-way ANOVA ($F_{BW\text{gain}}(2,18) = 0.464$; $p = 0.6359$ and $F_{Beverage}(2,18) = 181.303$; $p < 0.0001$) followed by Newman–Keuls post hoc test. * and ^a $p < 0.05$; * refers to SUC and ^a refers to HDOeS.

3.3.2. GTT and Serum Parameters

The analysis of AUC did not show significant differences between the groups (Figure 5a,b). No significant differences were observed in the levels of BG of HDOeS and SUC. In contrast, DOeS presented a decrease in BG levels compared to SUC (14.67%; $p < 0.01$) and HDOeS (12.86%; $p < 0.001$; Figure 5c). The levels of TC in HDOeS and DOeS were lower than those of SUC (17.44% and 11.69%, respectively; $p < 0.05$). Also, no significant differences were observed between HDOeS and DOeS (Figure 5d). The analysis of TG levels did not show significant differences between the HDOeS and SUC groups. However, DOeS presented lower levels than SUC and HDOeS (18.44% and 16.61%, respectively; $p < 0.01$; Figure 5e).

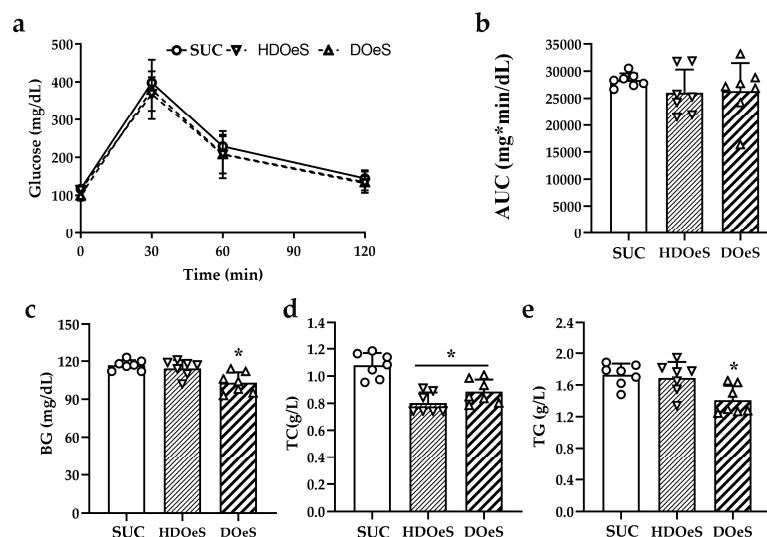


Figure 5. Levels of glucose during the GTT (a) and its AUC values (b), levels of BG (c), TC (d) and TG (e) after 40 days of administration of SUC, HDOeS and DOeS. Results are expressed as mean $\pm$ SD from 2 independent assays ($n = 7$ animals/group). Significant differences were determined by one-way ANOVA ($F_{AUC}(2,18) = 1.348$; $p = 0.2847$; $F_{BG}(2,18) = 7.603$; $p = 0.0040$; $F_{TC}(2,18) = 5.864$; $p = 0.0109$ and $F_{TG}(2,18) = 6.868$; $p = 0.0061$) followed by Newman–Keuls post hoc test. $p < 0.05$, * refers to SUC.

3.3.3. TBAR Assay

The analysis did not reveal significant differences between HDOeS and DOeS and SUC in any of the tissues evaluated (Table 3).

Table 3. Levels of MDA (fmol/μg of protein).

	Cerebral Cortex	Hippocampus	Hypothalamus
SUC	40.45 ± 13.82	34.67 ± 18.11	60.24 ± 7.83
HDOeS	44.39 ± 14.95	32.26 ± 7.27	46.64 ± 12.56
DOeS	46.21 ± 17.85	46.97 ± 10.65	56.20 ± 9.13

Results are presented as mean ± SD from two independent assays ($n = 7$ animals per group). The comparison between HDOeS, DOeS and SUC was performed by one-way ANOVA ($F_{\text{Cerebral cortex}}(2,18) = 0.248$; $p = 0.7829$; $F_{\text{Hippocampus}}(2,18) = 2.643$; $p = 0.0985$ and $F_{\text{Hypothalamus}}(2,18) = 3.388$; $p = 0.0564$).

3.3.4. Behavioral Tests

OFT and EPM

In the OFT, the analysis of total distance and lines crossing did not show significant differences between the groups ($F_{\text{Total distance}}(2,27) = 0.646$; $p = 0.5322$; $F_{\text{Lines}}(2,27) = 0.822$; $p = 0.4504$). In the zone 1, HDOeS entered more and spent more time than SUC (67.74%; $p < 0.001$ and 70.42%; $p < 0.05$, respectively, Figure 6a,b). However, the performance of DOeS was similar to SUC. Also, DOeS entered less and spent less time in the zone than HDOeS (50.77%; $p < 0.001$ and 43.57%; $p < 0.05$, respectively, Figure 6a,b). In zones 2 and 3, no significant differences were observed between the groups (Figure 6c,d). However, HDOeS spent less time in zone 2 (38.55%; $p < 0.05$) and more time in zone 3 (6.63%; $p = 0.05$) than HDOeS (Figure 6c,d). The analysis of the remaining parameters within each zone did not reveal significant differences between the groups ($F_{\text{Distancezone1}}(2,27) = 2.277$; $p = 0.1219$; $F_{\text{Entrieszone2}}(2,27) = 2.388$; $p = 0.1110$; $F_{\text{Distancezone2}}(2,27) = 2.185$; $p = 0.1319$; $F_{\text{Entrieszone3}}(2,27) = 0.944$; $p = 0.4016$ and $F_{\text{Distancezone3}}(2,27) = 2.193$; $p = 0.1311$).

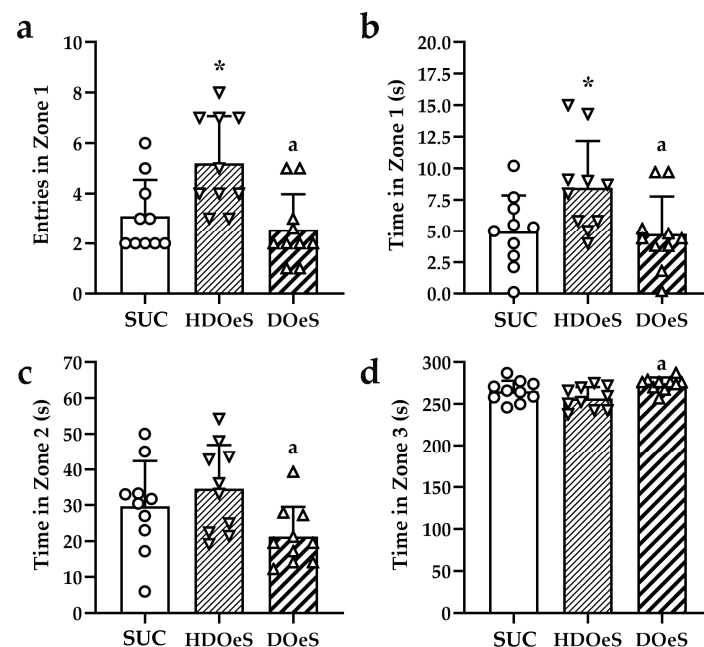


Figure 6. Entries in zone 1 (a), time spent in zones 1 (b), 2 (c) and 3 (d) in the OFT after 40 days of administration of SUC (empty circles), HDOeS (empty inverted triangles) and DOeS (empty triangles). Results are presented as mean ± SD from two independent assays ($n = 10$ animals per group). Significant differences were assessed using one-way ANOVA ($F_{\text{Entrieszone1}}(2,27) = 7.642$; $p = 0.0023$; $F_{\text{Timezone1}}(2,27) = 4.165$; $p = 0.0265$; $F_{\text{Timezone2}}(2,27) = 3.576$; $p = 0.0419$ and $F_{\text{Timezone3}}(2,27) = 5.349$; $p = 0.0110$) followed by Newman–Keuls post hoc test. *, $p < 0.05$, * refers to SUC and ^a refers to HDOeS.

The analysis of grooming behavior did not indicate significant differences in either the number of events or the duration of the activity ($F_{\text{Events}}(2,22) = 2.201$; $p = 0.1344$ and $F_{\text{Time}}(2,22) = 0.081$; $p = 0.9222$). It is important to note that only half of HDOeS performed the activity. Similarly, the analysis of rearing did not reveal significant differences in the number of events and/or time spent on the activity ($F_{\text{Events}}(2,27) = 0.119$; $p = 0.8886$ and $F_{\text{Time}}(2,27) = 2.154$; $p = 0.1356$).

In the EPM, the analysis did not reveal significant differences between the groups in any of the parameters evaluated in closed ($F_{\text{Entries}}(2,27) = 2.536$; $p = 0.0979$; $F_{\text{Time}}(2,27) = 0.712$; $p = 0.4996$ and $F_{\text{Distance}}(2,27) = 0.588$; $p = 0.5625$) and open arms ($F_{\text{Entries}}(2,27) = 3.218$; $p = 0.0558$; $F_{\text{Time}}(2,27) = 2.312$; $p = 0.1183$ and $F_{\text{Distance}}(2,27) = 2.312$; $p = 0.1183$), and center ($F_{\text{Entries}}(2,27) = 2.815$; $p = 0.776$; $F_{\text{Time}}(2,27) = 0.847$; $p = 0.4398$ and $F_{\text{Distance}}(2,27) = 1.059$; $p = 0.3608$). The activities of grooming and rearing were only observed in the closed arms. The analysis did not reveal significant differences in grooming ($F_{\text{Events}}(2,22) = 0.691$; $p = 0.5114$ and $F_{\text{Time}}(2,22) = 3.118$; $p = 0.0642$) and rearing ($F_{\text{Events}}(2,27) = 0.079$; $p = 0.9246$ and $F_{\text{Time}}(2,27) = 1.755$; $p = 0.1921$). It is important to note that not all the animals performed grooming (SUC $n = 7$; HDOeS $n = 9$ and DOeS $n = 9$). The analysis of head dip events did not show significant differences among the groups ($F_{\text{Events}}(2,27) = 2.241$; $p = 0.1258$).

NOL

The HDOeS group presented an increase in ER compared to SUC (47.03%; $p = 0.0037$). Also, no differences were observed in the amount of crossed lines in T1 and T2 and the time dedicated to exploring each object in T1 (Figure 7). It is important to note that the DOeS group was excluded from the analysis because only two animals explored both objects for the required duration during the trials.

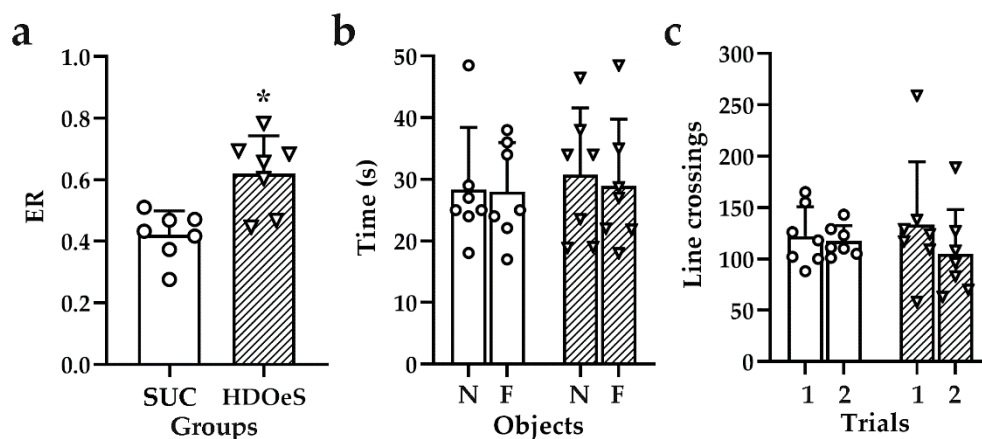


Figure 7. ER (a), time dedicated to exploring objects N and F in T1 (b) and lines crossing in T1 and T2 (c) in NOL test after 40 days of administration of SUC (empty circles) and HDOeS (empty inverted triangles). Results are presented as mean $\pm$ SD from two independent assays ($n = 7$ animals per group). Significant differences were assessed using Student's t -test (ER: $p = 0.0037$; time for object N: $p = 0.6661$ and for object F: $p = 0.8597$; and line crossing in T1: $p = 0.6629$ and in T2: $p = 0.4890$). $p < 0.05$, * refers to SUC.

4. Discussion

In this work, forty compounds and twenty-nine compounds were tentatively identified in MGEOe and DOe, respectively, including flavones (6, 7, 10 and 11), C-glycosyl flavones (4 and 5), isoflavone (8), fatty acids (2, 14–18, 20–26, 28–35 and 37), terpenes (27, 38), phenolic acids (4' and 19), aromatics (12 and 13), furane (9) and sterols (36 and 39), using UHPLC-ESI-QTOF-MS analysis in negative mode. These identifications represent the first report for *O. erythrorhiza*, providing an updated account of the chemical composition of this Andean species. Recent studies have reported the metabolite profiling of various medicinal plants and fruits collected in Argentina and Chile, utilizing two powerful techniques:

UHPLC-ESI-OT-MS and UHPLC-ESI-QTOF-MS. These exhaustive analyses have significantly contributed to updating the chemical composition of all the Andean medicinal plants studied, providing a more comprehensive understanding of their bioactive compounds. On the other hand, it has allowed us to provide solid support for some of the evaluated activities by identifying the potential compounds responsible for the biological effects, including the strong antioxidant properties of the decoctions and polar extracts from most of the analyzed species [14–16,22,23,31–35].

In the last decade, there has been an increase in research focused on identifying new sources of antioxidant compounds derived from natural products, such as fruits and medicinal plants, in their various forms of consumption. These compounds have the potential to serve as therapeutic options for treating pathological conditions where free radicals play a recognized role [36]. In this study, antioxidant activity was evaluated using assays based on electron transfer mechanisms, including FCR, TEAC, FRAP, and DPPH assays, which can also be neutralized by hydrogen atom donation [36,37]. Among these methods, the DPPH assay is particularly notable for its strengths; it is simple, cost-effective, and rapid, with reproducible results that are comparable to other radical scavenging methods. It is highly sensitive and often shows good correlation with bioactive compounds such as phenols and flavonoids, typically yielding a regression factor $R > 0.8$. However, these strengths are counterbalanced by certain limitations, including its sensitivity to the presence of Lewis bases, which can affect DPPH absorbance. Additionally, the analysis must be conducted in the dark or protected from light to prevent absorbance decay. Moreover, the absence of DPPH free radicals in the human body limits its physiological relevance [36].

The *in vitro* studies of MGEOe and DOe revealed significant antioxidant activity, highlighting the effect on ILP process. This could be explained at least in part by the presence of chlorogenic acid (4'), isoorientin (5), rutin (7), isovitexin (8), 4',5,7-trihydroxy-3,6-dimethoxyflavone (10), pinoleic acid (22), alpha-boswellic acid (25), and daucosterol (39) among others, which have been reported to have antioxidant activity [38–49]. However, the *in vivo* studies of HDOeS and DOeS did not reveal any significant effects on MDA levels in the tissues evaluated.

The Andean flora has been scarcely investigated for its cytotoxic properties. Recently, extracts and decoctions of two species growing in Argentina have demonstrated cytotoxic potential, supported in part by the comprehensive chemical characterization of these extracts and decoctions [16,35]. The cytotoxicity assays revealed that DOe produced a selective cytotoxic effect against a tumoral cell line, with no effects in non-tumoral cells, whereas MGEOe produced cytotoxic effects in both cell lines. Also, the effect of MGEOe was higher than that of DOe. These cytotoxic effects may be linked to the presence of vicentin II (4), chlorogenic acid (4'), isoorientin (5), rutin (7), meliartenin (9), alpha-boswellic acid (25), and daucosterol (39), which have been reported to have antitumoral properties [38,39,42,49–56].

The analysis of COX inhibition revealed that DOe produced a significant anti-inflammatory effect. This may be related to the presence of certain compounds tentatively identified in the UHPLC-MS analysis, including vicentin II (4), chlorogenic acid (4'), isoorientin (5), isovitexin (8), ricinoleic acid (18), pinoleic acid (22), alpha-boswellic acid (25), and daucosterol (39), which has reported anti-inflammatory properties [38,40,43–45,47–49,57–62].

On the other hand, the selected *in vivo* model of insulin resistance is characterized by alterations in glucose and lipid metabolism, oxidative state, as well as cognitive impairments, including anxiety-like behavior and memory deficits [20]. In this work, the administration of HDOeS and DOeS did not modify the BW gain. In contrast, several reports describe that compounds like vicentin II (4), chlorogenic acid (4') and rutin (7), among others, could modify the BW gain [63–67]. Although these treatments did not modify the response to glucose overload, the animals presented a decrease in BG. This effect may be linked to the presence of compounds such as quinic acid (3), chlorogenic acid (4') and bis-3-(4',5,7-trimethoxy-flavanone)-5'-(2',4,4'-trimethoxy-chalcone), (6), which can play vital functions in the process of regulating glucose metabolism [38,68,69]. Additionally, daucosterol (39) may inhibit key glucose metabolism enzymes, helping to maintain

blood sugar levels within normal ranges [45]. Regarding the lipid parameters, the administration of the decoction reduces the levels of TC and TG. The hypocholesterolemic properties can be attributed to the combined action of flavonoids, fatty acids, phenolic compounds, and sesquiterpenes, all of which have hypolipidemic effects through several mechanisms [22,23,38,43,48,49,61,70–72].

In the evaluation of anxiety levels, only the administration of HDOeS produced an anxiolytic effect, which could be attributed to the action of compounds like chlorogenic acid (4') [73], rutin (7) [74], isovitexin (8) [75], vicienin II (4) [76], ricinoleic acid isomer (20) [77], over different pathways. In addition, only the administration of HDOeS improved the spatial memory performance of the animals. This could be attributed to the content of chlorogenic acid (4') [78], rutin (7) [79], vicienin II (4) [80], daucosterol (39) [81], among others, which are capable of improving the cognitive state through different mechanisms. In the animal model reported in this work, the variation in results of the doses employed could be attributed to the difference in the beverage intake. Furthermore, it is important to highlight that administering both doses of decoction to the control animals did not affect their well-being or the evaluated parameters (see Supplementary Materials).

Although the results are promising, additional research is needed to establish whether *O. erythrorhiza* can be regarded as a new source of phytochemicals. The demonstrated antioxidant, anti-tumoral, anti-inflammatory, hypoglycemic, hypocholesterolemic, anxiolytic, and memory-enhancing activities of *O. erythrorhiza* open the way for future studies, including the incorporation of its extracts into polymeric systems, which can preserve the integrity of drugs and biomacromolecules, such as nanoparticles [82,83].

5. Conclusions

In summary, the findings presented in this study provide an updated chemical profile of the Andean medicinal species *O. erythrorhiza*. Several of the reported compounds could explain, at least partially, the demonstrated antioxidant, anti-tumoral, anti-inflammatory, hypoglycemic, hypocholesterolemic, anxiolytic and memory improvement activities of *O. erythrorhiza*. While mechanistic and molecular assays are necessary to clarify the action pathways, the current study provides valuable information about this species that may be beneficial in addressing various health conditions. Additionally, to our knowledge, this is the first work that involves the study of *O. erythrorhiza* on in vitro and in vivo assays. Although the results are promising, additional research is needed to determine whether *O. erythrorhiza* can be regarded as a new source of beneficial phytochemicals relevant to human health.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/antiox13121494/s1>, Gómez et al.: Table S1. BW gain (g) and average volume intake (mL/g rat); Table S2. AUC values from GTT; Table S3. Levels of BG (mg/dL), TC (g/L) and TG (g/L); Table S4. Levels of MDA (fmol/μg of protein); Table S5. OFT; Table S6. EPM; Table S7. NOL and Figure S1. MSn spectra of compounds detected by HR-MS in DOe and MGEe (extracted using Bruker data analysis 4.0). Quinic acid (3); Rutin (7); Isovitexin (8); 8-hydroxy-5-methoxyflavanone (11); Diffraitaic acid (13); Roccellaric acid (24) and Pristimerin (27).

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References

1. Barbinta-Patrascu, M.E.; Bitá, B.; Negut, I. From Nature to Technology: Exploring the Potential of Plant-Based Materials and Modified Plants in Biomimetics, Bionics, and Green Innovations. *Biomimetics* **2024**, *9*, 390. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Bustos, D.A.; Tapia, A.A.; Feresin, G.E.; Ariza Espinar, L. Ethnopharmacobotanical survey of Bauchazeta district, San Juan Province, Argentina. *Fitoterapia* **1996**, *67*, 411–415.
3. Lourteig, A. Oxalidaceae. In *Flora Patagónica, Colección Científica*; Correa, M.N., Ed.; Instituto Nacional de Tecnología Agropecuaria: Buenos Aires, Argentina, 1988; Volume 8, pp. 1–29.
4. Feresin, G.E.; Tapia, A.; López, S.N.; Zacchino, S.A. Antimicrobial Activity of Plants Used in Traditional Medicine of San Juan Province, Argentine. *J. Ethnopharmacol.* **2001**, *78*, 103–107. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Feresin, G.E.; Tapia, A.; Sortino, M.; Zacchino, S.; Arias, A.R.D.; Inchausti, A.; Yaluff, G.; Rodriguez, J.; Theoduloz, C.; Schmeda-Hirschmann, G. Bioactive Alkyl Phenols and Embelin from *Oxalis erythrorhiza*. *J. Ethnopharmacol.* **2003**, *88*, 241–247. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Gudasi, S.; Gharge, S.; Koli, R.; Patil, K. Antioxidant properties and cytotoxic effects of *Oxalis corniculata* on human Hepatocarcinoma (Hep-G2) cell line: An in vitro and in silico evaluation. *Futur. J. Pharm. Sci.* **2023**, *9*, 25. [\[CrossRef\]](#)
7. Gholipour, A.R.; Jafari, L.; Ramezani, M.; Evazalipour, M.; Chavoshi, M.; Yousefbeyk, F.; Kargar Moghaddam, S.J.; Yekta Kooshali, M.H.; Ramezani, N.; Daei, P.; et al. Apoptosis Effects of *Oxalis corniculata* L. Extract on Human MCF-7 Breast Cancer Cell Line. *Galen Med. J.* **2022**, *11*, e2484.
8. Zhang, J.; Shen, W.; He, H. Exploring the action mechanism of *Oxalis corniculata* L. decoction in treating osteoarthritis utilizing liquid chromatography-mass spectrometry technology combined with network pharmacology. *Medicine* **2024**, *103*, e39515. [\[CrossRef\]](#)
9. Jifar, W.W.; Debele, G.R.; Kanfe, S.G.; Mule, C.T. Evaluation of in vivo Antidiabetic, Antidyslipidemic and in vitro Anti-Oxidant Activity of Extract and Solvent Fractions of *Discopodium penninervium* Hoschst Leaf in Mice: Normoglycemic and Streptozocin-Induced Model. *J. Exp. Pharmacol.* **2022**, *28*, 317–330. [\[CrossRef\]](#)
10. Kabach, I.; Bouchmaa, N.; Zouaoui, Z.; Ennoury, A.; El Asri, S.; Laabar, A.; Oumeslakht, L.; Cacciola, F.; El Majdoub, Y.O.; Mondello, L.; et al. Phytochemical Profile and Antioxidant Capacity, α -Amylase and α -Glucosidase Inhibitory Activities of *Oxalis Pes-Caprae* Extracts in Alloxan-Induced Diabetic Mice. *Biomed. Pharmacother.* **2023**, *160*, 114393. [\[CrossRef\]](#)
11. Abhilash, P.A.; Nisha, P.; Prathapan, A.; Nampoothiri, S.V.; Lijo Cherian, O.; Sunitha, T.K.; Raghu, K.G. Cardioprotective Effects of Aqueous Extract of *Oxalis Corniculata* in Experimental Myocardial Infarction. *Exp. Toxicol. Pathol.* **2011**, *63*, 535–540. [\[CrossRef\]](#)
12. Gupta, G.; Kazmi, I.; Afzal, M.; Rahman, M.; Anwar, F. Anxiolytic Effect of *Oxalis Corniculata* (Oxalidaceae) in Mice. *Asian Pac. J. Trop. Dis.* **2012**, *2*, S837–S840. [\[CrossRef\]](#)
13. Aruna, K.; Rajeswari, P.D.R.; Sankar, S.R. The Effect of *Oxalis Corniculata* Extract against the Behavioral Changes Induced by 1-Methyl- 4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) in Mice. *J. Appl. Pharm. Sci.* **2017**, *7*, 148–153.

14. Manrique, S.; Gómez, J.; Piñeiro, M.; Sampietro, B.A.; Peschiutta, M.L.; Tapia, A.; Simirgiotis, M.J.; Lima, B. *Zuccagnia punctata* Cav., a Potential Environmentally Friendly and Sustainable Bionematicide for the Control of Argentinean Horticultural Crops. *Plants* **2023**, *12*, 4104. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Luna, L.; Simirgiotis, M.J.; Lima, B.; Bórquez, J.; Feresin, G.E.; Tapia, A. UHPLC-MS Metabolome Fingerprinting: The Isolation of Main Compounds and Antioxidant Activity of the Andean Species *Tetraglochin ameghinoi* (Speg.) Speg. *Molecules* **2018**, *23*, 793. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Gómez, J.; Simirgiotis, M.J.; Lima, B.; Paredes, J.D.; Villegas Gabutti, C.M.; Gamarra-Luques, C.; Bórquez, J.; Luna, L.; Wendel, G.H.; Maria, A.O.; et al. Antioxidant, gastroprotective, cytotoxic activities and UHPLC PDA-Q orbitrap mass spectrometry identification of metabolites in *Baccharis grisebachii* decoction. *Molecules* **2019**, *24*, 1085. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Kulmacz, R.J.; Lands, W.E. Requirements for Hydroperoxide by the Cyclooxygenase and Peroxidase Activities of Prostaglandin H Synthase. *Prostaglandins* **1983**, *25*, 531–540. [\[CrossRef\]](#)
18. Torres Carro, R.; D’Almeida, R.E.; Isla, M.I.; Alberto, M.R. Antioxidant and Anti-Inflammatory Activities of *Frankenia Triandra* (J. Rémy) Extracts. *S. Afr. J. Bot.* **2016**, *104*, 208–214. [\[CrossRef\]](#)
19. Chou, T.C.; Martin, N. *CompuSyn for Dose–Effect Analysis and for Quantitation of Synergism and Antagonism. Software and Manual*; ComboSyn: Paramus, NJ, USA, 2004.
20. Rey, M.; Coirini, H.; Marchena, A.; González-Deniselle, M.C.; Kruse, M.S. Effects of metformin on behavioral alterations produced by chronic sucrose consumption in male rats. *J. Neuroendocrinol.* **2024**, *36*, e13362. [\[CrossRef\]](#)
21. Rey, M.; Kruse, M.S.; Magrini-Huamán, R.N.; Coirini, H. High-Fat Diets and LXRs Expression in Rat Liver and Hypothalamus. *Cell. Mol. Neurobiol.* **2019**, *39*, 963–974. [\[CrossRef\]](#)
22. Rey, M.; Kruse, M.S.; Magrini-Huamán, R.N.; Gómez, J.; Simirgiotis, M.J.; Tapia, A.; Feresin, G.E.; Coirini, H. *Tessaria absinthioides* (Hook. & Arn.) DC. (Asteraceae) Decoction Improves the Hypercholesterolemia and Alters the Expression of LXRs in Rat Liver and Hypothalamus. *Metabolites* **2021**, *11*, 579. [\[CrossRef\]](#)
23. Rey, M.; Kruse, M.S.; Gómez, J.; Simirgiotis, M.J.; Tapia, A.; Coirini, H. Ultra-High-Resolution Liquid Chromatography Coupled with Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometry Analysis of *Tessaria absinthioides* (Hook. & Arn.) DC. (Asteraceae) and Antioxidant and Hypocholesterolemic Properties. *Antioxidants* **2024**, *13*, 50.
24. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Sturman, O.; Germain, P.L.; Bohacek, J. Exploratory Rearing: A Context- and Stress-Sensitive Behavior Recorded in the Open-Field Test. *Stress* **2018**, *21*, 443–452. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Veloso, A.W.N.; Filgueiras, G.B.; Lorenzo, P.; Estanislau, C. Modulation of Grooming Behavior in Rats by Different Test Situations. *Psychol. Neurosci.* **2016**, *9*, 91–104. [\[CrossRef\]](#)
27. Cruz, A.D.M.; Frei, F.; Graeff, F.G. Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacol. Biochem. Behav.* **1994**, *49*, 171–176. [\[CrossRef\]](#)
28. Rodgers, R.J.; Johnson, N.J. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacol. Biochem. Behav.* **1995**, *52*, 297–303. [\[CrossRef\]](#)
29. Espejo, E.F. Effects of weekly or daily exposure to the elevated plus-maze in male mice. *Behav. Brain Res.* **1997**, *87*, 233–238. [\[CrossRef\]](#)
30. Holmes, A.; Rodgers, R.J. Responses of Swiss-Webster mice to repeated plus-maze experience: Further evidence for a qualitative shift in emotional state? *Pharmacol. Biochem. Behav.* **1998**, *60*, 473–488. [\[CrossRef\]](#)
31. Gómez, J.; Simirgiotis, M.J.; Lima, B.; Gamarra-Luques, C.; Bórquez, J.; Caballero, D.; Feresin, G.E.; Tapia, A. UHPLC–Q/Orbitrap/MS/MS fingerprinting, free radical scavenging, and antimicrobial activity of *Tessaria absinthioides* (Hook. & Arn.) DC. (Asteraceae) lyophilized decoction from Argentina and Chile. *Antioxidants* **2019**, *8*, 593. [\[CrossRef\]](#)
32. Gómez, J.; Simirgiotis, M.J.; Manrique, S.; Piñeiro, M.; Lima, B.; Bórquez, J.; Feresin, G.E.; Tapia, A. UHPLC-ESI-OT-MS Phenolics Profiling, Free Radical Scavenging, Antibacterial and Nematicidal Activities of “Yellow-Brown Resins” from *Larrea* spp. *Antioxidants* **2021**, *10*, 185. [\[CrossRef\]](#)
33. Gómez, J.; Simirgiotis, M.J.; Manrique, S.; Lima, B.; Bórquez, J.; Feresin, G.E.; Tapia, A. UHPLC-HESI-OT-MS-MS biomolecules profiling, antioxidant and antibacterial activity of the “orange-yellow resin” from *Zuccagnia punctata* Cav. *Antioxidants* **2020**, *9*, 123. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Ramos, L.C.; Palacios, J.; Barrientos, R.E.; Gómez, J.; Castagnini, J.M.; Barba, F.J.; Tapia, A.; Paredes, A.; Cifuentes, F.; Simirgiotis, M.J. UHPLC-MS Phenolic Fingerprinting, Aorta Endothelium Relaxation Effect, Antioxidant, and Enzyme Inhibition Activities of *Azara dentata* Ruiz & Pav Berries. *Foods* **2023**, *12*, 643. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Persia, F.A.; Troncoso, M.E.; Rinaldini, E.; Simirgiotis, M.; Tapia, A.; Bórquez, J.; Mackern-Oberti, J.P.; Hapon, M.B.; Gamarra-Luques, C. UHPLC–Q/Orbitrap/MS/MS Fingerprinting and Antitumoral Effects of *Prosopis Strombulifera* (LAM.) BENTH. Queous Extract on Allograft Colorectal and Melanoma Cancer Models. *Heliyon* **2020**, *6*, e03353. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Bibi Sadeer, N.; Montesano, D.; Albrizio, S.; Zengin, G.; Mahomoodally, M.F. The versatility of antioxidant assays in food science and safety—Chemistry, applications, strengths, and limitations. *Antioxidants* **2020**, *9*, 709. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Huang, D.; Ou, B.; Prior, R.L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856. [\[CrossRef\]](#)

38. Naveed, M.; Hejazi, V.; Abbas, M.; Kamboh, A.A.; Khan, G.J.; Shumzaid, M.; Xiao Hui, Z. Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomed. Pharmacother.* **2018**, *97*, 67–74. [\[CrossRef\]](#)
39. Bender, O.; Atalay, A. Chapter 28—Polyphenol chlorogenic acid, antioxidant profile, and breast cancer. In *Cancer*, 2nd ed.; Academic Press: Cambridge, MA, USA, 2021; pp. 311–321.
40. Liang, N.; Kitts, D.D. Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. *Nutrients* **2015**, *8*, 16. [\[CrossRef\]](#)
41. Yuan, L.; Wang, J.; Wu, W.; Liu, Q.; Liu, X. Effect of isoorientin on intra-cellular antioxidant defense mechanisms in hepatoma and liver cell lines. *Biomed. Pharmacother.* **2016**, *81*, 356–362. [\[CrossRef\]](#)
42. Ziqubu, K.; Dlodla, P.V.; Joubert, E.; Muller, C.J.; Louw, J.; Tiano, L.; Mazibuko-Mbeje, S.E. Isoorientin: A dietary flavone with the potential to ameliorate diverse metabolic complications. *Pharmacol. Res.* **2020**, *158*, 104867. [\[CrossRef\]](#)
43. Yuan, L.; Han, X.; Li, W.; Ren, D.; Yang, X. Isoorientin prevents hyperlipidemia and liver injury by regulating lipid metabolism, antioxidant capability, and inflammatory cytokine release in high-fructose-fed mice. *J. Agric. Food Chem.* **2016**, *64*, 2682–2689. [\[CrossRef\]](#)
44. He, M.; Min, J.W.; Kong, W.L.; He, X.H.; Li, J.X.; Peng, B.W. A review on the pharmacological effects of vitexin and isovitexin. *Fitoterapia* **2016**, *115*, 74–85. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Hu, J.J.; Wang, H.; Pan, C.W.; Lin, M.X. Isovitexin alleviates liver injury induced by lipopolysaccharide/d-galactosamine by activating Nrf2 and inhibiting NF- κ B activation. *Microb. Pathog.* **2018**, *119*, 86–92. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Durmaz, L. Antioxidant, antiepileptic, and anticholinergic properties of 4', 5, 7-Trihydroxy-3, 6-dimethoxyflavone as natural phenolic compound: A toxicology approach. *Toxin. Rev.* **2021**, *40*, 292–299. [\[CrossRef\]](#)
47. Zhang, Y.; Jia, J.; Ding, Y.; Ma, Y.; Shang, P.; Liu, T.; Wen, A. Alpha-boswellic acid protects against ethanol-induced gastric injury in rats: Involvement of nuclear factor erythroid-2-related factor 2/heme oxygenase-1 pathway. *J. Pharm. Pharmacol.* **2016**, *68*, 514–522. [\[CrossRef\]](#)
48. Baker, E.J.; Miles, E.A.; Calder, P.C. A Review of the Functional Effects of Pine Nut Oil, Pinolenic Acid and Its Derivative Eicosatrienoic Acid and Their Potential Health Benefits. *Prog. Lipid Res.* **2021**, *82*, 101097. [\[CrossRef\]](#)
49. El Omari, N.; Jaouadi, I.; Lahyaoui, M.; Benali, T.; Taha, D.; Bakrim, S.; El Menyiy, N.; El Kamari, F.; Zengin, G.; Bangar, S.P.; et al. Natural Sources, Pharmacological Properties, and Health Benefits of Daucosterol: Versatility of Actions. *Appl. Sci.* **2022**, *12*, 5779. [\[CrossRef\]](#)
50. Ganeshpurkar, A.; Saluja, A.K. The pharmacological potential of rutin. *Saudi Pharm. J.* **2017**, *25*, 149–164. [\[CrossRef\]](#)
51. Ghanbari-Movahed, M.; Shafiee, S.; Burcher, J.T.; Lagoa, R.; Farzaei, M.H.; Bishayee, A. Anticancer Potential of Apigenin and Isovitexin with Focus on Oncogenic Metabolism in Cancer Stem Cells. *Metabolites* **2023**, *13*, 404. [\[CrossRef\]](#)
52. Joray, M.B.; Villafañez, F.; González, M.L.; Crespo, M.I.; Laiolo, J.; Palacios, S.M.; Carpinella, M.C. P53 tumor suppressor is required for efficient execution of the death program following treatment with a cytotoxic limonoid obtained from *Melia azedarach*. *Food Chem. Toxicol.* **2017**, *109*, 888–897. [\[CrossRef\]](#)
53. Roy, N.K.; Deka, A.; Bordoloi, D.; Mishra, S.; Kumar, A.P.; Sethi, G.; Kunnumakkara, A.B. The potential role of boswellic acids in cancer prevention and treatment. *Cancer Lett.* **2016**, *377*, 74–86. [\[CrossRef\]](#)
54. Zhao, C.; She, T.; Wang, L.; Su, Y.; Qu, L.; Gao, Y.; Xu, S.; Cai, S.; Shou, C. Daucosterol Inhibits Cancer Cell Proliferation by Inducing Autophagy through Reactive Oxygen Species-Dependent Manner. *Life Sci.* **2015**, *137*, 37–43. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Zeng, J.; Liu, X.; Li, X.; Zheng, Y.; Liu, B.; Xiao, Y. Daucosterol Inhibits the Proliferation, Migration, and Invasion of Hepatocellular Carcinoma Cells via Wnt/ β -Catenin Signaling. *Molecules* **2017**, *22*, 862. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Zingue, S.; Gbaweng Yaya, A.J.; Michel, T.; Ndinteh, D.T.; Rutz, J.; Auberger, F.; Maxeiner, S.; Chun, F.K.H.; Tchinda, A.T.; Njamen, D.; et al. Bioguided Identification of Daucosterol, a Compound That Contributes to the Cytotoxicity Effects of *Crateva Adansonii* DC (Capparaceae) to Prostate Cancer Cells. *J. Ethnopharmacol.* **2020**, *247*, 112251. [\[CrossRef\]](#)
57. Chen, Z.; Yang, Y.; Mi, S.; Fan, Q.; Sun, X.; Deng, B.; Wu, G.; Li, Y.; Zhou, Q.; Ruan, Z. Hepatoprotective Effect of Chlorogenic Acid against Chronic Liver Injury in Inflammatory Rats. *J. Funct. Foods* **2019**, *62*, 103540. [\[CrossRef\]](#)
58. Lee, W.; Ku, S.K.; Bae, J.S. Vascular Barrier Protective Effects of Orientin and Isoorientin in LPS-Induced Inflammation in Vitro and in Vivo. *Vascul. Pharmacol.* **2014**, *62*, 3–14. [\[CrossRef\]](#)
59. Li, Y.; Zhao, Y.; Tan, X.; Liu, J.; Zhi, Y.; Yi, L.; Bai, S.; Du, Q.; Li, Q.X.; Dong, Y. Isoorientin Inhibits Inflammation in Macrophages and Endotoxemia Mice by Regulating Glycogen Synthase Kinase 3 β . *Mediators Inflamm.* **2020**, *2020*, 8704146. [\[CrossRef\]](#)
60. Lin, C.M.; Huang, S.T.; Liang, Y.C.; Lin, M.S.; Shih, C.M.; Chang, Y.C.; Chen, T.Y.; Chen, C.T. Isovitexin Suppresses Lipopolysaccharide-Mediated Inducible Nitric Oxide Synthase through Inhibition of NF-Kappa B in Mouse Macrophages. *Planta Med.* **2005**, *71*, 748–753. [\[CrossRef\]](#)
61. Takala, R.; Ramji, D.P.; Choy, E. The Beneficial Effects of PineNuts and Its Major Fatty Acid, Pinolenic Acid, on Inflammation and Metabolic Perturbations in Inflammatory Disorders. *Int. J. Mol. Sci.* **2023**, *24*, 1171. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Vieira, C.; Evangelista, S.; Cirillo, R.; Lippi, A.; Maggi, C.A.; Manzini, S. Effect of ricinoleic acid in acute and subchronic experimental models of inflammation. *Mediators Inflamm.* **2000**, *9*, 223–228. [\[CrossRef\]](#)
63. Seo, S.; Oh, S.; Shin, Y.; Jung, S.; Kim, Y. Reduction of body weight by rutin is associated with an increase of brown adipose tissue mitochondrial biogenesis in high-fat diet induced obese rat (LB430). *FASEB J.* **2014**, *28*, LB430. [\[CrossRef\]](#)

64. Watanabe, T.; Kobayashi, S.; Yamaguchi, T.; Hibi, M.; Fukuhara, I.; Osaki, N. Coffee Abundant in Chlorogenic Acids Reduces Abdominal Fat in Overweight Adults: A Randomized, Double-Blind, Controlled Trial. *Nutrients* **2019**, *16*, 1617. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Yang, J.; Lee, J.; Kim, Y. Effect of Deglycosylated Rutin by Acid Hydrolysis on Obesity and Hyperlipidemia in High-Fat Diet-Induced Obese Mice. *Nutrients* **2020**, *25*, 1539. [\[CrossRef\]](#)
66. Qiao, Y.; Zhang, Z.; Zhai, Y.; Yan, X.; Zhou, W.; Liu, H.; Guan, L.; Peng, L. Apigenin Alleviates Obesity-Associated Metabolic Syndrome by Regulating the Composition of the Gut Microbiome. *Front. Microbiol.* **2022**, *12*, 805827. [\[CrossRef\]](#)
67. Kanchanasurakit, S.; Saokaew, S.; Phisalprapa, P.; Duangjai, A. Chlorogenic Acid in Green Bean Coffee on Body Weight: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Syst. Rev.* **2023**, *12*, 163. [\[CrossRef\]](#)
68. Heikkilä, E.; Hermant, A.; Thevenet, J.; Bermont, F.; Kulkarni, S.S.; Ratajczak, J.; Santo-Domingo, J.; Dioum, E.H.; Canto, C.; Barron, D.; et al. The plant product quinic acid activates Ca²⁺-dependent mitochondrial function and promotes insulin secretion from pancreatic beta cells. *Br. J. Pharmacol.* **2019**, *176*, 3250–3263. [\[CrossRef\]](#) [\[PubMed\]](#)
69. Salehi, B.; Quispe, C.; Chamkhi, I.; El Omari, N.; Balahbib, A.; Sharifi-Rad, J.; Bouyahya, A.; Akram, M.; Iqbal, M.; Docea, A.O.; et al. Pharmacological Properties of Chalcones: A Review of Preclinical Including Molecular Mechanisms and Clinical Evidence. *Front. Pharmacol.* **2021**, *11*, 592654. [\[CrossRef\]](#)
70. Meng, S.; Cao, J.; Feng, Q.; Peng, J.; Hu, Y. Roles of chlorogenic Acid on regulating glucose and lipids metabolism: A review. *Evid. Based Complement. Alternat. Med.* **2013**, *2013*, 801457. [\[CrossRef\]](#)
71. Ding, L.; Zhang, L.; Wen, M.; Che, H.; Du, L.; Wang, J.; Xue, C.; Xu, J.; Wang, Y. Eicosapentaenoic acid-enriched phospholipids improve atherosclerosis by mediating cholesterol metabolism. *J. Funct. Foods* **2017**, *32*, 90–97. [\[CrossRef\]](#)
72. Clandinin, M.T.; Cook, S.L.; Konard, S.D.; French, M.A. The effect of palmitic acid on lipoprotein cholesterol levels. *Int. J. Food Sci. Nutr.* **2000**, *51* (Suppl. S1), S61–S71. [\[CrossRef\]](#)
73. Bouayed, J.; Rammal, H.; Dicko, A.; Younos, C.; Soulimani, R. Chlorogenic acid, a polyphenol from *Prunus domestica* (Mirabelle), with coupled anxiolytic and antioxidant effects. *J. Neurol. Sci.* **2007**, *262*, 77–84. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Foudah, A.I.; Alqarni, M.H.; Alam, A.; Devi, S.; Salkini, M.A.; Alam, P. Rutin Improves Anxiety and Reserpine-Induced Depression in Rats. *Molecules* **2022**, *27*, 7313. [\[CrossRef\]](#) [\[PubMed\]](#)
75. de Oliveira, D.D.; da Silva, C.P.; Iglesias, B.B.; Belebony, R.O. Vitexin Possesses Anticonvulsant and Anxiolytic-Like Effects in Murine Animal Models. *Front. Pharmacol.* **2020**, *11*, 1181. [\[CrossRef\]](#) [\[PubMed\]](#)
76. Salehi, B.; Venditti, A.; Sharifi-Rad, M.; Kregiel, D.; Sharifi-Rad, J.; Durazzo, A.; Lucarini, M.; Santini, A.; Souto, E.B.; Novellino, E.; et al. The Therapeutic Potential of Apigenin. *Int. J. Mol. Sci.* **2019**, *20*, 1305. [\[CrossRef\]](#)
77. Nisbett, K.E.; Vendruscolo, L.F.; Koob, G.F. Indulging Curiosity: Preliminary Evidence of an Anxiolytic-like Effect of Castor Oil and Ricinoleic Acid. *Nutrients* **2024**, *16*, 1527. [\[CrossRef\]](#)
78. Munawaroh, F.; Arfian, N.; Saputri, L.A.A.W.S.; Kencana, S.M.S.; Sari, D.C.R. Chlorogenic acid may improve memory function and decrease inflammation of frontal lobe in diabetic rat. *Med. J. Malays.* **2023**, *78*, 476–483.
79. Xu, P.X.; Wang, S.W.; Yu, X.L.; Su, Y.J.; Wang, T.; Zhou, W.W.; Zhang, H.; Wang, Y.J.; Liu, R.T. Rutin improves spatial memory in Alzheimer's disease transgenic mice by reducing A β oligomer level and attenuating oxidative stress and neuroinflammation. *Behav. Brain Res.* **2014**, *264*, 173–180. [\[CrossRef\]](#)
80. Olasehinde, T.A.; Olaokun, O.O. The Beneficial Role of Apigenin against Cognitive and Neurobehavioural Dysfunction: A Systematic Review of Preclinical Investigations. *Biomedicines* **2024**, *12*, 178. [\[CrossRef\]](#)
81. Ji, Z.H.; Xu, Z.Q.; Zhao, H.; Yu, X.Y. Neuroprotective effect and mechanism of daucosterolpalmitate in ameliorating learning and memory impairment in a rat model of Alzheimer's disease. *Steroids* **2017**, *119*, 31–35. [\[CrossRef\]](#) [\[PubMed\]](#)
82. Cortez Tornello, P.R.; Feresin, G.E.; Tapia, A.; Dzieciuch, M.; Cuadrado, T.R.; Abraham, G.A. Effect of processing techniques on new poly (ϵ -caprolactone)-embelin microparticles of biomedical interest. *Adv. Polym. Technol.* **2018**, *37*, 1570–1580. [\[CrossRef\]](#)
83. Wan, Z.; Gan, L.; Wang, W.N.; Jiang, Y. Rapid membrane surface functionalization with Ag nanoparticles via coupling electro-spray and polymeric solvent bonding for enhanced antifouling and catalytic performance: Deposition and interfacial reaction mechanisms. *J. Colloid Interface Sci.* **2023**, *639*, 203–213. [\[CrossRef\]](#)

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