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HPLC–NMR-Based Chemical Profiling of *Matricaria pubescens* (Desf.) Schultz and *Matricaria recutita* and Their Protective Effects on UVA-Exposed Fibroblasts

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Abstract: The present study aimed to investigate the chemical profile and the protective activity on fibroblasts of two *Matricaria* species: *M. pubescens*, which grows wild in Algeria, and *M. recutita*, which is cultivated in Greece. A comparative phytochemical investigation using High-Performance Liquid Chromatography, coupled with Photodiode Array Detection and Mass Spectrometry (HPLC–PDA–MS) combined with Nuclear Magnetic Resonance (NMR), was performed for the identification of the main constituents in the flowerheads of these medicinal plants. In *M. pubescens* more than 25 compounds were identified and/or isolated; among them are quercetagenin-3-O-glucopyranoside, reported for the first time in *Matricaria* sp., and two polyamines previously reported in other Asteraceae species. In *M. recutita*, which is the officially recognized species in Europe, 19 constituents were identified. To minimize time analysis, the structure elucidation was based on a multi-analytical approach directly on subfractions. Two representative polar extracts from each species were characterized chemically and further screened for their protective effects on 3T3 fibroblasts. The cells were exposed to a mild toxic dose of UVA light (6 J/cm²), in the presence of different concentrations of the extracts. Both *M. recutita* and *M. pubescens* extracts were effective. The methanolic extract was the best protective agent at lower concentrations (0.1 to 10 µg/mL), and hydromethanolic was best at higher ones (100–200 µg/mL). *M. recutita* exhibited the most enhanced cell viability in relation to those not exposed to UV control; it ranged from of 28 to 49% higher viability, depending on the dose, leading to the conclusion that the latter seems to exhibit potent cytoprotective activity and significant regeneration activity.

Keywords: *Matricaria pubescens*; *Matricaria recutita*; HPLC–PDA–MS; BALbC 3T3 fibroblasts



Citation: Ignatiadou, M.-E.; Kostaki, M.; Kabouche, Z.; Chatzopoulou, P.; Rallis, M.C.; Karioti, A. HPLC–NMR-Based Chemical Profiling of *Matricaria pubescens* (Desf.) Schultz and *Matricaria recutita* and Their Protective Effects on UVA-Exposed Fibroblasts. *Sci* **2022**, *4*, 14. <https://doi.org/10.3390/sci4010014>

Academic Editor: Gavino Sanna

Received: 5 January 2022

Accepted: 14 March 2022

Published: 18 March 2022

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1. Introduction

Matricaria pubescens (Desf.) Schultz (Asteraceae), known as hairy chamomile, is endemic to North Africa and is used to treat rheumatic and muscular pains, coughs, allergies, ocular affections, dysmenorrhea, scorpion stings, and toothaches [1]. Ethnobotanical studies [1,2] have put in evidence the important role that this species holds in Algerian traditional medicine. Previous phytochemical work on *M. pubescens* [3] showed the presence of flavonoids, mainly apigenin and luteolin derivatives, and the same was shown for the well-known European species *Matricaria recutita* L. The latter is the officially recognized medicinal species in Europe. According to the European Medicines Agency (EMA),

chamomile preparations are used for mild gastrointestinal disorders, ulcers and inflammations of the mouth and throat, for irritated skin and mucosae, and the relief of common cold [4]. A variety of constituents are responsible for these activities, such as the essential oil components—bisabolol and chamazulene—as well as polar phenolic compounds such as apigenin-7-O-glucoside. Chamomile flowers have a broad spectrum of applications in the pharmaceutical and cosmetic industries, mainly due to their anti-inflammatory properties, which have been well known since antiquity. Chamomile products are often used to treat several skin conditions such as ultraviolet (UV)-induced erythema, pityriasis alba, peristomal lesions, contact dermatitis, eczema, atopic eczema, erythema induced by removal of adhesive tape, radiodermatitis, induced contact dermatitis, and wounds. In some cases, chamomile preparations have been shown to be superior to corticosteroids [5]. However, in many of these studies the specific species is not defined. For example, other members of the Asteraceae family, such as *Chamaemelum* spp. (known as Roman chamomile or *Anthemis* spp.), have similar chemistry and are often confused with *Matricaria* spp.; these might induce allergic cross-reactions with other Asteraceae members [6].

In the framework of a project aiming to study the chemical profile of *Matricaria* spp., we carried out chemical characterization of extracts of *M. pubescens* and *M. recutita*. The particular environmental conditions under which *M. pubescens* grows—a hot desert environment with mild winters and little rainfall—made this species an attractive target for studying its chemical and biological properties. In previous work Gherboudj et al. [3], reported the presence of apigenin and luteolin derivatives, showing chemical similarity to the European species *M. recutita*.

Fibroblasts, the main components of the dermis, have a crucial role in the wound healing process, and they also prevent photoaging by releasing tropocollagen and tropoelastin, precursors of the elastic skin fibers. Elevated reactive oxygen and nitrogen species (ROS and RNS), which occur in chronic wounds and after exposure to UV radiation and oxidative stress, lead to skin inflammation disrupting fibroblasts' normal functions [7]. When exposed to oxidative stress, the effectiveness of the skin's endogenous antioxidant system is decreased [8]. Plant extracts, rich in phenolic compounds, possess antioxidant properties and may inhibit ROS production, thus leading to decreased or non-inflammation and to the restoration of fibroblasts. In the present work, a detailed phytochemical profile of *M. pubescens* is reported for the first time. In parallel, *M. recutita* cultivated in Greece was chosen for comparison reasons. Comparative phytochemical and pharmacological investigations concerning their protective effect on fibroblast viability were designed. The identification of the constituents was based on chromatographic investigations and assisted by the application of two analytical platforms: the High-Performance Liquid Chromatography coupled with Photodiode Array Detection and Mass Spectrometry (HPLC–PDA–MS) and Nuclear Magnetic Resonance (NMR) techniques. Two polar extracts from each species, one methanolic and one hydromethanolic, were characterized qualitatively and quantitatively and their dried forms were tested for their activity on BALbC 3T3 mouse skin fibroblasts.

2. Materials and Methods

2.1. General Experimental Procedures

^1H , ^{13}C and 2D NMR experiments were recorded at 295 K in CD_3OD on an Agilent DD2 500 (500.1 MHz for ^1H -NMR and 125.5 MHz ^{13}C -NMR) spectrometer (Palo Alto, CA, USA). Chemical shifts are given in parts per million (ppm) and were referenced to the solvent signals at 3.31 ppm and 49.5 ppm for ^1H and ^{13}C NMR, respectively. COSY, HSQC, HMBC and HSQC-TOCSY were performed using standard Varian microprograms. Column chromatography (CC) was performed on Sephadex LH-20 (Sigma-Aldrich, Darmstadt, Germany) and Amberlite XAD7HP resin (Supelco, Bellefonte, PA, USA) with the solvent mixtures indicated in each case; TLC analyses were carried out using aluminum-coated silica gel plates 60 F₂₅₄ (Merck, Art. 5554, Darmstadt, Germany). Detection was performed using UV light and Naturstoff reagent [9].

2.2. Plant Samples

The aerial parts of *Matricaria pubescens* (Desf.) Schultz. were collected during flowering (April 2008) at Ghardaia (Algerian Septentrional Sahara). Authentication was performed by Prof. Gérard De Belair and a voucher specimen (ZKLOST Cc03/08) was deposited at Annaba University, Algeria. Samples of *Matricaria recutita* flowers were collected at the full-bloom stage from a cultivated population native to Greece (voucher specimen ch. 19/2009), at IPGRB-ELGO DIMITRA [10].

2.3. Isolation of the Compounds from *M. pubescens*

The dried and powdered aerial parts (347 g) of *M. pubescens* were extracted according to a protocol developed by Bohlmann and slightly modified [11]. According to this scheme, a 1:1:1 cyclohexane: diethylether: methanol (chex: Et₂O: MeOH, 3 times, 3Lt in total) solvent mixture is used with the aim of extracting medium polarity constituents such as sesquiterpene lactones, which are often present in plants of the Asteraceae family. The extract was condensed to dryness (27.9 g) and then redissolved in 100 mL of the above system and partitioned with an equal volume of brine to obtain an organic layer (organic phase A, 15.2 g) and an aqueous phase. The aqueous phase was further extracted twice with ethylacetate 100% (EtOAc) and Butanol 100% (BuOH) and yielded two extracts: organic phase B (EtOAc, 5.10 g) and organic phase C (BuOH, 4.39 g). The plant material was further extracted three times with MeOH 100% and MeOH:H₂O 75:25 and produced two extracts of 16.1 g and 20.8 g, respectively (Figure S1, Supplementary Material).

M. recutita flowers (117.7 g) were treated in the same way and they produced the following extracts: organic phase A (9.0 g), organic phase B (EtOAc, 2.19 g), organic phase C (BuOH, 2.2 g), MeOH extract (7.17 g) and MeOH:H₂O 50:50 extract (7.66 g). A scheme of the extraction procedure is available as supplementary material. From the above extracts, the MeOH and MeOH:H₂O extracts of both plants were characterized qualitatively and quantitatively using HPLC–PDA–MS and tested for their protective activity in 3T3 fibroblasts.

The organic phases B and C of *M. pubescens* were subjected to classical phytochemical fractionations as follows: Organic phase B (EtOAc, 5.10 g) was subjected to vacuum liquid chromatography (10 cm × 6.5 cm) over silica gel (Merck 1511) with mixtures of solvents of increasing polarity, petroleum ether (PE), EtOAc and acetone (Ac), and produced 11 fractions (MPB-A to MPB-K). Fraction MPB-D (487.5 mg, eluted with PE:EtOAc 25:75) was subjected to Sephadex LH-20 with DM:MeOH 50:50 and yielded ten fractions (MPB-DA to MPB-DK). Part (18.9 mg) of fraction MPB-DI was subjected to RP-HPLC with MeOH:H₂O 60:40 and produced pure apigenin (**21**) (2.1 mg) and luteolin (**19**) (2.6 mg). Fraction MPB-E (355.8 mg, eluted with EtOAc 100%), was subjected to Sephadex LH-20 with DM:MeOH 50:50 and yielded quercetin (**18**) (4.5 mg). Fraction MPB-E (355.8 mg, eluted with EtOAc 100%) was fractionated over non-polar Sephadex LH-20 with DM:MeOH 1:1 and yielded a mixture of apigenin (**21**), luteolin (**19**) and p-coumaric acid (**25**), as well as a mixture of quercetin (**18**) and quercetagenin (**23**). Fraction MPB-F (353.8 mg, eluted with EtOAc:Ac 90:10) was fractionated over non-polar Sephadex LH-20 with PE:DM:MeOH (from 5:3:1 to 3:3:1) and yielded impure hispidulin, which was further purified by RP-HPLC with MeOH:H₂O 1:1 to give pure hispidulin (**22**) (0.6 mg). Fraction MPB-H (339.3 mg, eluted with EtOAc:Ac 75:25) was fractionated over Sephadex LH-20 with MeOH 100% and yielded 3,5-O-dicaffeoylquinic acid (**11**) (10.9 mg) and 1,5-O-dicaffeoylquinic acid (**12**) (34.4 mg). *M. pubescens* organic phase C (BuOH, 2.2 g) was initially partitioned with DM to eliminate non-polar constituents, and the aqueous phase (2.0 g) was subjected to Sephadex LH-20 with MeOH 90%; it yielded 21 subfractions (ALG-CA to ALG-CU). Subfraction ALG-CS (6.4 mg) was identified as luteolin-4'-O-glucoside by NMR (**13**). Subfraction ALG-CK (15.0 mg) was subjected to preparative RPC18-HPLC (MeOH 50%) and produced polyamine **26** (N1(E)-N5(E)-N9(E)-N14(E)-tetra-trans-p-coumaroyl thermospermine, 5.5 mg). Subfractions ALG-CL (33.5 mg), ALG-CP (8.4 mg) and ALG-CQ (12.9 mg) were studied as such by a combination of HPLC–PDA–MS and NMR. In subfraction

ALG-CL (33.5 mg), the presence of apigenin-7-O-glucopyranoside (**14**) and isoorientin (**3**, luteolin-6-C-glucopyranoside) was confirmed. Subfraction ALG-CQ was identified as a mixture of mainly luteolin-4'-O-glucoside (**13**) and quercetagenin-3-O-glucoside (**2**), and also contained traces of apigenin (**21**), hispidulin (**22**), quercetin-7-O-glucoside (**6**) and 6-hydroxykaempferol-3-O-hexoside (**24**). In ALG-CP (8.4 mg), the presence of luteolin-7-O-glucoside (**10**) and 6-hydroxyluteolin-7-O-glucoside (**5**) was confirmed by NMR and MS (Table 1, Figures 1 and S10, Table S3 of Supplementary Material).

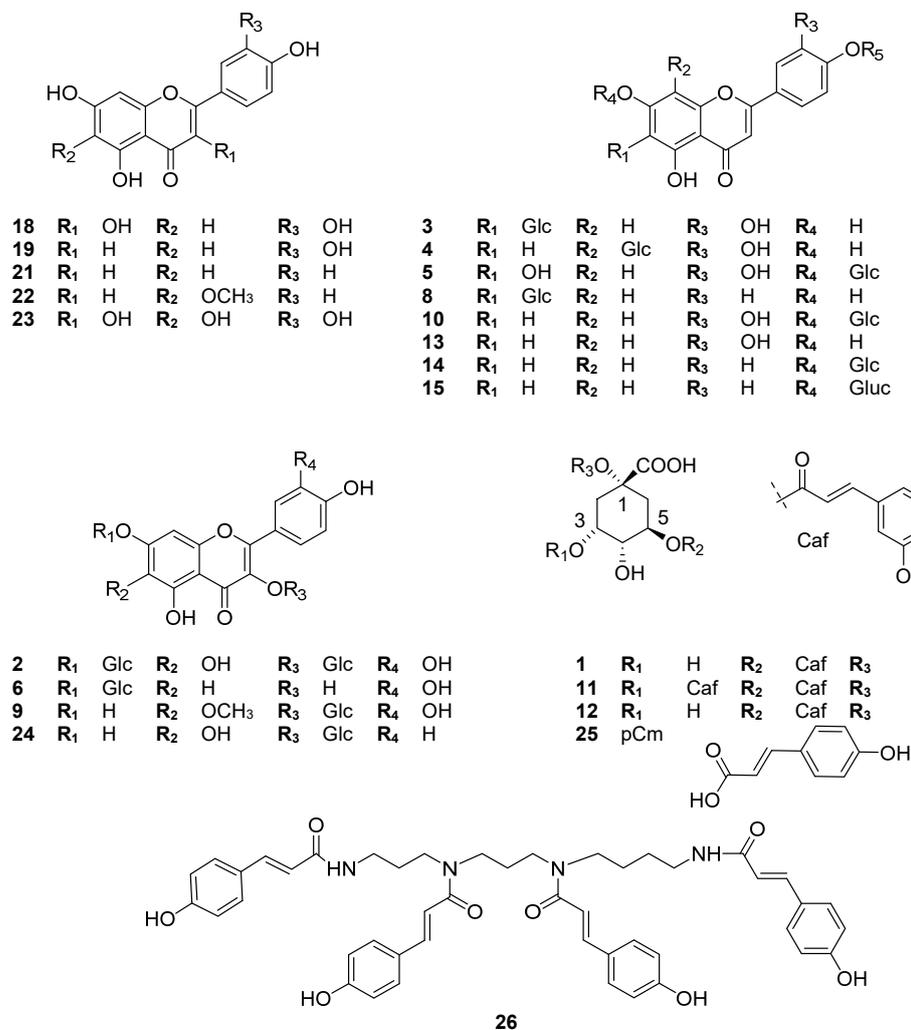


Figure 1. Constituents isolated and/or detected in *Matricaria pubescens* flowers. Glc: glucose; Gluc: glucuronic acid; Caf: caffeic acid.

Chromatographic isolations of the extracts of *M. recutita* were not carried out, because the plant is well characterized for its chemical content by many researchers. Since we aimed to perform biological tests with extracts of *M. recutita*, their chemical analysis was mandatory and, therefore, was carried out exclusively using HPLC–PDA–MS.

2.4. Sample Preparation for HPLC Quantitative Analysis of Methanol and Hydromethanolic Extracts

Approximately 50 mg of each extract was diluted in a 100 mL volumetric flask with 70% methanol. The samples were filtered through Nylon filters (0.45 µm pore size) and immediately injected.

2.5. Chemicals and Standards

The solvents used for the isolation of the flavonoids were of reagent grade, whereas the solvents used for HPLC analysis were HPLC grade. All solvents were purchased from Sigma-Aldrich (Amersham, Sweden). Water was purified using a Milli-Q plus system from Millipore (Milford, MA, USA). Sephadex LH-20 was purchased from Sigma-Aldrich. Nylon filters (0.45 μm pore size) were from Agilent (Agilent Technologies, Palo Alto, CA, USA). Apigenin-7-O-glucoside (99% purity) was purchased from Extrasynthese (Genay, France). Rutin (95% purity) was purchased from Sigma-Aldrich, and apigenin (97% purity) and chlorogenic acid (98% purity) from Alfa Aesar (Kandel, Germany). A series of stock solutions were prepared and kept at $-20\text{ }^{\circ}\text{C}$ in 100% methanol. From these stock solutions, a series of fresh working solutions were prepared immediately prior to analysis.

2.6. HPLC–PDA–MS Analysis Instrumentation

Analysis was carried out using an HPLC–PDA–MS Thermo Finnigan system (LC Pump Plus, Autosampler, Surveyor PDA Plus Detector) interfaced with an ESI MSQ Plus (Thermo Finnigan) and equipped with Xcalibur software (2.1, Thermo Finnigan, MA, USA). The same column, timetable and flow rate were used during the HPLC-MS analyses. The mass spectrometer operated in both negative and positive ionization modes, scan spectra were from m/z 100 to 1000, gas temperature was at $350\text{ }^{\circ}\text{C}$, nitrogen flow rate at 10 L/min, and capillary voltage at 3000 V. The cone voltage was in the range 60–100 V. The column was an SB-Aq (Agilent) RP-C18 column (150 mm \times 3 mm) with a particle size of 5 μm , maintained at $30\text{ }^{\circ}\text{C}$. The eluents were H_2O at pH 2.8 by formic acid (0.05% v/v) (A) and acetonitrile (B), with a flow rate of 0.4 mL/min. Samples were analyzed using a gradient program as follows: 0–15 min, 85–79%A; 15–25 min, 79–77%A; 25–45 min, 77–65%A; 45–53 min, 65–35%A; 53–56 min, 35–85%A; 56–60 min, 85%A. The injected volume of the samples was 5 μL of solution. The UV–vis spectra were recorded between 220 and 600 nm and the chromatographic profiles were registered at 315, 330 and 350 nm.

2.7. Qualitative and Quantitative Determination of Flavonoids

Identification of the constituents of *M. pubescens* and *M. recutita* was performed by examining their retention time, UV and MS data, and by comparing them to authentic reference samples and consulting literature references (Figures 1–4). For *M. pubescens* and *M. recutita*, methanol extracts results are shown in Tables 1 and 2. Hydromethanolic and butanol extracts are shown in Supplementary data, Tables S4–S6. Identification of compounds 1, 3, 5, 6, 10, 11, 12, 13, 14, 18, 19, 21, 22, 23 and 24–26 from *M. pubescens* was carried out using 1D and 2D NMR and using HPLC–PDA–MS wherever needed, as described above. The identification of the rest of the constituents was performed using HPLC–PDA–MS. For the quantitative determination of flavonoids, the method of external standard was applied. The linearity range of responses of the standards was determined on five concentration levels with two injections for each level. Calibration graphs for HPLC were recorded with amounts ranging from 0.22 ng to 0.09 μg stock solutions of the standards, prepared at different concentrations ranging from 0.11×10^{-3} mg/mL to 4.6×10^{-3} mg/mL and injected into HPLC (injection volumes varying from 2 to 8 μL). Measurements were performed at 330 nm for the caffeoylquinic acid derivatives and for flavones, and at 350 nm for 3-O-substituted flavonols. Results were adjusted using a molecular weight correction factor.

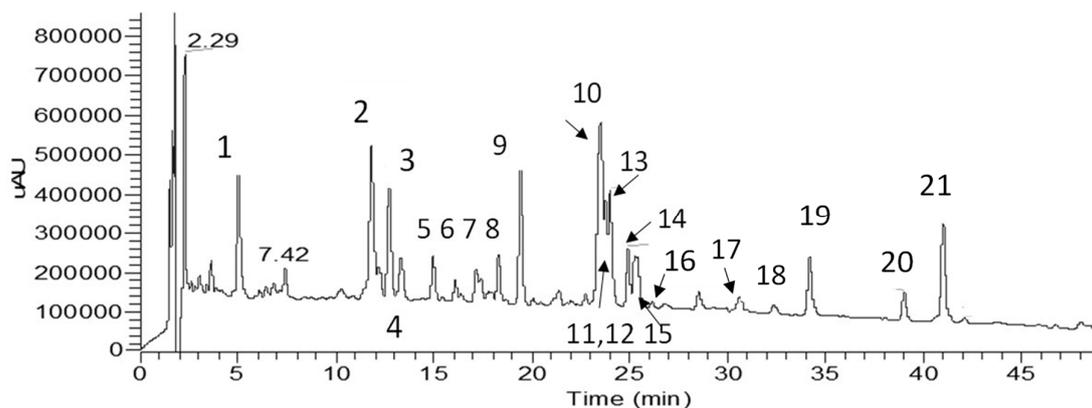


Figure 2. Representative HPLC–PDA–MS chromatogram of the MeOH extract of *Matricaria pubescens* flowers. Experimental conditions: column: Zorbax SbAq RP-C18 (150 × 3.0 mm), particle size of 5 μm (Agilent) at 30 °C. Compounds detected: chlorogenic acid (1), quercetagenin-3-O-glucoside (2), isoorientin (3), orientin (4), 6-hydroxyluteolin-7-O-glucoside (5), quercetin-7-O-glucoside (6), 6/8-hydroxyluteolin-4'-O-glucoside (7), isovitexin (8), patuletin-3-O-glucoside (9), luteolin-7-O-glucoside (10), 3,5-O-dicaffeoylquinic acid (11), eluted together with 1,5-O-dicaffeoylquinic acid (12), luteolin-4'-O-glucoside (13), apigenin-7-O-glucoside (14), apigenin-7-O-glucuronide (15), caffeic acid derivative (16), tri-p-coumaroyl derivative of spermine/thermospermine (17), quercetin (18), luteolin (19), polyamine derivative (20), and apigenin (21).

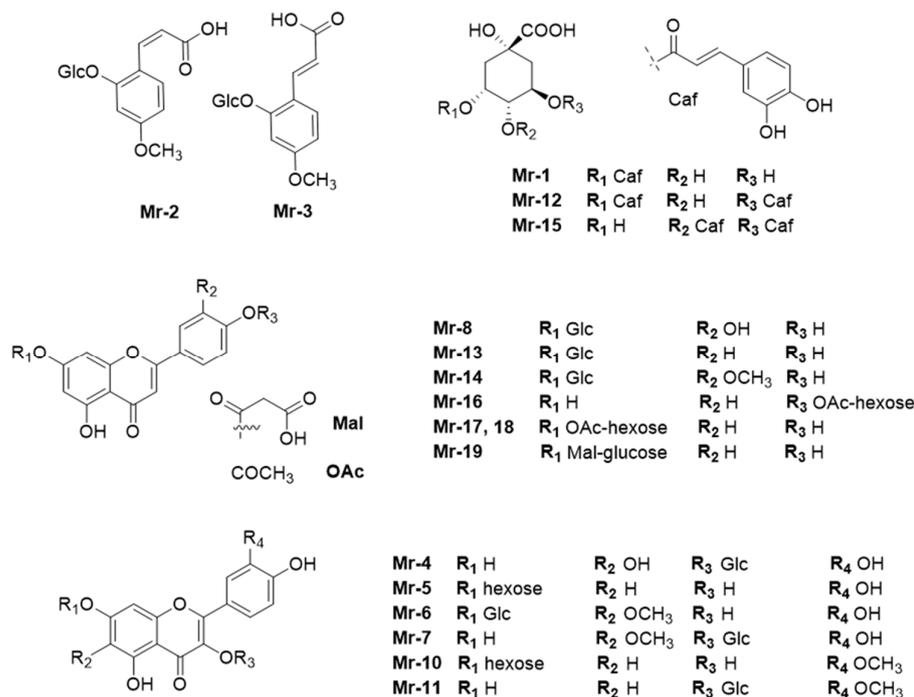


Figure 3. Constituents (Mr-1–Mr-19) detected in *Matricaria recutita* flowers.

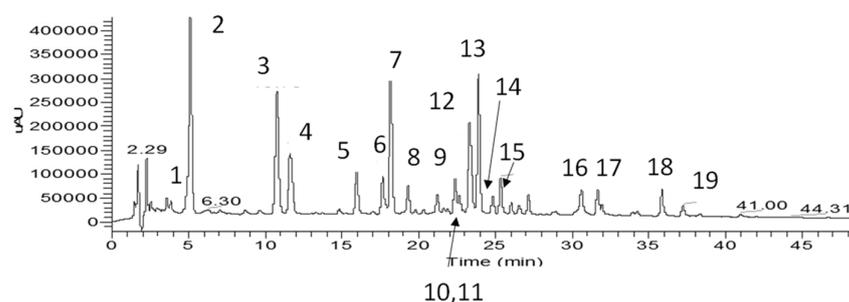


Figure 4. Representative HPLC–PDA–MS chromatogram of the MeOH extract of *Matricaria recutita* flowers. Experimental conditions: column: Zorbax SbAq RP-C18 (150 × 3.0 mm), particle size of 5 μm (Agilent) at 30 °C. Compounds detected: chlorogenic acid (1), cis-2-hydroxy-4-methoxycinnamic-oxo-2-O-β-D-glucopyranoside (2), trans-2-hydroxy-4-methoxycinnamic-oxo-2-O-β-D-glucopyranoside (3), quercetagenin-3-O-glucoside (4), quercetin-7-O-glucoside (5), patuletin-7-O-glucoside (6), patuletin-3-O-glucoside (7), luteolin-7-O-glucoside (8), unknown (9), isorhamnetin-7-O-hexoside (10), isorhamnetin-3-O-glucoside (11), 3,5-O-dicaffeoylquinic acid (12), apigenin-7-O-glucoside (13), chrysoeriol-7-O-glucoside (14), 4,5-O-dicaffeoylquinic acid (15), apigenin-4'-acetylhexoside (tentatively identified) (16), apigenin-7-acetylhexoside (17), apigenin-7-acetyl hexoside isomer (18), apigenin-7-O-(6''-malonyl)-glucoside (19).

NMR data quercetagenin-3-O-glucoside (2): ¹H-NMR (CD₃OD): 7.74 (1H, d, J = 2.0 Hz H-2'), 7.64 (1H, dd, J = 8.5, 2.0 Hz H-6'), 6.90 (1H, s, H-8), 6.87 (1H, d, J = 8.5 Hz H-5'), 5.04 (1H, d, J = 7.4 Hz H-1''), 3.97 (1H, dd, J = 12.2, 2.0 Hz H-6''a), 3.74 (1H, dd, J = 12.2, 5.8 Hz H-6''b), 3.58 (1H, H-2''), 3.56 (1H, H-5''), 3.43 (1H, H-4''). ¹³C NMR: 177.7 (C-4), 153.1 (C-7), 150.5 (C-9), 150.4 (C-3), 149.1 (C-4'), 146.2 (C-3'), 131.1 (C-6), 124.1 (C-1'), 122.2 (C-6'), 116.5 (C-2' and C-5'), 106.6 (C10), 102.9 (C-1''), 95.6 (C-8), 78.9 (C-5''), 77.9 (C-3''), 75.1 (C-2''), 71.7 (C-4''), 62.8 (C-6'').

2.8. Activity of *M. pubescens* and *M. recutita* Extracts on Fibroblasts

Equipment and Reagents: The incubator was an InCO₂ Memmert (Schwabach, Germany), and the abductor a Telstar PV100 (Terrassa, Spain). An Axiovert 25 ZEISS (Schwabach, Switzerland) inverted microscope and a Fluostar Galaxy BMG Microplate Photometer (Ortenberg, Germany) were used. The UVA source was an Astralux Type UVA MED, (UK), and the centrifugal was a Hettich Roto Silenta III (Tuttlingen, Germany). The Laboratory Oven was a Memmert (Schwabach, Germany) and the liquid nitrogen freezing cell container was a 34XT Taylor-Wharton (Cambridge Scientific, Merck KGaA, Darmstadt, Germany). The plate shaker was an MS2 Minishaker, Vortex–IKA, Staufen, Germany). The following reagents were used: DMEM 1X, FBS (Fetal Bovine Serum), PBS, Trypsin-EDTA and antibiotic–antimycotic solution were all purchased from Gibco (Thermo Fisher Scientific Inc., Palo Alto, CA, USA), whereas dimethyl sulfoxide, absolute ethanol and glacial acetic acid were of analytical grade and were purchased from Sigma (Merck KGaA, Darmstadt, Germany). Chlorpromazine hydrochloride was purchased from Thermo Fisher Scientific Inc. (Palo Alto, CA, USA). Neutral red solution was from Sigma (Merck KGaA, Darmstadt, Germany), and distilled water or purified water suitable for cell culture was from Millipore–Sigma (Merck KGaA, Darmstadt, Germany). BALB/c 3T3, Fibroblasts, were a gift from Biological Laboratory, Demokritos, Greece.

The possible protective role against cell-induced necrosis of methanolic and hydromethanolic extracts of the two chamomile species (*M. pubescens* and *M. recutita*) was tested in vitro. Both extracts were dried prior to use. More specifically, using BALBc 3T3 mouse skin fibroblasts (ATCC cell line) and UVA irradiation (6 J/cm²), the effect of the two chamomile species was studied in a mild UVA-induced phototoxicity test. The selected UVA dose was slightly cytotoxic for the specific cell line, in order to reveal the possible cytoprotective efficacy of the extracts. The irradiance was adjusted to reach 6 J/cm² within a time period of 60 min. The chamomile extracts were incorporated both in the culture

medium (DMEM/2d) and in the irradiation phosphate buffer saline (PBS) solution. Six concentrations of the chamomile extracts were tested, covering a broad spectrum (0.1, 1, 10, 50, 100 and 200 µg/mL). Cells incubated with DMEM medium served as a control, and positive control cells incubated with Chlorpromazine hydrochloride were used as a positive control at the same concentrations as the extracts (0.1–200 µg/mL). Cell viability was evaluated by neutral red absorption (540 nm) (Supplementary Material—In vitro protective activity on BALbC 3T3 mouse skin fibroblasts).

3. Results

In the present work, the chemical constitution and biological activity of *M. pubescens* from Algeria and *M. recutita* from Greece were assessed. Both plants were subjected to successive extractions, and the polar extracts, once characterized for their chemical content (Tables 1 and 2; Figures 1–4; Figures S1, S13–S15 and Tables S4–S6 of Supplementary Material), were tested for their activity on fibroblasts. *M. pubescens* is a sub-Saharan species used in Algerian traditional medicine, but its chemical content has not been sufficiently studied [3]. To fill this gap, the plant was further analyzed, and the results are herein reported. In contrast, *M. recutita*, which is widely distributed in Europe, is well-characterized and was chosen for comparison reasons. A cultivated population from Greece was used, which recently demonstrated rich polyphenolic content [12]. In the present study, polar methanol and hydromethanolic extracts were prepared and quantified in order to perform biological assays in a comparative manner with the Algerian species. To this end, a successive extraction scheme was applied for both plants.

3.1. Extraction, Isolation and Identification of the Constituents

The solvent system that was initially applied was chex:Et₂O:MeOH 1:1:1. This system is appropriate for the removal of triterpenoids and sesquiterpene lactones, as well as medium-polarity compounds (Figure S1, Supplementary Material) [11] and has been used extensively in the past for plants of the Asteraceae family containing sesquiterpene lactones [13]. The more polar fractions of this extract, which were obtained through liquid-liquid extractions, served as a reservoir for the phytochemical isolations in order to create a small chemical and spectral library to use in further steps of this study (Figure S1). Phytochemical isolations, although not initially considered, were mandatory; this is because several compounds were not commercially available as reference standards and the phytochemical profile of *M. pubescens* has been little explored. Fractionations of the ethyl acetate (organic phase B) and butanol phase (organic phase C) (Figure S1, Supplementary Material) from *M. pubescens* produced eight compounds, which were identified by 1D and 2D NMR, namely: apigenin (21), luteolin (19), hispidulin (22), quercetin (18), quercetagenin (23), luteolin-4'-O-glucoside (13), p-coumaric acid (25) and the p-coumaroyl polyamine derivative 26 (Figure 1). In order to minimize the time of the analysis, a multi-analytical approach was applied, and selected subfractions were studied spectroscopically using a combination of NMR, HPLC–PDA–MS, reference standards where available. Subfractions ALG-CQ, ALG-CL and ALG-CP, obtained from the *M. pubescens* organic phase C, were studied in this way. Detailed information on the identification process is provided in the Supplementary Material (Figures S2–S12). Using this dereplication methodology, the following compounds were identified: (isoorientin) (3), 6-hydroxyluteolin-7-O-glucopyranoside (5), quercetin-7-O-glucoside (6), luteolin-7-O-glucopyranoside (10), luteolin-4'-O-glucoside (13), 6-hydroxykaempferol-3-hexoside (24). Compound 24 has been reported several times in plants of the Asteraceae family [14,15], while the presence of 6-hydroxyluteolin-7-O-glucopyranoside is considered characteristic of the European chamomile *M. recutita* [16]. Based on the in-house-created chemical library, the analysis of the polar extracts (methanolic, hydromethanolic) of *M. pubescens* and *M. recutita* was then performed (Tables 1 and 2, Tables S4–S6 and Figures S13–S15, Supplementary Material). The discrimination between the isomers orientin (4) and isoorientin (3) was feasible by co-chromatography with the lab isolate. A series of caffeoylquinic acid derivatives such as chlorogenic acid (1), 3,5-dicaffeoyl

quinic acid (**11**) and 1,5-dicaffeoyl quinic acid (**12**) were confirmed by examining of the UV and MS data, and by using reference standards [17]. Indeed, it seems that compounds **11** and **12** co-elute under the present HPLC conditions. The presence of isovitexin (**8**) was suggested due to the lack of shoulder at 302 nm, which is observed in the isobaric vitexin. Instead, compound **7** was identified as a hydroxyluteolin-4'-O-glucoside derivative on the basis of its molecular weight and the hypsochromic shift of Band I at 337 nm, just like the similar luteolin-4'-O-glucoside (**13**), also isolated from this plant. However, the exact hydroxylation site could not be deduced. The peak at 30.72 min (**17**) had an absorption maximum typical of p-coumaroyl moiety and its fragmentation pattern was similar to that of polyamine **26**. From its molecular weight, it is suggested that it is a tri-p-coumaroyl derivative of spermine/thermospermine. Unfortunately, the compound was isolated only in a small amount (1.5 mg), which did not permit further elucidation of its structure. Finally, for compound **20**, UV, MS data and retention time suggested a caffeoyl-substituted polyamine derivative, but the structure needs further isolations and study.

Table 1. MS fragmentation and UV-vis absorption data of the compounds detected in the MeOH extract of *Matricaria pubescens* flowers.

	Rt (min)	UV (nm)	m/z (–) Negative Mode	Identification	Mode of Identification
1	5.04	297, 326	191 [quinic acid-H] [–] , 353 [M-H] [–]	chlorogenic acid	UV/MS, std
2	11.83	259, 274, 357	317 [A-H] [–] , 479 [M-H] [–]	quercetagenin-3-O-glucoside	NMR, UV/MS
3	12.73	255, 269, 349	327 [M-120-H] [–] , 357 [M-90-H] [–] , 447 [M-H] [–]	isoorientin	NMR, UV/MS
4	13.40	255, 268, 342	327 [M-120-H] [–] , 357 [M-90-H] [–] , 447 [M-H] [–]	orientin	UV/MS
5	14.96	281, 343	301 [A-H] [–] , 463 [M-H] [–]	6-hydroxyluteolin-7-O-glucoside	NMR, UV/MS
6	16.11	255, 369	301 [A-H] [–] , 463 [M-H] [–]	quercetin-7-O-glucoside	NMR, UV/MS
7	17.20	276, 337	301 [A-H] [–] , 463 [M-H] [–]	6/8-hydroxyluteolin-4'-O-glucoside	UV/MS, tentatively
8	17.45	269, 336	311 [M-120-H] [–] , 341 [M-90-H] [–] , 431 [M-H] [–]	isovitexin (lack of shoulder at 302 nm)	NMR, UV/MS
9	18.32	259, 276sh, 356	331 [A-H] [–] , 493 [M-H] [–]	patuletin-3-O-glucoside	UV/MS, [18]
10	19.45	254, 266sh, 347	285 [A-H] [–] , 447 [M-H] [–]	luteolin-7-O-glucoside	NMR, UV/MS
11–12	23.55	245, 300, 328	179 [caffeic acid-H] [–] , 191 [quinic acid-H] [–] , 353 [M-caffeoyl group-H] [–] , 515 [M-H] [–]	3,5-O-dicaffeoylquinic acid + 1,5-O-dicaffeoylquinic acid	UV/MS, std, lab isolate [17]
13	23.80	268, 336	285 [A-H] [–] , 447 [M-H] [–]	luteolin-4'-O-glucoside	NMR, UV/MS
14	24.02	267, 335	269 [A-H] [–] , 431 [M-H] [–]	apigenin-7-O-glucoside	UV/MS, std
15	24.93	267, 338	269 [A-H] [–] , 445 [M-H] [–]	apigenin-7-O-glucuronide	UV/MS lab isolate [19]
16	25.20	298, 327	161, 179, 381, 543 [M-H] [–]	derivative of caffeic acid	UV/MS
17	30.72	308	269, 639 [M-H] [–]	tri-p-coumaroyl derivative of spermine/thermospermine	NMR, UV/MS
18	32.56	254, 368	301 [M-H] [–]	quercetin	NMR, UV/MS, std
19	34.22	253, 266, 348	285 [M-H] [–]	luteolin	NMR, UV/MS

Table 1. Cont.

	Rt (min)	UV (nm)	m/z (–) Negative Mode	Identification	Mode of Identification
20	39.02	295, 326	705 [M-H] [–]	polyamine derivative—not identified	UV/MS
21	41.02	267, 337	269 [M-H] [–]	apigenin	NMR, UV/MS

A: aglycon.

Table 2. MS fragmentation and UV–vis absorption data of the compounds detected in the methanol extract of *Matricaria recutita* flowers.

Mr	Rt (min)	UV (nm)	m/z (–) Negative Mode	Identification	Mode of Identification
Mr-1	5.16	296, 326	191 [quinic acid-H] [–] , 353 [M-H] [–]	chlorogenic acid	UV/MS, std
Mr-2	5.40	279, 301	134 [A-CO ₂ -CH ₃ -H] [–] , 149 [A-CO ₂ -H] [–] , 193 [A-H] [–] , 355 [M-H] [–] , 711 [2M-H] [–]	cis-2-hydroxy-4-methoxycinnamic-oxo-2-O-β-D-glucopyranoside	UV/MS, [20]
Mr-3	11.08	295, 318	135 [A-CO ₂ -CH ₂ -H] [–] , 149 [A-CO ₂ -H] [–] , 193 [A-H] [–] , 355 [M-H] [–] , 711 [2M-H] [–]	trans-2-hydroxy-4-methoxycinnamic-oxo-2-O-β-D-glucopyranoside	UV/MS, [20]
Mr-4	11.90	259, 354	317 [A-H] [–] , 479 [M-H] [–]	quercetagenin-3-O-glucoside	NMR/UV/MS
Mr-5	15.98	255, 370	301 [A-H] [–] , 463 [M-H] [–]	quercetin-7-O-hexoside	UV/MS, [12,20]
Mr-6	17.90	258, 369	331 [A-H] [–] , 493 [M-H] [–]	patuletin-7-O-glucoside	UV/MS, [12]
Mr-7	18.47	259, 356	331 [A-H] [–] , 493 [M-H] [–]	patuletin-3-O-glucoside	UV/MS, [18]
Mr-8	19.59	255, 347	285 [A-H] [–] , 447 [M-H] [–]	luteolin-7-O-glucoside	NMR/UV/MS, std
Mr-9	21.07	-	711	not identified	
Mr-10	22.61	254, 370	315 [A-H] [–] , 477 [M-H] [–]	isorhamnetin-7-O-hexoside	UV/MS, [12]
Mr-11	22.94	254, 352	314 [A-H] [–] , 477 [M-H] [–]	isorhamnetin-3-O-glucoside	UV/MS, std
Mr-12	23.34	298, 327	179 [caffeic acid-H] [–] , 191 [quinic acid-H] [–] , 353 [M-caffeoyl-H] [–] , 515 [M-H] [–]	3,5-O-dicaffeoylquinic acid	UV/MS, std
Mr-13	23.93	267, 336	268 [A-2H] [–] , 431 [M-H] [–]	apigenin-7-O-glucoside	NMR/UV/MS, std
Mr-14	25.10	252, 266, 347	299 [A-H] [–] , 446 [M-CH ₃ -H] [–] , 461 [M-H] [–]	chrysoeriol-7-O-glucoside	UV/MS, [12]
Mr-15	25.36	298, 327	179 [caffeic acid-H] [–] , 191 [quinic acid-H] [–] , 353 [M-caffeoyl-H] [–] , 515 [M-H] [–]	4,5-O-dicaffeoylquinic acid	UV/MS, std
Mr-16	30.63	267, 329	269 [A-H] [–] , 473 [M-H] [–]	apigenin-4'-acetyl-hexoside (tentatively)	UV/MS
Mr-17	31.93	267, 336	269 [A-H] [–] , 473 [M-H] [–]	apigenin-7-acetyl-hexoside	UV/MS, [21]
Mr-18	35.88	267, 336	269 [A-H] [–] , 473 [M-H] [–]	apigenin-7-acetyl hexoside isomer	UV/MS, [21]
Mr-19	37.20	267, 336	269 [A-H] [–] , 515 [M-H] [–]	apigenin-7-O-(6''-malonyl)-glucoside	UV/MS, [21]

A: aglycon; numbering is set as Mr-X, according to the retention time and in order to discriminate from the constituents of *M. pubescens*.

Concerning *M. recutita*, its identification (Table 2) was based on reference standards, isolated compounds (where available) and data from the literature. Quercetagenin-3-O-glucoside (corresponding to peak number Mr-4 in Table 2 and Figure 3) was detected for the first time in *M. recutita* and was confirmed by co-elution of the isolated compound. Peaks

Mr-16, 17, 18 and **19** were identified as acetylated apigenin hexosides [21]. Constituents **Mr-16** and **Mr-19** had identical spectral data, suggesting that the isobaric constituents had different acetyl substitutions on the sugar moiety. Compound **Mr-16** however, presented many differences. Its UV spectrum had an hypsochromic shift of Band I at 329 nm, indicating a 4'-substitution on ring B of the flavonoid. Likewise, its MS fragmentation pattern was different and the fragment at $m/z = 473$ $[M-H]^-$ was merely observable, while the fragment at $m/z = 269$ $[A-H]^-$ had the higher intensity. Therefore, the compound **Mr-16** was tentatively identified as apigenin-4'-O-acetylhexoside, reported here for the first time in *Matricaria* spp. Overall, 26 compounds were characterized (isolated and/or identified using HPLC–PDA–MS) in the extracts of *M. pubescens*, and 19 compounds were detected using HPLC–PDA–MS in hydromethanolic and methanolic extracts of *M. recutita*, obtained under the extraction scheme described above.

3.2. Quantitative Data

Quantitation of the major phenolic acids and flavonoids in the examined extracts (methanolic and hydromethanolic 50%) showed marked qualitative and quantitative differences (Table 3). *M. pubescens* extracts had a luteolin derivatives' content, with the main representative, luteolin-7-O-glucoside, rising up to 1.1% (*w/w*). On the contrary, *M. recutita* extracts had notably higher concentrations of the anti-inflammatory apigenin-7-O-glucoside (1.91% vs. 0.50% in *M. pubescens*). Concerning flavonol glycosides, *M. recutita* extracts contained a higher variety of flavonols (5.06 vs. 4.00%), especially a considerably higher amount of patuletin-3-O-glucoside (1.91% vs. 0.49%). *M. pubescens* had a lower content of dicaffeoylquinic acid derivatives (2.43 and 2.79% vs. 2.70 and 3.04%), but it contained no *cis*- and *trans*- 2-hydroxy-4-methoxycinnamic-oxo-2-O- β -D-glucopyranoside, in contrast with *M. recutita*. This is of importance, since these two latter constituents have been linked to allergic reactions [22].

Table 3. Amounts of flavonoids and phenolics in the polar (MeOH and MeOH 50%) extracts of *M. pubescens* (Mp) and *M. recutita* (Mr) flowers ($n = 3$). Results expressed as % *w/w*.

Name	MrM	MrHM	MpM	MpHM
apigenin	0.20 ± 0.01	<0.001	0.24 ± 0.01	0.10 ± 0.01
Apigenin-7-O-glucoside	1.20 ± 0.03	0.59 ± 0.01	0.50 ± 0.01	0.36 ± 0.01
Apigenin-7-O-glucuronide	-	-	0.24 ± 0.01	0.34 ± 0.01
Luteolin	-	-	0.60 ± 0.01	0.31 ± 0.01
6-hydroxyluteolin-glucoside	-	-	0.60 ± 0.01	0.64 ± 0.04
Luteolin-7-O-glucoside	-	-	1.13 ± 0.02	0.73 ± 0.03
Patuletin-3-O-glucoside	1.91 ± 0.06	1.89 ± 0.03	0.49 ± 0.02	0.38 ± 0.01
Quercetin-7-O-glucoside	0.47 ± 0.01	0.34 ± 0.01	0.21 ± 0.02	0.52 ± 0.01
Patuletin-7-O-glucoside	0.46 ± 0.02	0.33 ± 0.02	-	-
Isorhamnetin-3-O-glucoside	0.81 ± 0.04	0.60 ± 0.01	-	-
Total flavonoids	5.06 ± 0.01	3.75 ± 0.05	4.00 ± 0.06	3.38 ± 0.08
3,5- +1,5- dicaffeoylquinic acids	0.90 ± 0.03	1.13 ± 0.02	1.78 ± 0.01	1.67 ± 0.02
4,5-dicaffeoylquinic acid	0.68 ± 0.01	1.02 ± 0.01	0.65 ± 0.03	1.11 ± 0.03
<i>cis</i> -2-hydroxy-4-methoxycinnamic-oxo-2-O- β -D-glucopyranoside	0.49 ± 0.01	0.41 ± 0.02	-	-
<i>trans</i> -2-hydroxy-4-methoxycinnamic-oxo-2-O- β -D-glucopyranoside	0.63 ± 0.01	0.48 ± 0.01	-	-
Total phenolic acids	2.70 ± 0.05	3.04 ± 0.04	2.43 ± 0.04	2.79 ± 0.13

M: methanolic; HM: hydromethanolic.

3.3. In Vitro Protective Activity on BALbC 3T3 Mouse Skin Fibroblasts

The administration of 6J/cm² of UVA induced a 10% mean fibroblast viability decrease.

The mean positive control decrease in viability was 60% in the higher tested doses (Figure 5). Both dried methanolic extracts provided significant cell protection at the lower

concentrations, while in the higher ones, the viability in most cases slightly decreased (Figure 5). Upon addition of *M. recutita* methanolic extract at doses of 0.1 to 10 µg/mL, a mean increase in viability is observed in relation to the control, ranging from of 28 to 49%, depending on the dose. In this case, apparently, in addition to the UVA protection there was an increase in the fibroblast mitosis rate. Correspondingly, *M. pubescens* methanolic extract showed an increase of 10% only with the lower dose of 0.1 µg/mL. Hydromethanolic extracts showed some protection at relatively higher doses; maximum viability was obtained by *M. recutita* at a dose of 100 µg/mL with a mean enhancement of 22%. Both extracts showed, at many concentrations, enhanced fibroblast cytoprotection. The phototoxicity protocol is apparently valid, as the addition of chlorpromazine showed an enhanced decrease in fibroblasts of 67% for the highest dose of 200 µg/mL. Qualitative viability appreciation under the microscope confirmed the quantitative measurements.

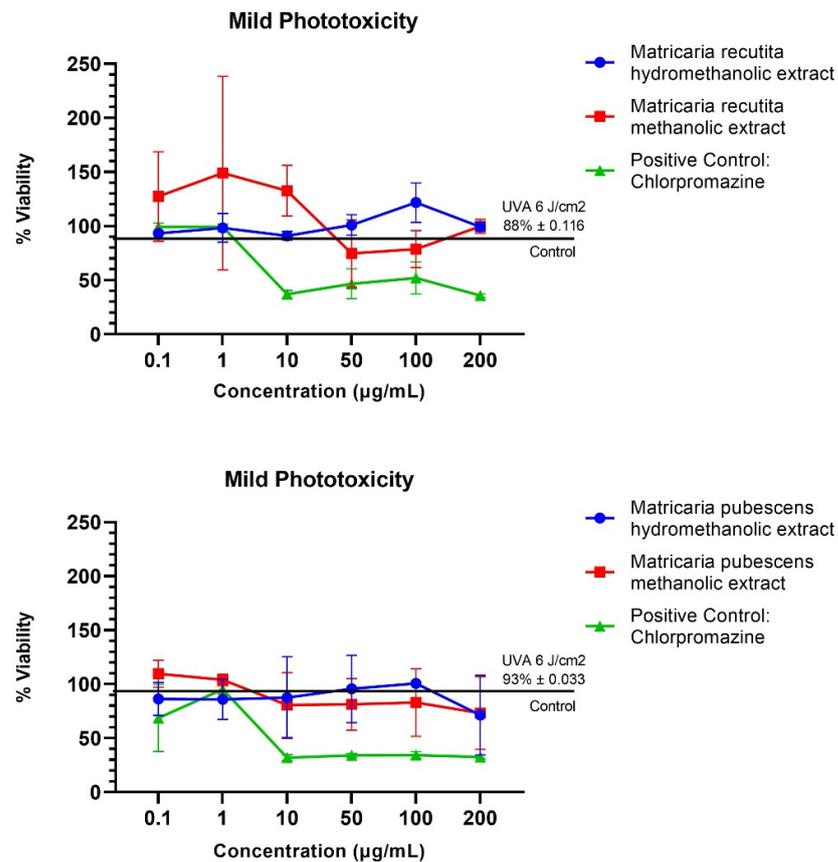


Figure 5. % Viability of *M. pubescens* and *M. recutita* methanolic and hydromethanolic (75%) extracts in UV-A-irradiated BALBc 3T3 fibroblasts. Viability of 100% corresponds to non-UV-exposed cells.

4. Discussion

In the present study, the phytochemical profile of *M. pubescens* was fully explored. In polar extracts, it consists mainly of flavonoid compounds, among which luteolin glycosides and luteolin prevail, and are followed by apigenin-7-O-glucoside and patuletin-3-O-glucoside. It should be noted that luteolin and its derivatives are present in negligible amounts in *M. recutita*, though the content of apigenin glycosides is almost twice that in *M. pubescens*. Quantitatively, the content of total phenolic acids is almost equal in both species.

Phenolic compounds as functional ingredients are considered an important tool with many applications in skin-care products. The antioxidant properties with which these compounds are endowed play a crucial role in the restoration of fibroblasts. When the latter are exposed to phenolic compounds, a decrease in ROS production and an increase in collagen expression is observed, resulting in the acceleration of wound healing and protection against UV-induced photoaging [7]. *Matricaria* species are traditionally used

for several skin ailments, such as ultraviolet (UV)-induced erythema, pityriasis alba, peritomal lesions, contact dermatitis, eczema, atopic eczema, radiodermatitis, induced contact dermatitis, and wounds. Recently, a dual-layered herbal biopolymeric patch based on chamomile extract (of which the chemical synthesis is not reported) increased collagen deposition and showed rapid re-epithelialization at a wound site as a potential wound dressing [23]. Chamomile hydroalcoholic extracts (ethanol: water 1:1, *v/v*) (no chemical analysis provided) have been found to improve wound healing by enhancing fibroblast proliferation and re-vascularization in diabetic skin injuries [24]. In another study (no chemical data provided) the wound-healing effects of chamomile have been demonstrated to be superior to those of corticosteroids [25]. All these effects are generally attributed to apigenin and its derivatives. Apigenin has notable anti-inflammatory activities such as inhibition of prostaglandin E2 (PG-E2), cyclooxygenase 2 (COX-2) and nitric oxide production (EMA). Furthermore, it has been found to interfere with leukocyte adhesion and adhesion-protein upregulation in human endothelial cells. It has also been shown to inhibit interleukin 1 α (IL-1)-induced prostaglandin synthesis and tumor necrosis factor α (TNF- α), among others. Choi et al. [26] reported that apigenin restored the viability of normal human dermal fibroblasts exposed to UVA irradiation through suppression of the expression of the collagenase, matrix metalloproteinase (MMP)-1. Further in vivo tests with an apigenin-containing cream showed increased dermal density and elasticity, improved skin evenness, improved moisture content, and improved trans-epidermal water loss in the subjects who used it. A literature survey showed that other phenolic compounds, which are also present in chamomile extracts, act in a similar manner. Chlorogenic acid from *Coffea arabica*, administered in a dose-dependent manner, inhibited intracellular reactive oxygen species production in CCRF cells stimulated by UV radiation; suppressed the expression of the matrix metalloproteinases-1, 3, and 9; and increased synthesis of type-I procollagen [27]. Flavonol derivatives from *Eriobotrya deflexa* and especially hyperin reduced matrix metalloproteinase I and intracellular reactive oxygen species, and increased procollagen type-I and TIMP-1 in UVB-irradiated human fibroblasts (WS-1 cells) [28].

In view of the above data, the activity of our extracts on UVA-exposed fibroblasts might be explained. A comparison between the relation of the biological activity and the quantitative results corroborates the hypothesis that apigenin-7-O-glucoside accounts mostly for the UVA-protective activity of chamomile. *M. pubescens* methanol extracts, rich in luteolin-7-O-glucoside (up to 1.1% *w/w*), also showed UVA-protective potential. Luteolin-7-O-glucoside anti-inflammatory activity [29,30] could contribute significantly to the cytoprotective properties of *M. pubescens* methanol extracts. The slight regeneration properties of *M. pubescens* methanol extracts at low doses (Figure 5) are in accordance with Ustuner et al. [31], who have shown that luteolin-7-O-glycoside was the major phenolic compound of *Thymus sipyleus* decoction and infusion; these were proven to be effective in the wound-healing process. Similarly, dicaffeoylquinic acid derivatives, which are also reported to have antioxidant, antiradical and hepatoprotective activities [32,33], are in accordance with the cytoprotective effect of *M. pubescens* methanolic extract.

M. recutita extracts contain a higher variety and content of flavonols (5.06 vs. 4.00% in *M. pubescens*), especially a considerable higher amount of apigenin-7-O-glucoside in methanolic extracts, and patuletin-3-O-glucoside in both the methanol and hydromethanolic extracts (1.91 and 1.89%, respectively); however, the phenolic acids content was almost equal in both extracts. This feature might explain the difference in the activity of the plant extracts on fibroblast protection in relation to *M. pubescens* extracts, as well in the difference obtained between *M. recutita* methanolic and hydromethanolic extract (Figure 5). Additionally, anti-inflammatory activity of extracts rich in patuletin derivatives has been previously reported [34]. The anti-inflammatory effect of patuletin 3-O- β -D-glucopyranoside in vivo has been proven to be almost equal to that of dexamethasone [35], though previous studies show that it significantly inhibits histamine-induced hind-paw edema [36]. Apigenin possessing notable anti-inflammatory activity and collagenase and MMP-1 downregulation [26]—as well caffeic acid and patuletin glycosides, endowed with antioxidant and

anti-inflammatory activity [37,38], which are contained in *M. recutita* methanolic extract—could explain its cytoprotective and regenerative activity (Figure 5). The fact that the methanolic extract at concentrations $>50 \mu\text{g/mL}$ showed relative cytotoxicity, up to a mean maximum of 27% for *M. pubescens* could be attributed to the phenolic antioxidants it contains, which often prevent pro-oxidant activity [39]. To sum up, the stronger protective potential against UV stress might be attributed to a combination of apigenin-7-O-glucoside with other flavonoids and phenolics present in the extracts. Further studies with the isolated constituents are needed in order to understand the contribution or the synergistic effect of each compound toward the protective outcome. Further studies are also needed, especially with *M. recutita* methanolic extract, in the field of UV-induced skin damage and wound healing.

5. Conclusions

In the present work, *M. pubescens* growing wild in Algeria was studied for the first time by a combination of HPLC–PDA–MS and chromatographic isolations, followed by NMR. For comparison reasons, extracts of *Matricaria recutita*, the officially recognized European species, were prepared under the same experimental conditions and analyzed using HPLC–PDA–MS. Overall, 26 compounds were characterized (isolated and/or identified using HPLC–PDA–MS) in the extracts of *M. pubescens*, and 19 compounds were detected using HPLC–PDA–MS in the hydromethanolic and methanolic extracts of *M. recutita*. Quantitation using HPLC–PDA–MS showed that *M. pubescens* extracts had a higher content of luteolin derivatives, while *M. recutita* extracts had notably higher concentrations of the anti-inflammatory apigenin-7-O-glucoside, as well as a higher variety of flavonols and caffeoylquinic acid derivatives. Two representative polar extracts from each species were screened for their protective effects on UVA-induced 3T3 fibroblast cytotoxicity. Both *M. recutita* and *M. pubescens* extracts were cytoprotective. The methanolic extracts had the best protective effect at the lower concentrations, while the hydromethanolic extracts had the best protective effect at the higher ones. *M. recutita* exhibited the higher cell viability, leading to the conclusion that the latter seems to exhibit potent cytoprotective activity and significant regeneration activity. The stronger protective potential against UV stress might be attributed to a combination of apigenin-7-O-glucoside with other flavonoids and phenolics present in the extracts. Further studies with the isolated constituents are underway in order to understand the contribution of each compound to the protective effect. Further studies are also needed, especially with *M. recutita* methanolic extract, in the field of UV-induced skin damage such as ageing, irritation, skin cancer and wound healing.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/sci4010014/s1>, Figure S1. Scheme of the whole extraction protocol, analysis and biological assays; Figure S2. HPLC-PDA MS chromatogram of fraction ALG-CQ; Table S1. MS fragmentation and UV-vis absorption data of fraction ALG-CQ; Figure S3. $^1\text{H-NMR}$ spectrum (CD_3OD , 500 MHz) of subfraction ALG-CQ, *Matricaria pubescens*; Figure S4. $^1\text{H-NMR}$ spectrum (CD_3OD , 500 MHz); Figure S5. HSQC spectrum (CD_3OD , 500 MHz); Figure S6. COSY spectrum of subfraction ALG-CQ (CD_3OD , 500 MHz); Figure S7. HPLC-PDA MS chromatogram of fraction ALG-CL; Table S2. MS fragmentation and UV-vis absorption data of fraction ALG-CL; Figure S8. $^1\text{H-NMR}$ spectrum (CD_3OD , 500 MHz) of subfraction ALG-CL; Figure S9. HSQC spectrum (CD_3OD , 500 MHz) of subfraction ALG-CL; Figure S10. HPLC-PDA MS chromatogram of fraction ALG-CP; Table T3. MS fragmentation and UV-vis absorption data of fraction ALG-CP; Figure S11. $^1\text{H-NMR}$ spectrum (CD_3OD , 500 MHz) of subfraction ALG-CP; Figure S12. HMBC spectrum (CD_3OD , 500 MHz) of subfraction ALG-CP; Figure S13. HPLC–PDA–MS chromatogram of the MeOH 50% extract of *Matricaria pubescens* flowers; Table S4. MS fragmentation and UV-vis absorption data of the compounds detected in the MeOH:H₂O 50:50 extract of *Matricaria pubescens* flowers; Figure S14. HPLC–PDA–MS chromatogram of the Butanol extract of *Matricaria pubescens* flowers; Table S5. MS fragmentation and UV-vis absorption data of the compounds detected in the butanol extract of *Matricaria pubescens* flowers; Figure S15. HPLC–PDA–MS chromatogram of the MeOH 50% extract of *Matricaria recutita* flowers; Table S6. MS fragmentation and UV-vis absorption data of the compounds

detected in the MeOH:H₂O extract of *Matricaria recutita* flowers; In vitro protective activity on BALBc 3T3 mouse skin fibroblasts.

Author Contributions: Conceptualization, A.K.; methodology, A.K. and M.C.R.; validation, A.K. and M.C.R.; formal analysis, M.-E.I., A.K., M.K. and M.C.R.; investigation, M.-E.I., A.K., M.K. and M.C.R.; resources, Z.K., P.C., A.K. and M.C.R.; data curation, P.C., M.C.R. and A.K.; writing—original draft preparation, P.C., A.K., M.K. and M.C.R.; writing—review and editing, P.C., A.K., M.K. and M.C.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Cherif, H.S.; Ferrah, R.; Bennacer, A.; Tail, G.; Saidi, F. Traditional use of *Matricaria pubescens* (Desf.) Schultz in two regions of Southern Algeria and contribution to study the antioxidant activity. *Indian J. Trad. Knowl.* **2017**, *16*, 562–567.
- Hammiche, V.; Maiza, K. Traditional medicine in Central Sahara: Pharmacopoeia of Tassili N'ajjer. *J. Ethnopharmacol.* **2006**, *105*, 358–367. [CrossRef]
- Gherboudj, O.; Benkiki, N.; Seguin, E.; Tillequin, F.; Kabouche, Z. Components of *Matricaria pubescens* from Algerian septentrional Sahara. *Chem. Nat. Comp.* **2012**, *48*, 470–471. [CrossRef]
- EMA/HMPC/55843/2011. European Union Herbal Monograph on *Matricaria recutita* L., Fflos. Available online: https://www.google.com.hk/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKewiKjluBrM_2AhWhyYsBHcoeDb8QFnoECAgQAQ&url=https%3A%2F%2Fwww.ema.europa.eu%2Fdocuments%2Fherbal-monograph%2Ffinal-european-union-herbal-monograph-matricaria-recutita-l-flos-first-version_en.pdf&usq=AOvVaw2PiRhwaUwdNzyCBuaJGM9u (accessed on 3 December 2021).
- Ferreira, E.B.; Vasques, C.I.; Jesus, C.A.C.; Reis, P.E.D. Topical effects of *Chamomilla recutita* in skin damage: A literature review. *Pharmacologyonline* **2015**, *3*, 123–130.
- Barnes, J.; Anderson, L.A.; Phillipson, J.D. Chamomile, German and Chamomile, Roman. In *Herbal Medicines*, 3rd ed.; Pharmaceutical Press: London, UK, 2007; pp. 152–158.
- Merecz-Sadowska, A.; Sitarek, P.; Kucharska, E.; Kowalczyk, T.; Zajdel, K.; Cegliński, T.; Zajdel, R. Antioxidant Properties of Plant-Derived Phenolic Compounds and Their Effect on Skin Fibroblast Cells. *Antioxidants* **2021**, *10*, 726. [CrossRef] [PubMed]
- Zillich, O.V.; Schweiggert-Weisz, U.; Eisner, P.; Kersch, M. Polyphenols as active ingredients for cosmetic products. *Int. J. Cosmet. Sci.* **2015**, *37*, 455–464. [CrossRef]
- Wagner, H.; Bladt, S. *Plant Drug Analysis: Thin Layer Chromatography Atlas*, 2nd ed.; Springer: Berlin/Heidelberg, Germany, 1996; p. 262.
- Tsivelika, N.; Sarrou, E.; Gusheva, K.; Pankou, C.; Koutsos, T.; Chatzopoulou, P.; Mavromatis, A. Phenotypic variation of wild Chamomile (*Matricaria recutita* L.) populations and their evaluation for medicinally important essential oil. *Biochem. Syst. Ecol.* **2018**, *80*, 21–28. [CrossRef]
- Saroglou, V.; Karioti, A.; Heilmann, J.; Kypriotakis, Z.; Skaltsa, H. Sesquiterpene Lactones from *Anthemis melanolepis*. *Helv. Chim. Acta* **2007**, *90*, 171–175. [CrossRef]
- Tsivelika, N.; Irakli, M.; Mavromatis, A.; Chatzopoulou, P.; Karioti, A. Phenolic Profile by HPLC-PDA-MS of Greek Chamomile Populations and Commercial Varieties and Their Antioxidant Activity. *Foods* **2021**, *10*, 2345. [CrossRef]
- Zdero, C.; Bohlmann, F. Sesquiterpene lactones and other terpenes from *Geigeria* species. *Phytochemistry* **1989**, *28*, 3105–3120. [CrossRef]
- Bhave, A.; Schulzová, V.; Mrnka, L.; Hajšlová, J. Influence of Harvest Date and Postharvest Treatment on Carotenoid and Flavonoid Composition in French Marigold Flowers. *J. Agric. Food Chem.* **2020**, *68*, 7880–7889. [CrossRef] [PubMed]
- Gong, Y.; Liu, X.; He, W.H.; Xu, H.G.; Yuan, F.; Gao, Y.X. Investigation into the antioxidant activity and chemical composition of alcoholic extracts from defatted marigold (*Tagetes erecta* L.) residue. *Fitoterapia* **2012**, *83*, 481–489. [CrossRef]
- Greger, H. Laubblatt-Flavonoide und Systematik bei *Matricaria* und *Tripleurospermum* (Asteraceae-Anthemideae). *Plant Syst. Evol.* **1975**, *124*, 35–55. [CrossRef]
- Karioti, A.; Bolognesi, L.; Vincieri, F.F.; Bilia, A.R. Analysis of the constituents of aqueous preparations of *Stachys recta* by HPLC-DAD and HPLC-ESI-MS. *J. Pharm Biomed. Anal.* **2010**, *53*, 15–23. [CrossRef] [PubMed]
- Barron, D.; Ibrahim, R.K. Ombuin 3-sulphate from *Flaveria chloraefolia*. *Phytochemistry* **1988**, *27*, 2362–2363. [CrossRef]

19. Govari, S.; Paloukopoulou, C.; Soulioti, A.; Tasi, G.; Karioti, A. *Origanum dictamnus*, an important Greek species with long ethnomedicinal history as anti-inflammatory agent, is rich in phenolic content as confirmed by HPLC-PDA-MS and phytochemical analyses. In Proceedings of the 19th International Congress of International Society for Ethnopharmacology (ISE), Dresden, Germany, 12–14 June 2019.
20. Avula, B.; Wang, Y.H.; Wang, M.; Avonto, C.; Zhao, J.; Smillie, T.J.; Rua, D.; Khan, I.A. Quantitative determination of phenolic compounds by UHPLC-UV-MS and use of partial least-square discriminant analysis to differentiate chemo-types of Chamomile/*Chrysanthemum* flower heads. *J. Pharm. Biomed. Anal.* **2014**, *88*, 278–288. [[CrossRef](#)] [[PubMed](#)]
21. Lin, L.Z.; Harnly, J.M. LC-PDA-ESI/MS Identification of the Phenolic Components of Three Compositae Spices: Chamomile, Tarragon, and Mexican Arnica. *Nat. Prod. Commun.* **2012**, *7*, 749–752. [[CrossRef](#)] [[PubMed](#)]
22. Avonto, C.; Rua, D.; Lasonkar, P.B.; Chittiboyina, A.G.; Khan, I.A. Identification of a compound isolated from German chamomile (*Matricaria chamomilla*) with dermal sensitization potential. *Toxicol. Appl. Pharm.* **2017**, *318*, 16–22. [[CrossRef](#)] [[PubMed](#)]
23. Sellappan, L.; Sanmugam, A.; Manoharan, S. Fabrication of dual layered biocompatible herbal biopatch from biological waste for skin—Tissue regenerative applications. *Int. J. Biol. Macromol.* **2021**, *183*, 1106–1118. [[CrossRef](#)]
24. Nematollahi, P.; Mohammadi Aref, N.; Zahmatkesh Meimandi, F.; Rozei, S.L.; Zareé, H.; Mirlohi, S.M.; Rafiee, S.; Mohsenikia, M.; Soleymani, A.; Ashkani-Esfahani, S.; et al. *Matricaria chamomilla* Extract Improves Diabetic Wound Healing in Rat Models. *Trauma Mon.* **2019**, *24*, e14318. [[CrossRef](#)]
25. Martins, M.D.; Marques, M.M.; Bussadori, S.K.; Martins, M.A.; Pavesi, V.C.; Mesquita-Ferrari, R.A.; Fernandes, K.P. Comparative analysis between *Chamomilla recutita* and corticosteroids on wound healing. An in vitro and in vivo study. *Phytother. Res.* **2009**, *23*, 274–278. [[CrossRef](#)] [[PubMed](#)]
26. Choi, S.; Youn, J.; Kim, K.; da Joo, H.; Shin, S.; Lee, J.; Lee, H.K.; An, I.S.; Kwon, S.; Youn, H.J.; et al. Apigenin inhibits UVA-induced cytotoxicity in vitro and prevents signs of skin aging in vivo. *Int. J. Mol. Med.* **2016**, *38*, 627–634. [[CrossRef](#)] [[PubMed](#)]
27. Cho, Y.H.; Bahuguna, A.; Kim, H.H.; Kim, D.I.; Kim, H.J.; Yu, J.M.; Jung, H.G.; Jang, J.Y.; Kwak, J.H.; Park, G.H.; et al. Potential effect of compounds isolated from *Coffea arabica* against UV-B induced skin damage by protecting fibroblast cells. *J. Photochem. Photobiol. B* **2017**, *174*, 323–332. [[CrossRef](#)] [[PubMed](#)]
28. Huang, C.Y.; Lin, Y.T.; Kuo, H.C.; Chiou, W.F.; Lee, M.H. Compounds isolated from *Eriobotrya deflexa* leaves protect against ultraviolet radiation B-induced photoaging in human fibroblasts. *J. Photochem. Photobiol. B* **2017**, *175*, 244–253. [[CrossRef](#)] [[PubMed](#)]
29. De Stefano, A.; Caporali, S.; Di Daniele, N.; Rovella, V.; Cardillo, C.; Schinzari, F.; Minieri, M.; Pieri, M.; Candi, E.; Bernardini, S.; et al. Anti-Inflammatory and Proliferative Properties of Luteolin-7-O-Glucoside. *Int. J. Mol. Sci.* **2021**, *22*, 1321. [[CrossRef](#)] [[PubMed](#)]
30. Aziz, N.; Kim, M.Y.; Cho, J.Y. Anti-inflammatory effects of luteolin: A review of in vitro, in vivo and in silico studies. *J. Ethnopharmacol.* **2018**, *225*, 342–358. [[CrossRef](#)] [[PubMed](#)]
31. Naveed, M.; Hejazi, V.; Abbas, M.; Kamboh, A.A.; Khan, G.J.; Shumzaid, M.; Ahmad, F.; Babazadeh, D.; FangFang, X.; Modarresi-Ghazani, F.; et al. Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomed. Pharm.* **2018**, *97*, 67–74. [[CrossRef](#)] [[PubMed](#)]
32. Ustuner, O.; Anlas, C.; Bakirel, T.; Ustun-Alkan, F.; Diren Sigirci, B.; Ak, S.; Akpulat, H.A.; Donmez, C.; Koca-Caliskan, U. In Vitro Evaluation of Antioxidant, Anti-Inflammatory, Antimicrobial and Wound Healing Potential of *Thymus Sipyleus* Boiss. Subsp. *Rosulans* (Borbas) Jalas. *Molecules* **2019**, *24*, 3353. [[CrossRef](#)] [[PubMed](#)]
33. Tian, D.; Yang, Y.; Yu, M.; Han, Z.Z.; Wei, M.; Zhang, H.W.; Jia, H.M.; Zou, Z.M. Anti-inflammatory chemical constituents of Flos *Chrysanthemi Indici* determined by UPLC-MS/MS integrated with network pharmacology. *Food Funct.* **2020**, *11*, 6340–6351. [[CrossRef](#)] [[PubMed](#)]
34. De Araújo, E.; Félix-Silva, J.; Xavier-Santos, J.B.; Fernandes, J.M.; Guerra, G.; de Araújo, A.A.; Araújo, D.; de Santis Ferreira, L.; da Silva, A.A., Jr.; Fernandes-Pedrosa, M.F.; et al. Local anti-inflammatory activity: Topical formulation containing *Kalanchoe brasiliensis* and *Kalanchoe pinnata* leaf aqueous extract. *Biomed. Pharmacother.* **2019**, *113*, 108721. [[CrossRef](#)]
35. Corrêa, W.R.; Serain, A.F.; Aranha Netto, L.; Marinho, J.V.N.; Arena, A.C.; de Santana Aquino, D.F.; Kuraoka-Oliveira, Â.M.; Júnior, A.J.; Bernal, L.P.T.; Kassuya, C.A.L.; et al. Anti-Inflammatory and Antioxidant Properties of the Extract, Tiliroside, and Patuletin 3-O- β -D-Glucopyranoside from *Pfaffia townsendii* (Amaranthaceae). *Evid. Based Complement. Altern. Med.* **2018**, *2018*, 6057579. [[CrossRef](#)] [[PubMed](#)]
36. Yasukawa, K.; Kasahara, Y. Effects of Flavonoids from French Marigold (Florets of *Tagetes patula* L.) on Acute Inflammation Model. *Int. J. Inflamm.* **2013**, *2013*, 309493. [[PubMed](#)]
37. Dilshara, M.G.; Lee, K.T.; Jayasooriya, R.G.; Kang, C.H.; Park, S.R.; Choi, Y.H.; Choi, I.W.; Hyun, J.W.; Chang, W.Y.; Kim, Y.S.; et al. Downregulation of NO and PGE2 in LPS-stimulated BV2 microglial cells by trans-isofेरulic acid via suppression of PI3K/Akt-dependent NF-kappaB and activation of Nrf2-mediated HO-1. *Int. Immunopharmacol.* **2014**, *18*, 203–211. [[CrossRef](#)] [[PubMed](#)]
38. Su, P.; Shi, Y.; Wang, J.; Shen, X.; Zhang, J. Anticancer agents derived from natural cinnamic acids. *J. Anticancer. Agents Med. Chem.* **2015**, *15*, 980–987. [[CrossRef](#)]
39. Do Carmo, M.A.V.; Granato, D.; Azevedo, L. Antioxidant/pro-oxidant and antiproliferative activities of phenolic-rich foods and extracts: A cell-based point of view. *Adv. Food Nutr. Res.* **2021**, *98*, 253–280.