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Understanding local Mediterranean diets: A multidisciplinary pharmacological and ethnobotanical approach

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Abstract

Epidemiological data indicate a beneficial effect of Mediterranean diets on human health, especially on the prevalence of cardiovascular disease. These observations are supported by recent intervention studies. However, very little is known about the current role of local Mediterranean food products, which are consumed on a less regular basis and their contribution to a healthy diet. The European consortium "Local Food-Nutraceuticals" collected 127 locally consumed wild or semi-wild plants in three Mediterranean countries, i.e. Greece, Italy, and Spain, in order to assess their ethnobotanical features as well as their biological activities. The project also includes a second line of research, the study of local conceptions about these food resources. All pharmacological assays were conducted with ethanolic extracts prepared from the dried plant material. The biological activities of the extracts were assessed with the following 12 different assays covering a broad range of mechanisms considered crucial in the pathology of chronic, aging-related diseases. Four antioxidant tests: DPPH scavenging, prevention of oxyhaemoglobin bleaching, prevention of lipid peroxidation (malondialdehyde formation), and protection from DNA damage (Comet assay); three enzyme inhibition tests: inhibition of xanthine oxidase, inhibition of myeloperoxidase-catalysed guaiacol oxidation as well as the inhibition of acetylcholine esterase; one test investigating the inhibition of cytokine-induced cell activation (including the extracts' potential cytotoxicity); one assay measuring the anti-proliferation potential; one test assessing the anti-diabetic activity (PPARγ) as well as one assay investigating the extracts' effect on mood disorder-related biochemical parameters (hSERT). Furthermore, the polyphenol content of all extracts was determined using the Folin–Ciocalteaus method. The assays revealed diverse biological effects for the tested extracts ranging from no activity to almost complete inhibition/activation. Moreover, the experimental matrix led to the identification of a sub-set of extracts, i.e. *Berberis vulgaris*, *Reichardia picroides*, *Scandix australis*, *Satureja montana*, *Thymus piperella*, *Lythrum salicaria* and *Vitis vinifera*, showing high activity in a broad range of assays. In summary, the in vitro observed modulations and effects exerted by extracts derived from local food plants suggest that these plants may contribute to the observed better aging of rural Mediterranean populations.

Keywords: Mediterranean diet(s); Local food; Ethnopharmacology; Food plants; Antioxidants; Polyphenols; Nutraceuticals

1. Introduction

Generally, the consumption of vegetables and fruits is considerably higher in Mediterranean countries than in North and Central Europe and the United States. Several of the main elements of these Mediterranean diets, like olive oil and red wine, have been studied in considerable detail from a pharmacological perspective and have increased our understanding of the beneficial effects of certain plant constituents [1]. Epidemiological studies also clearly demonstrated the impact of various elements of these Mediterranean traditions on longevity and health, including the prevention of diverse chronic, aging-related diseases. The effects are due to different classes of natural products in these foods and include essential unsaturated fatty acids [2], polyphenols as well as various vitamins (especially E and C) [3–5]. While an ethnopharmacological approach has been used

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very extensively in the search for novel leads for licensable medicines [6], the potential of similar approaches in the area of nutraceuticals has not been explored very systematically. Crucial pharmacological targets of such an approach centre on inflammatory pathways, targets relating to brain function, cardiovascular effects as well as antioxidant effects in a broad sense.

Traditionally, people all over the Mediterranean consume a diversity of plants, which are often gathered from the wild [7]. These hitherto neglected minor elements of Mediterranean diets are the focus of an EU-funded project "Local Food-Nutraceuticals" (LFN), which includes specialists in many fields like pharmacology, ethnobotany, pharmacognosy and nutrition. This project is unique in the framework of the EU's programmes in being the first with a very strong focus on ethnopharmacological and ethnobotanical questions and having a truly multidisciplinary socio-cultural and pharmacological approach.

On the one hand the Consortium intends to contribute to the development of leads for new nutraceuticals by characterizing plant extracts derived from plant species with potential health-beneficial effects traditionally used in rural communities of Southern Italy, Greece (Crete) and South-Eastern Spain. As importantly, the project wants to give new (cultural, social, and economic) value to local food products, which have been used for many generations and which now are at the brink of becoming forgotten. Therefore, benefit sharing agreements have been put in place in case that a commercial product be developed as a result of the research activities within this project (for details see Ref. [8]).

This paper provides a general overview on the outcome of the pharmacological evaluation of the plant extracts forming the basis for numerous (and in part ongoing) more detailed investigations of specific extracts.

2. Materials and methods

2.1. Ethnobotanical methods

2.1.1. Data collection and plant identification

Traditional knowledge regarding food plants was assessed using standard ethnobotanical tools, as for example participant observation [9,10]. Participant observation is a data collection technique that requires the researcher to be present during, involved in, and record, the daily activities of people. Moreover, classical cognitive anthropological series of queries were used in order to analyse and classify such knowledge [11]. All information was gathered through consented semi-structured interviews (ethnobotanical questionnaire) with elderly members of selected communities in Spain, Greece (Crete) and Italy, who still retain considerable knowledge about these practices. We used a similar methodology and a comparable approach in all the regions, we worked in which allows a comparison of the information collected.

During the first phase of the field research participants were asked to freely recall all non-cultivated food plants used both on a regular basis and those that were used in the past. Particular attention was given to processes, which may be of relevance for detoxifying traditionally used food species. For each of the identified species the local name, information about the plant parts used, the culinary process, taste perception and frequency of use were asked and tape-recorded. Participant observation techniques were also utilized to better understand the cultural implications of plant gathering, preparation and distribution of foods within the community.

2.1.2. Plant collection

Voucher specimens of all the reported food plants were collected and identified following the standard botanical works of each country. These specimens constitute the herbarium of the "Local Food-Nutraceuticals" project, which is located at the Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London. Nomenclature follows the current botanical principles and we used the current names for plant families as opposed to the historical ones (e.g. Asteraceae instead of Compositae). Plants were collected depending on the plant parts used either in spring (e.g. aerial parts) or autumn (e.g. fruits).

In addition to the voucher specimen, bulk samples of some species were obtained. Only the traditionally consumed plant parts (flowering tops, seeds, stems, leaves, petals, or leaf stalks) were collected and directly dried in the region of fieldwork for 24–48 h in the shadow at room temperature. About 500 g of dried plant material was collected in order to have an adequate amount of plant material from one season to be investigated in the laboratory.

2.1.3. Plant extraction

Extraction of plants from Spain and Greece took place in Murcia (Spain), whereas the Italian plants were extracted in London (UK). A standardised extraction protocol was used at both locations. Briefly, 50 g of air dried material were extracted by reflux with ethanol (90%) for 30 min where upon the plant material was pressed and the resultant liquids were pooled and cooled at room temperature before being filtered through filter paper Whatman No. 1 (Whatman, Maidstone, England). The extracts were then concentrated with a rotary evaporator (40 °C) to facilitate its further freeze-drying process. Finally, the extracts were freeze-dried at −50 °C and stored at −20 °C. Extracts were redissolved in ethanol or DMSO prior usage in the individual assays.

2.2. Pharmacological methods

2.2.1. Antioxidant assays

2.2.1.1. Measurement of FRSA in the DEPPD assay (DPPH). The Free Radical Scavenging Activity (FRSA) assay was carried out according to the method used by Pieroni et al. [7]. 10 mg of the crude extract were dissolved in 5 ml of

ethanol:water (4:1) and 1.5 ml of this solution was added to 1.5 ml of DPPH (1,1-diphenyl-2-picrylhydrazil) 0.1 mM dissolved in DMSO. Absorbance was read at 517 nm after 2 min. The FRSA was calculated as $[1-(A_i-A_j)/A_c]-100$, where A_i is the absorbance of 1.5 ml of the crude extract solution mixed with an equal volume of the DPPH solution; A_j the absorbance of 1.5 ml of the crude extract solution mixed with an equal volume of DMSO, and A_c the absorbance of a blank prepared mixing 1.5 ml of the DPPH with an equal volume of ethanol:water (4:1). Quercetin (a frequently occurring and well studied flavonoid) and trolox (water-soluble Vitamin E) were used as standard antioxidant. In addition, an extract of bulbs of *Leopoldia comosa* (*Muscari comosum*; *Liliaceae*) and an extract of the aerial parts of *Rhodiola rosea* (Crassulaceae) widely sold in health food stores because of its reported radical scavenging activity were used as controls.

2.2.1.2. Measurement of hypochlorous acid-induced oxyhaemoglobin bleaching (OxyHb). Haemoglobin was reduced and oxygen-loaded according to manufacturer's instructions, with slight modifications. Briefly, a Sephadex G-25 column was equilibrated with phosphate buffer saline (PBS; 20 mM; pH 7.0) containing EDTA (1 mM). Sodium dithionite (200 mg) was added to the column and drained into the gel by adding 2 ml of PBS. After dissolving 100 mg haemoglobin in 10 ml PBS, the haemoglobin solution was applied to the column and eluted. The reduced haemoglobin was saturated with oxygen and dialyzed against oxygen-saturated PBS without EDTA to eliminate excess dithionite and to achieve complete conversion to oxyhaemoglobin (OxyHb). The obtained OxyHb solution was stored at −20 °C. Concentration of HOCl was determined at 292 nm using a molar extinction coefficient of 142. Samples and OxyHb were added to 24- or 96-well plates and the bleaching reaction was started by adding a bolus of HOCl (400 μM). Change in absorbance was recorded at 405 nm using a microplate reader (Wallac Victor^2 1420 Multilabel Counter, Perkin-Elmer, Rodgau-Jügesheim, Germany). Luteolin and trolox were used as standard antioxidants.

2.2.1.3. Measurement of lipid peroxidation in mouse brain tissue (MDA). Brain tissue from 3-month-old NMRI mice was homogenized in ice-cold Tris–HCl (20 mM). Brain homogenates were incubated with 150 μM $FeCl_3$, 1 mM hydrogen peroxide (H_2O_2) and with or without extracts for 4 h at 37 °C in a shaking water bath. After incubation the homogenates were centrifuged at 3000 × *g* for 10 min. The supernatants were collected and tested for lipid peroxidation by measuring the concentration of malondialdehyde (MDA). The commercially available 'Lipid Peroxidation Assay Kit' (Calbiochem, Darmstadt, Germany) was utilized. The assay takes advantage of a special chromogenic reagent, which reacts specifically with MDA at 45 °C. Quercetin and trolox were used as standard antioxidant. All experiments were carried out in accordance with the local laws for animal welfare.

2.2.1.4. Measurement of plant extracts' protective ability against hydrogen peroxide-induced DNA damage (Comet). After pre-incubating the lymphocytes with 10 μg ml^{-1} plant extracts for 1 h, they were centrifuged and washed with Ca^{2+}- and Mg^{2+}-free PBS. Subsequently, 85 μl of cell suspension containing 2×10^4 lymphocytes was added to 0.5% low melting point agarose and spread on microscope slides pre-coated with 100 μl of 0.5% normal melting point agarose. After solidification on ice, the microscope slides were immersed in cold 25 μM H_2O_2 for 5 min and washed. Then, following a quick rinsing in cold water, the slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10 and 1% Triton X-100) for 1 h, rinsed, covered with 50 μl of endonuclease III (0.1 U per slide) and incubated for 45 min at 37 °C. Before electrophoresis was conducted at 0.74 V cm^{-1} for 30 min (current adjusted to 300 mA) the slides were washed and stored for 40 min at 4 °C. For visualization, slides were placed in distilled water for 5 min and stained with 2.5 μg ml^{-1} propidium iodide). For the detection of DNA damage a 20× objective was used (the final magnification was ×200) on an epifluorescence microscope (Olympus IX-50) equipped with appropriate filters. The microscope was linked to a computer through a CCD camera (i.CAM-hrM; sensor SONY ICX) for transporting images to the computer software and the image analysis system (Comet Plus from Theta System GmbH, Germany) was used for the quantification of DNA damage.

The percentage of DNA in the tail was automatically generated. At least two slides per subject and 50 randomly selected cells per slide were analysed. Each experiment was at least run twice. The protective effect of each plant extract on induced DNA damage is presented as the percentage of changes in an extent of oxidized pyrimidines in the DNA as compared to the extend of DNA damage induced by hydrogen peroxide alone. Quercetin was used as a standard antioxidant.

2.2.2. Enzyme inhibition assays

2.2.2.1. Inhibition of the xanthine oxidase (XO). The measurement of the xanthine oxidase inhibition was carried out using a modified photometric method based on Noro et al. [12], Owen and Johns [13] and Cimanga et al. [14].

2.2.2.2. Inhibition of acetylcholine esterase assay (AChE). The inhibition of acetylcholine esterase activity was determined with a photometric method based on the description of Ellman et al. [15].

2.2.2.3. Inhibition of myeloperoxidase-catalysed guaiacol oxidation (G-OH). Lyophilised human MPO (Calbiochem, Darmstadt, Germany) was reconstituted according to manufacturer's recommendation and aliquots were stored at −20 °C. Samples, guaiacol (5 mM in 20 mM phosphate buffer, pH 7.0) and MPO (0.2 μg ml^{-1}) were added to 24-well or 96-well plates and the reaction initiated by addition

of H_2O_2 (200 μM). Absorbance was read over time at 485 nm using a microplate reader (Wallace Victor^2 1420 Multilabel Counter, Perkin Elmer, Rodgau-Jügesheim, Germany). Again quercetin and trolox were used as standard antioxidants.

2.2.3. Anti-inflammatory (Griess-) assay [including cytotoxicity assessment (Viability)]

2.2.3.1. Inhibition of cytokine-induced cell activation (Nitrite). Anti-inflammatory activity of the plant extracts was evaluated by examining their ability to inhibit cytokine-stimulated, iNOS-dependent synthesis of nitric oxide. Murine brain microvascular endothelial cells (MBE), a gift from Dr. R. Auerbach (Madison, WI, USA), were cultured in 96-well plates in complete medium (DMEM + 10% FCS). The medium was replaced with 100 μl of DMEM supplemented with 2% FCS and containing cytokines TNFα (10 ng ml^{-1}; Suntory Pharmaceuticals, Osaka, Japan) and IL-1β (10 ng ml^{-1}; PeproTech INC, Rocky Hill, NJ, USA) together with the plant extracts at a concentration of 0.1 mg ml^{-1}. The medium of the control group contained cytokines and DMSO. After 24 h of incubation, nitrite concentration in the media was determined by a microplate assay. Briefly, 100 μl aliquots of the culture media were incubated with equal volumes of Griess reagent (1% sulfanilic acid/0.1% *N*-(1-naphtyl)ethylenediamine dihydrochloride (Sigma Chemicals Co., St Louis, MO, USA) in 2.5% H_3PO_4) at room temperature for 10 min. The absorbance at 545 nm was measured with a microplate reader (SpectraMax, Molecular Devices, Sunnyvale, CA, USA). Nitrite concentration was determined by using dilutions of sodium nitrite in medium as a standard. The nitrite levels of the control cells were regarded as 100%.

2.2.3.2. Cytotoxicity assessment (Viability). The cytotoxic activity of the plant extracts was determined by the MTT test. After incubation of the MBE cells with plant extracts (as described for the nitrite assay), the media were replaced with the fresh DMEM containing 2% FCS and MTT (0.5 mg ml^{-1}; Sigma Chemicals Co., St. Louis, MO, USA) and the cells incubated for an additional 4 h. Formazan crystals were dissolved in 100 μl of isopropanol containing 5 mM HCl and the absorbance measured with a plate reader at 562 nm. The absorbance of control cells (incubated with DMSO instead of the plant extract) was taken as 100% viability.

2.2.4. Anti-proliferation assay

2.2.4.1. Determination of endothelial cell proliferation (BrdU). Incorporation of 5-bromo-2′-deoxyuridine (BrdU) in place of thymidine is monitored as a parameter for DNA synthesis and cellular proliferation. Experiments were performed on unsynchronised human microvascular endothelial cells (HMEC-1) at a density of 3000 cells per well, cultured in media with 10% foetal calf serum (FCS) in the absence and presence of plant extract (10–100 μg ml^{-1}). After a 48 h incubation period, BrdU was added for 2 h and proliferation was measured by ELISA for BrdU (Roche, Mannheim, Germany).

2.2.5. Anti-diabetic assay

2.2.5.1. PPARγ binding assay (PPARγ). For 96 reactions 2 mg of SPA-YSi beads (Amersham Biosciences, Freiburg, Germany) were incubated with 80 μg of bacterially expressed and affinity purified GST-PPARγ protein in 200 μl of binding buffer (50 mM Tris pH 8, 50 mM KCl, 2 mM EDTA pH 8.0, 0.015% BSA fatty acid free and 15 mM DTT). Binding occurs at 4 °C in an over night incubation. The slurry is centrifuged at 10,000 rpm in an Eppendorf centrifuge for 1 min, the pellet washed with 1 ml binding buffer, beads pelleted by centrifugation at 10,000 rpm for 1 min and the pellet re-suspended in 2.5 ml binding buffer (PPARγ-coated SPA beads).

As tracer, any known H-3 or C-14 labelled, specific PPARγ agonist can serve (e.g. Rosiglitazone). For the competition binding reaction, in a 96-well OptiPlate (Packard/Perkin-Elmer, Rodgau-Jügesheim, Germany), the following components are added per well: 120 μl Binding Buffer, 25 μl tracer (400 nM, 800 dpm μl^{-1}), 5 μl test compounds (40× stock in 100% DMSO), 50 μl PPARγ-coated SPA beads. The plate has been incubated for 1 h at room temperature on a thermomixer, shaking at 800 rpm. The plate has been centrifuged at 3000 rpm for 20 min, the supernatant discarded and the plate placed upside down on a paper towel for 5 min to drain residual liquid. The plate was sealed and read using the TopCount (Packard/Perkin-Elmer, Rodgau-Jügesheim, Germany).

2.2.6. Mood disorder-related assay

2.2.6.1. Serotonin re-uptake inhibition (hSERT). The primary screening assay consisting in measurement of tritiated serotonin-uptake into hSERT expressing HEK-293 cells (non-exclusive license obtained from Emory University) was performed essentially as described in the literature [16]. Briefly, cells were seeded (50,000 per well) 24 h prior to assay in poly-D-lysine coated 96-well plates (BD, Franklin Lakes, NJ, USA). Immediately prior to assay the medium was exchanged for KRH (130 mM NaCl, 1.3 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 10 mM HEPES, pH 7.4) supplemented with ascorbic acid (100 mM) and pargyline (100 mM), and test fractions added as applicable. After 10 min tritiated serotonin (Amersham Biosciences, Freiburg, Germany) was added (final concentration 30 nM), and incubated at 37 °C. Unincorporated radiolabel was removed by washing. Following addition of Micro-Scint 20 the incorporated serotonin was quantified by liquid scintillation counting. We have demonstrated that uptake of tritiated serotonin into these cells can be blocked by known hSERT inhibitors (Imipramine, Fluoxetine, Desimipramine, Clomipramine), with the correct rank order of potency, although the assay appears to be less sensitive to inhibition by these agents than the fluorescent assay.

2.2.7. Determination of polyphenol content present in plant extracts

The polyphenolic content was determined according to Visioli et al. [17].

3. Results

In all, 127 plants (Table 1) belonging to 42 different families have been collected by the botanical groups in Greece (36 plants), Spain (66 plants), and Italy (22 plants). All plants presented are spermatophytes except three (p# 1, p# 120 and p# 124), which are mushrooms, respectively. The plant parts used as food in the local communities were extracted using a standardized protocol. Interestingly, the ethnobotanical assessment revealed that the investigated communities nutritionally utilize different parts of certain plants. Whereas, for example, young stems of *Vitis vinifera* are consumed in Spanish communities, the leaves of this plant are used in Cretan cuisine.

The consortium applied a set of primary assays focusing mainly on aging-related, degenerative diseases, such as CVD, cancer, diabetes type II, and cognitive decline. Also, several EC_{50} values have been determined for standard antioxidants to allow a comparison of the results with data from the literature: EC_{50} for quercetin = 9.3 μM (MDA), 6.9 μM (G-OH), 2.25 μM (Comet); EC_{50} for luteolin = 77 μM (OxyHb); EC_{50} for trolox (water-soluble Vitamin E) = 120 μM (MDA), 118 μM (GOH), 380 μM (OxyHb). All screening data obtained in the various cell-free and cell-based assays are represented by symbols (Table 2) highlighting a high (●), medium (◖), or low (◯) biological activity in the specific assay and as compared to the controls of the extract. Furthermore, a total activity score (TAS) was calculated for each extract in order to identify those plants with the most promising profile of biological effects. For this, following activity scores were assigned: ● = 1, ◖ = 0.5, ◯ = 0. The TAS calculation for an extract was accomplished by adding up the activity scores from the 12 bioassays (Table 3).

3.1. Antioxidant activity

None of the tested extracts showed high activity in all four antioxidant tests; however, 13 extracts (10%) demonstrated high activity in at least two assays. Similarly, 16 extracts (13%) showed low activity in all four assays. The samples showing high or low activity are from 11 to 13 different plant families, respectively. Consequently, this distribution does not allow for the identification of particular plant families with an above average number of highly active plant species. Furthermore, a substantial number of extracts (i.e. 36%) showed high activity in terms of cellular protection from lipid peroxidation, whereas only 3% of all samples were able to substantially attenuate H_2O_2-induced DNA damage.

3.2. Enzyme inhibition activity

Only two of the 127 screened extracts showed high activity in all three enzyme inhibition tests. These plants are *Berberis vulgaris* (p# 51) and *Vitis vinifera* (p# 126) originating from Spain and Greece, respectively. A high activity in at least two of the assays was found for 15 extracts (12%). Moreover, 27 extracts (21%) demonstrated low enzyme inhibition effects in all three tests. The taxa of the Lamiaceae are examples highlighting the potential of this family as enzyme inhibitors. Five of the six plants investigated (p# 91 to 96) showed high activity in at least two of the three assays. Only the extract prepared from *Origanum heraclioticum* (p# 93) displayed twice medium and once low enzyme inhibition activity. In contrast, four of the five tested Liliaceae extracts did not significantly affect MPO-catalysed guaiacol oxidation, or AChE and XO activity, whereas one extract (p# 99) demonstrated medium activity in only one of the assays. Moreover, 50% of the extracts prepared from fruits (total of 14 plants) showed low activity in all three tests.

3.3. Anti-inflammatory activity

The MTT assay was performed to identify false positive results due to decreased cell viability or disturbed metabolism. Almost 10% of the plant extracts had a cytotoxic/pro-apoptotic effect (assessed by the mainly mitochondria-dependent reduction of the MTT dye) in MBE cells. Cytokine-induced inflammatory response was efficiently suppressed by 12 of the non-toxic extracts. The amount of PPs present in these samples scattered between 0 and 367 mg per g extract, indicating no correlation between PP concentration and the observed anti-inflammatory activity.

3.4. Anti-proliferation activity

The extracts' effect on cell proliferation has been investigated in HMEC-1 by the BrdU incorporation assay. High inhibitory effects on cell proliferation have been found with 17 extracts belonging to 8 different plant families. The PP content of the positively scored extracts ranged from 11 to 285 mg per g extract, again suggesting that the detected activities are not necessarily linked to the polyphenol content of the respective plant extracts. The latter observation is supported by the data obtained for the samples from *Papaver rhoeas*. Whereas both extracts prepared from plants coming from Greece (p# 104) and Italy (p# 106) affect BrdU incorporation by more than 50% (i.e. show high activity), the PP content differs by more than 100% (i.e. 120 mg/g versus 286 mg/g, respectively).

3.5. Anti-diabetic activity

The modulation of PPARγ binding activity has been suggested as a promising target for the prevention and treatment

Table 1
Overview on the taxa of local Mediterranean food plants used (including plant parts), investigated in this study, their origin, and content of polyphenols (PP = amount of polyphenols in mg g^{-1} ethanolic extract)

Number	Plant name	Family	Parts used	Origin	PP
1	*Agaricus campestris* L. ex Fr.	Agaricaceae	Caps	Italy	10.2
2	*Aizoon hispanicum* L.	Aizoaceae	Aerial parts	Spain	46.7
3	*Amaranthus* cf. *graecizams* L.	Amaranthaceae	Leaves	Greece	25.9
4	*Apium nodiflorum* L.	Apiaceae (Umbelliferae)	Aerial parts	Italy	80.9
5	*Daucus carota* L.	Apiaceae	Leaves	Greece	243.7
6	*Eryngium campestre* L.	Apiaceae	Sprouts	Spain	55.1
7	*Foeniculum vulgare* Mill.	Apiaceae	Leaves	Greece	178.87
8	*Oenanthe pimpinelloides* L.	Apiaceae	Leaves	Greece	17.4
9	*Scandix australis* L.	Apiaceae	Aerial parts (leaves and tender stems)	Spain	191.55
10	*Scandix pecten-veneris* L.	Apiaceae	Leaves	Greece	130.7
11	*Tordylium apulum* L.	Apiaceae	Leaves	Greece	57.8
12	*Phoenix dactylifera* L.	Arecacea (Palmae)	Tender leaves (very tender leaf rachis)	Spain	11.0
13	*Achillea ageratum* L.	Asteraceae (Compositae)	Aerial part	Spain	214.7
14	*Anacyclus clavatus* Pers.	Asteraceae	New shoots, tender leaves	Spain	54.5
15	*Arctium minus* Bernh.	Asteraceae	Tender leaves	Spain	189.1
16	*Artemisia alba* Asso	Asteraceae	Aerial parts	Spain	229.9
17	*Carlina acaulis* L.	Asteraceae	Flower receptacles	Italy	59.1
18	*Chiliadenus glutinosus* Fourr.	Asteraceae	Aerial parts	Spain	253.3
19	*Chondrilla juncea* L.	Asteraceae	Leaves	Italy	117.8
20	*Chrysanthemum coronarium* L.	Asteraceae	Leaves	Greece	235.1
21	*Chrysanthemum segetum* L.	Asteraceae	Young leaves	Italy	25.8
22	*Cichorium intybus* L.	Asteraceae	Leaves	Greece	48.1
23	*Cichorium intybus* L.	Asteraceae	Leaves	Spain	107.0
24	*Cichorium intybus* L.	Asteraceae	Leaves (whorls)	Italy	64.8
25	*Cichorium spinosum* L.	Asteraceae	Leaves	Greece	40.1
26	*Crepis commutata* (Sprengel) W. Greuter	Asteraceae	Leaves	Greece	115.4
27	*Crepis vesicaria* L.	Asteraceae	Leaves	Greece	148.5
28	*Crepis vesicaria* L. subsp. *haenselerii*	Asteraceae	Tender leaves	Spain	69.1
29	*Cynara cardunculus* L. subsp. *cardunculus*	Asteraceae	Stems, flowers and receptacles	Italy	448.8
30	*Cynara cardunculus* L. var. *ferocissima* Lowe	Asteraceae	Rachis (leaf rachis)	Spain	214.4
31	*Helmintotheca echioides* (L.) Holub.	Asteraceae	Leaves	Greece	50.2
32	*Hypochoeris radicata* L.	Asteraceae	Leaves	Italy	215.9
33	*Lactuca viminea* L.	Asteraceae	Leaves	Italy	210.2
34	*Mantisalca salmantica* L. Briq. & Cavill.	Asteraceae	Tender leaves	Spain	118.8
35	*Onopordum macracanthum* Schousboe	Asteraceae	Tender leaves	Spain	89.0
36	*Helmintotheca echioides* (L.) J. Holub	Asteraceae	Aerial parts	Italy	242.1
37	*Reichardia picroides* Roth	Asteraceae	Leaves	Greece	318.5
38	*Reichardia picroides* Roth	Asteraceae	Leaves	Italy	335.1
39	*Rhagadiolus stellatus* (L.) Gaertner	Asteraceae	Tender leaves	Spain	24.4
40	*Scolymus hispanicus* L.	Asteraceae	Rachis (tender leaf rachis)	Spain	110.8
41	*Scorzonera cretica* Will.	Asteraceae	Leaves	Greece	151.3
42	*Silybum marianum* (L.) Gaertner	Asteraceae	Tender leaf rachis	Spain	88.0
43	*Sonchus asper* L.	Asteraceae	Tender leaves	Spain	108.3
44	*Sonchus oleraceus* L.	Asteraceae	Tender leaves	Spain	75.2
45	*Sonchus oleraceus* L.	Asteraceae	Leaves	Greece	122.0
46	*Sonchus oleraceus* L.	Asteraceae	Leaves	Italy	157.2
47	*Sonchus tenerrimus* L.	Asteraceae	Tender leaves	Spain	99.2
48	*Urospermum dalechampii* Scop. ex. F.W. Schmidt	Asteraceae	Tender leaves	Italy	424.6
49	*Urospermum delechampii* Scop. ex. F.W. Schmidt	Asteraceae	Aerial parts	Italy	80.6
50	*Urospermum picroides* (L.) F. Scop. ex. W. Schmidt.	Asteraceae	Leaves	Greece	245.9
51	*Berberis vulgaris* L. subsp. *seroi* O. Bolos & Vigo	Berberidaceae	Leaves and tender stems	Spain	271.2
52	*Berberis vulgaris* L. subsp. *seroi* O. Bolos & Vigo	Berberidaceae	Fruits	Spain	178.1
53	*Anchusa officinalis* L.	Boraginaceae	Leaves	Greece	154.5
54	*Borago officinalis* L.	Boraginaceae	Leaves	Greece	36.3
55	*Borago officinalis* L.	Boraginaceae	Leaf petioles	Spain	5.3
56	*Lithodora fruticosa* (L.) Griseb.	Boraginaceae	Aerial parts	Spain	161.2
57	*Symphytum bulbosum* Schimp.	Boraginaceae	Leaves	Greece	145.56
58	*Brassica oleracea* L. var. *capitata*	Brassicaceae (Cruciferae)	Stems	Greece	58.9
59	*Eruca sativa* Mill.	Brassicaceae	Leaves	Greece	58.4

Table 1 (*Continued*)

60	*Eruca vesicaria* Cav.	Brassicaceae	Leaves	Spain	311.7
61	*Erucaria hispanica* Druce	Brassicaceae	Leaves	Greece	123.2
62	*Hirschfeldia incana* (L.) Lagr.- Fossat	Brassicaceae	Leaves	Greece	63.6
63	*Hirschfeldia incana* (L.) Lagr.-Fossat	Brassicaceae	Stems	Greece	83.6
64	*Moricandia arvensis* (L.) DC.	Brassicaceae	Tender leaves	Spain	23.1
65	*Nasturtium officinale* R. Br.	Brassicaceae	Leaves	Spain	26.0
66	*Raphanus raphanistrum* L.	Brassicaceae	Leaves	Greece	40.4
67	*Sinapis alba* L.	Brassicaceae	Leaves	Greece	149.6
68	*Sinapis arvensis* L.	Brassicaceae	Aerial parts	Italy	208.2
69	*Sisymbrium crassifolium* Cav.	Brassicaceae	Sprouts	Spain	34.1
70	*Opuntia ficus-indica* (L.) Mill.	Cactaceae	Fruits	Spain	9.2
71	*Capparis spinosa* L. subsp. *spinosa*	Capparidaceae	Stems	Spain	63.71
72	*Capparis spinosa* L. subsp. *spinosa*	Capparidaceae	Flowers	Spain	10.84
73	*Capparis spinosa* L. subsp. *spinosa*	Capparidaceae	Fruits	Spain	52.0
74	*Silene vulgaris* (Moench) Garke subsp. *vulgaris*	Caryophyllaceae	Leaves	Italy	162.2
75	*Silene vulgaris* (Moench) Garke subsp. *vulgaris*	Caryophyllaceae	Leaves	Spain	119.4
76	*Beta vulgaris* L. subsp. *cicla* (L.) *Arcangeli*	Chenopodiaceae	Tender leaves	Spain	185.8
77	*Chenopodium bonus-henricus* L.	Chenopodiaceae	Leaves	Greece	59.3
78	*Merendera montana* Lange	Colchicaceae (Liliaceae)	Bulbs	Spain	206.7
79	*Fallopia convolvulus* (L.) A. Löve	Convulvulaceae	Leaves	Spain	118.2
80	*Bryonia dioica* Jacq.	Cucurbitaceae	Young shoots	Spain	49.7
81	*Cucurbita pepo* L.	Cucurbitaceae	Leaves	Greece	157.6
82	*Cyperus esculentus* L.	Cyperaceae	Tubers	Spain	22.5
83	*Arbutus unedo* L.	Ericaceae	Fruits	Spain	37.6
84	*Lotus edulis* L.	Fabaceae, s.str. (Leguminosae)	Young fruits	Italy	n.d.
85	*Lupinus albus* L.	Fabaceae, s.str.	Seeds	Spain	40.4
86	*Vicia faba* L.	Fabaceae, s.str.	Leaves	Greece	221.9
87	*Vicia faba* L.	Fabaceae, s.str.	Tender unripe fruits	Spain	76.3
88	*Quercus ilex* L. subsp. *Rotundifolia (Lam.) T. Morais* (Desf.) Samp.	Fagaceae	Fruits	Spain	102.5
89	*Globularia vulgaris* L.	Globulariaceae	Leaves	Spain	207.3
90	*Juglans regia* L.	Juglandaceae	Fruits	Spain	366.7
91	*Hissopus officinalis* subsp. *pilifer*	Lamiaceae (Labiatae)	Aerial parts	Spain	131.0
92	*Mentha aquatica* L.	Lamiaceae	Aerial parts	Spain	100.8
93	*Origanum heracleoticum* L.	Lamiaceae	Flowering tops	Italy	65.2
94	*Satureja montana* L.	Lamiaceae	Aerial parts	Spain	339.4
95	*Thymus piperella* L.	Lamiaceae	Aerial parts	Spain	119.4
96	*Thymus pulegioides* L.	Lamiaceae	Aerial parts	Italy	435.1
97	*Allium ampeloprasum* L.	Liliaceae, s.l. (Alliaceae)	Bulb	Spain	4.8
98	*Asparagus acutifolius* L.	Liliaceae, s.l. (Asparagaceae)	Young shoots	Italy	116.4
99	*Asparagus horridus* L.	Liliaceae, s.l. (Asparagaceae)	Young shoots	Spain	68.8
100	*Muscari spreitzehoferi* (Heldr.) Vierh.	Liliaceae, s.l. (Hyacinthaceae)	Bulbs	Greece	24.3
101	*Ornithogalum narbonense* L.	Liliaceae, s.l. (Hyacinthaceae)	Bulbs	Greece	32.8
102	*Lythrum salicaria* L.	Lythraceae	Aerial parts	Spain	628.7
103	*Ficus carica* L.	Moraceae	Fruits	Spain	1.3
104	*Papaver rhoeas* L.	Papaveraceae	Leaves	Greece	119.7
105	*Papaver rhoeas* L.	Papaveraceae	Aerial parts (leaves, tender stems and buds)	Italy	34.1
106	*Papaver rhoeas* L.	Papaveraceae	Aerial parts	Italy	285.8
107	*Roemeria hybrida* (L.) DC.	Papaveraceae	Tender leaves	Spain	83.9
108	*Pinus pinea* L.	Pinaceae	Seeds	Spain	197.2
109	*Plantago sempervirens* Crantz.	Plantaginaceae	Aerial parts	Spain	163.5
110	*Rumex thyrsyflorus* Fingerh.	Polygonaceae	Leaves	Greece	61.9
111	*Rumex pulcher* L.	Polygonaceae	Leaves	Greece	230.2
112	*Lysimachia ephemerum* L.	Primulaceae	Aerial parts	Spain	384.5
113	*Clematis vitalba* L.	Ranunculaceae	Young shoots	Italy	222.5
114	*Reseda alba* L.	Resedaceae	Aerial parts	Italy	186.3
115	*Ziziphus jujuba* Mill.	Rhamnaceae	Fruits	Spain	44.4
116	*Crataegus monogyna* Jacq.	Rosaceae	Fruits	Spain	70.4
117	*Prunus cerasifera* L. c.v. "*mayeras*"	Rosaceae	Fruits	Spain	79.3
118	*Pyrus rehderi c.v. "Peretas"*	Rosaceae	Fruits	Spain	126.4
119	*Rubus ulmifolius* Scott	Rosaceae	Sprouts	Spain	92.1
120	*Lactarius deliciosus* (L. ex Fr.) S.F. Gray	Russulaceae	Carpophore	Spain	30.9

Table 1 (*Continued*)

121	*Citrus limon* L. c.v. "Verna"	Rutaceae	Fruits	Spain	78.5
122	*Veronica beccabunga* L.	Scrophulariaceae	Aerial parts	Italy	n.d.
123	*Solanum nigrum* L.	Solanaceae	Leaves	Greece	11.3
124	*Terfezia arenaria* (Moris) Trappe	Terfeziaceae	Carpophore	Spain	13.6
125	*Celtis australis* L.	Ulmaceae	Fruits	Spain	0
126	*Vitis vinifera* L.	Vitaceae	Leaves	Greece	98.3
127	*Vitis vinifera* L.	Vitaceae	Tender shoots	Spain	235.9

Table 2
Definition of activity range (% of control) for the performed bioassays

Number	Assay	Activity			Concentration (mg extract ml^{-1})
		High (●)	Medium (◐)	Low (○)	
Antioxidant assays					
1	DPPH	≥50%	30%–49%	<30%	1.0
2	OxyHb	>50%	50%–25%	<25%	0.2
3	MDA	>75%	50%–75%	<50%	0.2
4	Comet	>40%	40%–20%	<20%	0.01
Enzyme inhibition assays					
5	XO	≥20%	10%–19%	<10%	0.1
6	AChE	≥20%	10%–19%	<10%	0.1
7	G-OH	>75%	75%–50%	<50%	0.2
Anti-inflammatory assay (including cytotoxicity assessment)					
8	Nitrite	<30%	30%–70%	>70%	0.1
9	Viability	>90%	90%–75%	<75%	0.1
Anti-proliferation assay					
10	BrdU	>50%	30%–50%	<30%	0.1
Anti-diabetic assay					
11	PPARγ	>75%	75%–30%	<30%	0.1
Mood disorder-related assay					
12	hSERT	>75%	75%–30%	<30%	0.1

of diabetes type II. A total number of 34 extracts has been identified to significantly affect PPARγ binding and confirmed to induce a luciferase reporter gene under the control of a PPARγ responsive element. These extracts belong to 13 different plant families and the PP content varied between 10 and 629 mg g^{-1} extract (mean: 185 mg g^{-1}). Interestingly, 4 of the 5 extracts prepared from plants belonging to the Liliaceae showed high or medium activity, whereas 5 of the 6 samples belonging to the Lamiaceae demonstrated only low modulatory effects on PPARγ binding activity.

3.6. Mood-disorder modulating activity

Less than 8% (i.e. a total of 10) of the extracts affected serotonin re-uptake by more than 75%, i.e. showed high activity. The PP content of these samples, belonging to 5 different plant families, ranged from 40 to 449 mg g^{-1} extract. Of note, both *Cynara cardunculus* samples, collected in Italy (p# 29) and Spain (p# 30), showed a high activity whereas their PP content differed by more than 100% (449 mg g^{-1} versus 214 mg g^{-1} extract, respectively).

4. Discussion

The term Mediterranean diet, as a model of a health-beneficial diet, has first been used by Ancel Keys [18]. The authors suggested a relationship between the dietary habits of populations of different geographical areas (e.g. Finland, Italy, Greece and USA) and the distribution of cardiovascular morbidity and mortality. Although differences in dietary traditions exist between the various Mediterranean populations [19], certain nutritional characteristics are common to all or most of the Mediterranean area, e.g. a high intake of bioactives, such as polyphenols (especially flavonoids) and unsaturated fatty acids (UFA) due to the regular consumption of large amounts of fruits, vegetables, and olive oil [20]. While the "classical" concept of the Mediterranean diet has been subject to numerous in vivo studies [21–23], the nutritional impact of locally grown and consumed, wild or semi-wild plants has largely been overlooked. Studying local plant foods is of particular interest as they often contain higher amounts of bioactive compounds than highly cultivated food plants [24], especially ones, which have been under cultivation for many generations. Therefore, the consortium "Local Food-Nutraceuticals" has undertaken a joint pharmacologi-

cal and ethnobotanical approach aiming

(i) at the evaluation of food plants, most of which with recorded additional health benefits, locally used in Mediterranean communities,
(ii) to characterize these plants with respect to their pharmacological effects, and
(iii) to contribute to the dissemination of knowledge about locally used food resources [8].

Table 3
Summary of primary screening data (TAS = total activity score; n.d. = not determined; ct = cytotoxic)

Numbers	DPPH	OxyHb	MDA	Comet	XO	AchE	G-OH	Viability	Nitrite	BrdU	PPARγ	hSERT	TAS
1	○	○	○	n.d.	○	○	●	n.d.	n.d.	ct	●	○	2
2	○	○	◖	n.d.	○	◖	○	◖	◖	○	◖	○	2.5
3	○	○	○	◖	○	●	○	●	●	○	○	○	3.5
4	◖	○	○	○	○	◖	○	○	ct	ct	◖	◖	2
5	○	◖	◖	○	●	○	◖	○	○	●	◖	○	4
6	○	○	◖	○	◖	○	●	●	●	●	○	○	5
7	◖	○	◖	○	○	○	○	○	◖	ct	●	○	2.5
8	○	○	●	○	○	●	●	●	●	n.d.	○	○	5
9	○	◖	○	◖	●	◖	●	●	○	◖	●	◖	6.5
10	○	◖	◖	○	○	○	◖	●	◖	●	●	○	5
11	○	●	○	○	◖	◖	◖	●	◖	◖	○	○	4.5
12	○	○	○	○	○	○	◖	●	○	●	○	○	2.5
13	○	○	●	n.d.	○	●	●	○	ct	n.d.	●	○	4
14	○	◖	●	○	○	◖	●	●	◖	○	○	○	4.5
15	○	○	●	○	○	○	●	○	ct	ct	●	○	3
16	◖	○	●	○	○	◖	●	●	●	n.d.	○	◖	5.5
17	○	○	◖	○	●	◖	◖	●	○	○	○	◖	4
18	◖	○	●	○	●	◖	●	◖	●	n.d.	○	○	5.5
19	◖	○	◖	○	○	○	○	◖	○	○	●	○	2.5
20	◖	○	●	○	○	○	◖	●	●	●	○	○	5
21	○	○	◖	n.d.	n.d.	●	●	n.d.	n.d.	n.d.	●	○	3.5
22	◖	○	◖	○	○	◖	●	●	●	n.d.	○	○	4.5
23	○	○	●	◖	○	◖	◖	○	○	○	◖	○	3
24	◖	○	○	○	○	○	○	◖	●	◖	●	◖	4
25	◖	○	○	n.d.	○	○	○	●	◖	n.d.	○	○	2
26	○	◖	◖	○	○	○	◖	●	◖	●	○	◖	4.5
27	◖	○	◖	○	○	◖	◖	●	○	◖	○	●	4.5
28	○	○	◖	●	○	○	○	●	◖	○	○	○	3
29	○	○	○	○	●	◖	●	○	ct	ct	◖	●	4
30	○	○	◖	n.d.	◖	○	●	n.d.	n.d.	n.d.	○	●	3
31	●	○	◖	○	○	○	○	●	◖	○	○	○	3

Table 3 (*Continued*)

32	○	○	●	○	○	○	○	◖	●	◖	◖	●	4.5
33	○	○	●	○	○	◖	○	◖	◖	◖	●	○	4
34	○	◖	●	◖	◖	◖	◖	●	○	●	○	◖	6
35	○	◖	●	○	●	◖	●	●	●	ct	○	○	6
36	◖	○	●	○	◖	○	○	●	◖	○	●	○	4.5
37	○	●	●	○	●	◖	●	●	○	●	●	○	7.5
38	◖	○	○	○	●	◖	●	●	◖	○	●	●	6.5
39	○	○	●	○	○	○	●	●	◖	○	○	○	3.5
40	○	◖	●	◖	○	○	◖	●	◖	◖	●	○	5.5
41	◖	◖	●	n.d.	○	○	●	●	◖	○	○	○	4.5
42	◖	○	○	○	◖	◖	○	○	○	◖	◖	○	2.5
43	○	○	◖	○	○	◖	○	●	○	○	●	○	3
44	○	○	◖	○	○	◖	○	●	○	◖	○	◖	3
45	◖	○	○	◖	◖	n.d.	○	●	○	◖	○	◖	3.5
46	○	○	◖	○	n.d.	○	○	◖	◖	●	●	○	3.5
47	○	○	○	○	○	◖	○	○	○	○	●	○	1.5
48	◖	○	○	○	○	○	●	○	○	◖	●	○	3
49	○	◖	●	○	●	○	●	○	○	○	●	○	4.5
50	○	◖	●	○	○	◖	●	●	●	○	○	○	5
51	●	◖	●	●	●	●	●	●	◖	○	●	○	9
52	●	○	◖	○	○	○	●	●	◖	n.d.	●	○	5
53	○	◖	●	○	○	◖	●	●	◖	○	○	○	4.5
54	○	○	○	○	○	◖	○	●	◖	n.d.	○	○	2
55	◖	○	●	○	○	○	◖	●	◖	○	○	○	3.5
56	○	○	●	○	○	○	◖	○	ct	n.d.	◖	○	2
57	○	●	●	○	○	○	●	●	◖	○	○	○	4.5
58	●	○	◖	○	○	◖	○	◖	○	○	○	●	3.5
59	◖	◖	◖	◖	○	◖	○	●	○	●	○	◖	5
60	◖	○	○	n.d.	○	○	○	○	○	○	○	○	0.5
61	◖	○	○	○	○	○	○	●	◖	◖	◖	◖	3.5
62	◖	○	●	○	○	◖	○	●	○	●	○	◖	4.5
63	○	○	○	n.d.	○	○	○	●	●	n.d.	○	◖	2.5
64	○	○	○	n.d.	○	○	○	●	◖	◖	○	○	2
65	○	○	○	n.d.	○	○	○	◖	◖	○	○	○	1

The pharmacological effect of polyphenols, a diverse class of natural products suggested to be the key bioactives present in plant foods, are attributed to their antioxidant (i.e. free radical and reactive oxygen species (ROS) scavenging activity), indirect antioxidant (e.g. enzyme inhibition), anti-inflammatory as well as gene expression-modifying effects [25–29]. Elevated levels of free radicals and ROS lead to the occurrence of oxidative stress, a hallmark of most ageing-related degenerative diseases. Deleterious modifications of fatty acids and proteins, due to elevated oxidative stress, are

Table 3 (*Continued*)

66	○	◖	○	○	○	◖	○	●	○	●	●	●	5
67	◖	○	○	○	○	◖	○	○	○	◖	●	◖	3
68	○	◖	◖	○	○	◖	○	◖	○	◖	●	◖	4
69	◖	○	○	○	○	◖	◖	●	◖	○	○	○	3
70	○	○	○	○	○	○	○	●	○	○	○	○	1
71	◖	○	◖	◖	○	○	◖	●	○	○	○	○	3
72	○	○	○	○	○	◖	○	◖	○	○	○	○	1
73	◖	◖	○	○	○	◖	○	◖	○	○	○	○	2
74	○	○	○	○	○	◖	○	◖	◖	◖	◖	○	2.5
75	○	○	◖	◖	○	◖	◖	●	◖	○	●	◖	5
76	○	○	○	○	○	○	○	◖	○	○	○	○	0.5
77	○	○	○	○	○	◖	◖	●	◖	●	◖	○	4
78	○	○	○	○	●	n.d.	○	●	●	n.d.	○	○	3
79	○	◖	●	●	○	◖	●	○	○	◖	●	○	5.5
80	○	○	●	○	○	◖	○	●	○	◖	○	◖	3.5
81	◖	◖	●	◖	○	○	○	●	○	○	○	◖	4
82	○	○	○	n.d.	○	○	○	●	◖	○	○	○	1.5
83	●	○	n.d.	◖	◖	○	●	◖	○	◖	○	○	4
84	◖	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5
85	○	○	○	◖	○	○	○	●	◖	○	○	○	2
86	○	◖	○	n.d.	●	◖	◖	●	●	n.d.	○	●	5.5
87	○	●	●	◖	○	◖	●	●	○	○	○	○	5
88	○	○	●	●	◖	○	●	●	○	○	○	◖	5
89	○	○	●	○	○	◖	●	●	◖	n.d.	○	○	4
90	○	◖	◖	○	○	○	●	●	◖	n.d.	●	◖	5
91	○	○	●	○	●	●	◖	●	○	n.d.	○	◖	5
92	○	○	●	○	◖	●	◖	●	◖	n.d.	◖	○	5
93	○	●	●	○	◖	◖	○	●	○	●	○	○	5
94	○	◖	●	○	●	○	●	●	◖	n.d.	○	●	6
95	◖	●	●	○	●	◖	●	●	◖	◖	○	○	7
96	○	◖	○	○	◖	◖	●	◖	◖	◖	○	○	4
97	○	○	○	○	○	○	○	◖	○	○	◖	○	1
98	○	◖	○	○	○	○	○	○	ct	○	●	●	2.5
99	○	○	○	○	○	◖	○	●	◖	◖	●	◖	4
100	○	○	○	n.d.	○	○	○	○	ct	●	●	○	0.5
101	○	◖	○	○	○	○	○	○	ct	n.d.	○	○	0.5
102	◖	●	●	○	○	◖	●	●	◖	n.d.	●	○	6.5
103	○	○	○	○	○	○	○	●	○	○	○	○	1
104	◖	○	○	○	○	●	○	●	○	●	◖	◖	4.5

Table 3 (*Continued*)

105	○	○	○	n.d.	●	◖	●	●	◖	○	n.d.	n.d.	4
106	○	◖	○	○	○	●	●	○	◖	●	●	○	5
107	○	○	○	○	○	◖	○	●	◖	○	○	○	2
108	◖	◖	●	◖	○	○	●	○	○	○	●	○	4.5
109	○	○	●	○	○	◖	●	●	○	n.d.	○	○	3.5
110	●	◖	●	○	○	◖	●	●	○	◖	○	○	5.5
111	○	○	●	n.d.	○	◖	◖	n.d.	n.d.	◖	○	◖	3
112	◖	●	●	○	○	○	●	○	ct	n.d.	○	○	3.5
113	○	n.d.	◖	○	○	◖	●	◖	◖	◖	●	◖	5
114	○	◖	●	○	○	◖	○	◖	○	◖	◖	◖	4
115	●	○	○	○	○	○	○	●	○	○	○	○	2
116	○	○	◖	○	○	○	◖	●	◖	n.d.	◖	○	3
117	●	○	○	○	○	○	○	○	○	○	○	◖	1.5
118	◖	○	○	○	○	○	○	○	○	○	○	○	0.5
119	●	◖	●	○	◖	○	●	●	◖	○	○	○	5.5
120	○	○	○	○	○	◖	○	●	◖	○	○	○	2
121	●	○	◖	○	○	○	○	●	○	○	○	○	2.5
122	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
123	○	◖	○	○	○	●	○	●	●	n.d.	◖	○	4
124	○	○	○	○	○	○	○	◖	◖	○	○	○	1
125	○	○	○	○	○	○	○	●	◖	n.d.	○	◖	2
126	●	◖	●	○	●	●	●	●	○	n.d.	○	○	6.5
127	◖	○	●	○	◖	◖	●	○	○	◖	○	○	4

For details, see Table 2.

known to occur in patients suffering from cardiovascular and neurodegenerative diseases, respectively [30–32]. Polyphenols have been repeatedly shown to attenuate the formation of oxidative stress markers in various cell-free, cell-based as well as in animal and, though to a lesser degree, human studies [33–38]. Surprisingly, in this study none of the primary assays revealed a clear correlation between the observed biological activity and the PP content, suggesting that the amount of PPs is not necessarily a predictive indicator for the potential antioxidant activity. This observation, however, is in contrast to recent studies showing good correlations between total PPs and focusing specifically on the total antioxidant activity [39,40]. The ethanol-based extraction procedure employed in the present study aimed to optimise the amount of lipophilic bioactives in the freeze-dried extracts. The authors, however, are aware that highly lipophilic compounds will not be extracted with this procedure. Also, the data for the PP content suggest that substantial amounts of non-phenolic constituents, such as bioactive terpenes and alkaloids, fatty acids or vitamins, are present in the extracts. Hence, since we used 'crude' extracts, the low correlation between the total PP content and the observed biological activities in fact provides interesting leads for further nutritional-pharmacological studies. Therefore, extracts showing high activity despite a low PP content are of considerable interest for subsequent studies.

Taken together, a number of plant species, i.e. *Berberis vulgaris* (TAS: 9; p# 51; Berberidaceae), *Reichardia picroides* (TAS: 7.5 and 6.5; p# 37 and 38; Asteraceae), *Scandix australis* (TAS: 6.5; p# 9; Apiaceae), *Satureja montana* (TAS: 6; p# 94; Lamiaceae), *Thymus piperella* (TAS: 7; p# 95; Lamiaceae), *Lythrum salicaria* (TAS: 6.5; p# 102; Lythraceae), and *Vitis vinifera* (*TAS:* 6.5; *p#* 126; *Vitaceae*), with very promising pharmacological profiles have been identified based on a TAS $\geq$ 6. Only a few number of studies investigated the biological activities of such wild gathered or semi-cultivated plants [2,24,41,42] and species, such as *Reichardia picroides*, *Scandix australis* or *Lythrum salicaria* are largely unknown to the broader scientific medical and nutritional science community. Although the primary screen-

ing has only relied on in vitro assays, the detected broad activity profile, covering antioxidant, enzyme inhibition, anti-inflammatory, anti-proliferation, anti-diabetic as well as mood disorder-attenuating effects, make these plants very promising candidates for more detailed pharmacological in vitro as well as in vivo studies. Moreover, the detailed assessment of local food habits in the Mediterranean sheds light on a largely overlooked aspect of the Mediterranean diet, and furthermore helps not only to preserve but also to disseminate local traditional knowledge regarding the nutritional suitability of wild gathered and semi-cultivated fruits and vegetables. This study is based on a few selected regions in Southern Europe and many other variants of the Mediterranean diet (from such diverse countries like Portugal, France, Albania, Cyprus, Turkey, the Arabic Mediterranean countries, Lebanon, and Israel) remain undocumented and unstudied from an ethnobotanical and pharmacological perspective.

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Appendix A

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