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Phytochemical composition and bioactivity of edible *Taraxacum officinale*: potential as an ingredient in brain health-oriented functional foods

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Taraxacum officinale (dandelion) is a widely recognised medicinal plant that is entirely edible and nutritionally rich. Traditionally consumed raw and cooked, it is used in cuisine and in food industry as a healthy ingredient, thanks to its high content of bioactive compounds. For centuries, dandelion has been valued in folk medicine for its choleretic, diuretic, antioxidant, anti-inflammatory, anti-tumour, and hepatoprotective effects. While the biological activities of Taraxacum species have been extensively studied, the specific role of T. officinale in modulating neuroinflammation remains underexplored. This study investigated the chemical composition and neuroprotective potential of hydroalcoholic extracts from dandelion leaves (Dan L) and roots (Dan R), derived from three ecotypes: Land Spontaneous (LSE), Mountain Spontaneous (MSE), and Organically grown (OE). In particular, HPLC-DAD analysis revealed that the MSE ecotype contained the highest levels of key polyphenols, including chicoric, caftaric, chlorogenic, and caffeic acids. Notably, MSE extracts of both Dan L and Dan R mitigated the cytotoxic effects of H₂O₂induced oxidative stress in HypoE22 hypothalamic cells without causing toxicity. Ex vivo, these extracts modulated neuroinflammatory markers in lipopolysaccharide-treated mouse specimens (prefrontal cortex and hypothalamus) by downregulating TNF-α and NOS-2 and upregulating BDNF expression. These findings suggest that dandelion, especially the mountain ecotype, offers significant antioxidant and neuroprotective benefits. Taraxacum officinale thus emerges not only as a traditional edible plant but also as a functional candidate and polyphenol source for nutraceutical applications targeting neurodegeneration and cognitive decline.

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1. Introduction

Dandelion (*Taraxacum officinale*), a member of the Asteraceae (Compositae) family, is a perennial plant that comprises over 3000 species. This herbaceous plant grows anywhere worldwide; it is widely distributed in the northern hemisphere, particularly in Italy. The plant morphology consists of roots, leaves, and flowers. Generally, dandelion leaves are simple or lobed and form a basal rosette above the central taproot. The flower heads, when present, are characterised by a yellow colour due to the presence of carotenoids. These mature into

spherical seed heads containing many single-seeded fruits called achenes.

Dandelion and its derivates are widely used in the human diet as culinary raw material (*e.g.* salad, soup, and drinks), additive in the food industry^{4–6} or as food supplements consumed in various formulations, including ancient remedies like decoctions and infusions, which have been maintained over time. It is an entirely edible plant, with both its leaves and roots being consumable. It can be used for various applications on its own, as a whole plant or its single parts, or in combination with other herbs.⁷ For that reason, dandelion could be considered a phytoalimurgic species, a wild edible plant which represents a valuable yet underutilized component of traditional food systems and biodiversity. Their adaptability to marginal environments and their richness in bioactive compounds have prompted a recent interest, opening research avenues that shed light on their pharmacological properties and health benefits.

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In Italy, as well as in Europe (European Commission)8 and the United States, the dandelion has been used as food and remedy to help a wide range of symptoms of gastrointestinal diseases due to its hepatoprotective, cholagogue, and eupeptic properties.⁹⁻¹¹ For this reason, dandelion preparations are included in the Herbal Pharmacopoeia and have been deemed safe by the US Food and Drug Administration (FDA) for their dietary use. 12,13

Taraxacum officinale is a herbaceous species widely distributed in heterogeneous environments, from lowland to mountainous areas. A previous analysis, 14 conducted using nuclear magnetic resonance (NMR) spectroscopy, showed significant chemical diversity between three dandelion ecotypes: Land Spontaneous (LSE), Mountain Spontaneous (MSE), and Organically grown (OE). These differences are associated with variability in secondary metabolite profiles, suggesting specific biochemical adaptation to different environmental conditions. Indeed, it has been shown that spontaneous or less selected ecotypes are generally richer in bioactive phytochemical compounds than their commercial counterparts. This higher richness is attributed to the need to respond to environmental pressures by accumulating secondary metabolites. 15 As a result, they can be recognised as excellent sources of healthpromoting compounds, particularly due to their high polyphenol content and associated antioxidant properties.

Previous biological studies conducted on dandelion root and leaf extracts have demonstrated antioxidant and antiinflammatory activity, undoubtedly due to the diverse phytoconstituents present in it, among them phenolic compounds (phenolic acids, flavonoids, and coumarins)¹⁶ and polysaccharides.¹⁷ For these effects, dandelion can be re-evaluated to ameliorate or prevent particular diseases in which oxidative stress and the inflammatory cascade are essential contributors to their pathogenesis and development. Literature reports plenty of studies on dandelion phytochemical profile and its effects on chronic gastrointestinal disorders, 18 rheumatic disease, and skeletal muscle inflammation, 19 to help cancer treatment, 20,21 and metabolic syndrome and its co-existing diseases like diabetes, in which the diet has a main role in prevention and therapy support. 22-25

Furthermore, according to the ClinicalTrials.gov database (https://www.clinicaltrials.gov) supported by the U.S. National Library of Medicine, a clinical study (NCT00442091) was also carried out evaluating the beneficial effect of dandelion juice on dyshidrotic hand eczema.²⁶

However, the neurological properties of this plant have not been sufficiently investigated.²⁷ Frequently, studies refer to other species of the Taraxacum genus like Taraxacum coreanum.28-31 Taraxacum officinale has remained highly unexplored scientifically for its neuroprotective activity. Still, some phytochemicals like chicoric acid (also known as dicaffeyltartaric acid), characteristic of the Asteraceae family, showed an inhibitory effect on neuroinflammation, synaptic and cognitive impairment at the basis of neurodegenerative disorders (e.g. Alzheimer's amyloidogenesis).32,33 The literature lacks studies concerning both phytochemical characterisation and neuroprotective activity, assessed through ex vivo assays on brain tissue and the expression of various genes under an inflammatory stimulus.

The present study investigated the phytochemical and neuroprotective potential of hydroalcoholic extracts of both T. officinale leaves and roots. The phenolic profile of the hydroalcoholic extracts of three different ecotypes (LSE, OE, and MSE) of both dandelion leaves (Dan L) and root (Dan R) was analysed by application of targeted HPLC-DAD, considering that the variety of bioactive compounds and their concentration greatly depends on the species, the season and time of harvesting, pedoclimatic factors, as well as preparation method of plant-derived products. 10,25

Biological assays assessed the biocompatibility of the most promising hydroalcoholic extracts (MSE) using rat hypothalamic cells (HypoE22) even under conditions of tissue damage (H_2O_2) . Furthermore, the gene expression of inflammatory and neurotrophic markers—TNF-α, NOS-2, and BDNF—was investigated in prefrontal cortex and hypothalamus specimens (C57/ BL6 mice) after treatment with the hydroalcoholic plant extract.

This workflow, which links targeted phytochemical profiling with direct functional evaluation in ex vivo brain tissue, could bridge a gap in nutritional neuroscience by associating the diversity of naturally occurring phytochemicals with neuroprotective effects. This method allows for the discovery of plant-based nutraceutical resources that promote brain health, associated with specific environmental and pedoclimatic backgrounds.

2. Materials and methods

Plant material

Three ecotypes of dandelion were cultivated in Isola del Liri (150 m MSL, 41° 41′ N, 13° 34′ E) and Collepardo (800 m MSL, 41° 46′ N, 13° 22′ E), Lazio, Italy. Land Spontaneous Ecotype (LSE, 150 m) and Mountain Spontaneous Ecotype (MSE, 800 m) were grown without treatment. In contrast, the Organic Ecotype (OE, 150 m) was cultivated by removing other vegetable species from the sandy clay loam soil. The previous study described the environmental growing conditions in Collepardo and Isola del Liri. 14 Roots and leaves were collected in October 2022, before spring blossoming. To perform extraction procedures, the matrices were freeze-dried (Buchi Lyovapor L-200) following a previously reported experimental procedure.¹⁴

2.2 Chemicals

Ethanol (HPLC-grade) and distilled water were obtained from Carlo Erba Reagenti (Milan, Italy). Double-distilled water was purchased using a Millipore Milli-Q Plus water treatment system (Millipore Bedford Corp., Bedford, MA, USA). Formic acid and Methanol (HPLC-grade) were purchased from Aldrich-Fluka-Sigma S.r.l. (Milan, Italy). All the analytical standards listed below were purchased from Sigma-Aldrich (Milan, Italy) with a purity of $\geq 95\%$ and stored at -20 °C. The stan**Food & Function**

dards of phenolic compounds used were: gallic acid, 3-hydroxytyrosol, caftaric acid, (+)-catechin, gentisic acid, 4-hydroxybenzoic acid, loganic acid, chlorogenic acid, vanillic acid, caffeic acid, (-)-epicatechin, syringic acid, syringaldehyde, chicoric acid, p-coumaric acid, t-ferulic acid, benzoic acid, hyperoside, rutin, resveratrol, t-cinnamic acid, quercetin, naringenin, hesperidin, kaempferol, carvacrol, thymol, flavone, and 3-hydroxyflavone. Dimethyl sulfoxide (DMSO) was acquired from Merck (Milan, Italy). TRI reagent was obtained from Sigma-Aldrich (St Louis, MO, USA). All murine genes detailed below, PCR primers, TaqMan probes, and High-Capacity cDNA Reverse Transcription Kit were sourced from Thermo Fisher Scientific (Waltman, Massachusetts, USA). The genes included: GADPH, TNF-α, NOS-2, and BDNF.

2.3 Extraction procedure for HPLC-DAD analysis

Extractions for HPLC-DAD analysis were carried out by optimising a green extraction protocol.³⁴ In particular, 100 mg of freeze-dried samples were added with 2 mL of an H₂O/EtOH (30:70% v/v) mixture with a 1:20 ratio between the plant matrix and the extraction solvent. The obtained system was sonicated (thermostat ultrasonic bath ARGOLAB DU-100 (Rome, Italy)) at 30 °C for 10 min and then centrifuged (Eppendorf Centrifuge 5430 R (Milan, Italy)) for 5 min (30 °C, 7745g). The hydroalcoholic extract was separated, and the residual pellet was extracted twice using the same protocol previously described to ensure complete extraction of phenolic compounds. Collected supernatants were pooled and filtered through 0.45 µm syringe filters. The extracts were stored at 4 °C until HPLC-DAD analysis. Each sample was prepared and analysed in triplicate.

2.4 HPLC-DAD analysis

The dandelion hydroalcoholic extracts were analysed for polyphenols quantitative determination using a reversed-phase HPLC-DAD in gradient elution mode as previously described.³⁵ The HPLC analyses were conducted using an instrument (Jasco, Tokyo, Japan) equipped with PU-2080 PLUS chromatographic pumps, a DG-2080-54-line degasser, a mix-2080-32 mixer, UV, diode array (DAD) and detectors, an AS-2057 PLUS autosampler, and a CO-2060 PLUS column thermostat.

The separation of polyphenols was performed by employing an Infinity lab Poroshell 120-SB reverse phase column (C18, 150 × 4.6 mm i.d., 2.7 μm; Agilent, Santa Clara, CA, USA) as stationary phase and using a binary mobile phase: methanol for HPLC with 0.1% v/v formic acid (B) and water both with 0.1% v/v formic acid (A). The analysis was conducted with 60 min of the chromatographic run at flow rate of 0.6 mL min⁻¹. It started with 97% water with 0.1% v/v formic acid (phase A) and 3% methanol with 0.1% v/v formic acid (phase B), and the solvent change in percentage ratio between the two phases is reported in Table S1. Column temperature was set at 30 °C.

Quantitative determination of phenolic compounds was performed via a DAD detector. Quantification was done through 7-point calibration curves, with linearity coefficients

 $(R^2) > 0.999$, in the 2-140 µg mL⁻¹ concentration range. All assayed analyte detection limits were lower than 1 µg mL⁻¹. The area under the curve from HPLC chromatograms was used to quantify the analyte concentrations in each dandelion hydroalcoholic extract. Integration was performed ChromNAV2 Chromatography software.

Two-way ANOVA, followed by Tukey's multiple comparisons test, was applied to underline, among ecotypes, significant differences (p < 0.0001) for each metabolite according to the same plant section: (a) vs. MSE, (b) vs. OE. GraphPad Prism 8.0.2 software was used for this purpose.

2.5 Extraction procedure for biological assays

Biological activity was assessed only for dandelion MSE based on the phytochemical profile obtained by chromatographic analysis. The same hydroalcoholic extract, analysed by HPLC-DAD, was dried under nitrogen flow and stored at 4 °C until use in biological assays.

The dried extract was dissolved in a DMSO/Milli-Q water mixture to ensure the complete solubilisation of phenolic compounds extracted from plant matrices and to treat cells or tissues. As previously reported, the total volume of solvent was 6 mL to follow the matrix/solvent ratio; thus, the HPLC-DAD extract concentration was 16 mg mL⁻¹.

To avoid toxicity exerted by dandelion extract, the highest tested phytochemical concentration was 200 µg mL⁻¹, and the DMSO concentration was 1%. The stock solution to treat cells or incubate tissues was obtained by adding a DMSO/H2O mixture at 50:50% v/v to both Dan R and Dan L dried hydroalcoholic extracts (MSE). The obtained system was sonicated by a thermostat ultrasonic bath at room temperature for 10 min and then filtered through 0.22 µm syringe filters to get a sterile solution for biological safety. As reported in Table 1, the stock solution was used to make the subsequent dilution to perform in vitro and ex vivo assays.

2.5 In vitro studies

2.5.1 Assessment of cell toxicity by dandelion (MSE) hydroalcoholic extracts. Rat hypothalamic HypoE22 cells were cul-

Table 1 Stock solution and its dilutions of reconstituted dried hydroalcoholic extracts from Dan L and Dan R (MSE). All concentrations were tested on cells for in vitro assays, but only 100 and 200 μ g mL⁻¹ were used to incubate cortical and hypothalamic tissues in ex vivo assays

	Phytochemical concentration	DMSO %	
80 X	16 mg/mL	50	
10 X	2 mg/mL	6.25	
Highest tested	500 μg/mL	1.56^{a}	
Higher Int. tested	200 μg/mL	0.625	
Lower Int. tested	100 μg/mL	0.312	
Lowest tested	50 μg/mL	0.156	

^aThis percentage is higher than that tolerated by the cells, which is why the effects on cell viability were evaluated compared to two control lines (Ctrl) in 96-well culture plates (one line was at this DMSO **Paper Food & Function**

tured in DMEM supplemented with 10% (v/v) heat-inactivated fetal bovine serum and 1% (v/v) penicillin G/streptomycin in 75 cm² cell culture flasks. The cultured cells were maintained in a humidified incubator with 5% CO2 at 37 °C. When the confluency reached 80%, a viability test was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) test to assess the basal cytotoxicity. For this assay, cells were seeded (3 \times 10³ cells per well) onto flat-bottomed 96-well culture plates and incubated overnight. After 24 hours, cells were treated with either vehicle (culture medium) or dandelion (MSE) hydroalcoholic extracts at different concentrations (50, 100, 200, and 500 μg mL⁻¹) and incubated overnight. Thereafter, 20 µL of MTT (5 mg mL⁻¹ in PBS) was added to each well, and the plate was incubated for 3 h at 37 °C. After that, the formed formazan dye was solubilised with dimethyl sulfoxide, and the cells were incubated in the dark room on a plate shaker at 200 rpm for 3 h (37 °C). The absorbance was measured at $\lambda = 540$ nm by a multi-plate reader. Effects on cell viability were compared to the untreated control group (Ctrl), see Fig. 1A.

2.5.2 Evaluation of antioxidant activity exerted by dandelion (MSE) hydroalcoholic extracts against H2O2-induced cell damage. In a second set of experiments, HypoE22 cells grown to 80% confluence were plated (3 \times 10³ cells per well) onto flatbottomed 96-well culture plates and incubated overnight. After cell attachment, the treatment with both vehicle and dandelion (MSE) extracts at different concentrations (50, 100, 200, and 500 µg mL⁻¹) was performed, and the multi-well plate was incubated. After 24 hours, cells were post-treated with hydrogen peroxide (H₂O₂) 300 µM as pro-oxidant stimulus. After three incubation hours, an MTT assay was carried out, removing the previous treatment and adding 20 µL of MTT at a 5 mg mL⁻¹ concentration in each well. The plate was incubated for 3 h at 37 °C in the dark room. The formed formazan dye was solubilised with dimethyl sulfoxide, and its absorbance was recorded at 540 nm. Effects on cell viability were evaluated compared to the control (Ctrl: untreated cells) and expressed as a percentage of the control culture value. Each condition was run in triplicate, including the untreated and blank cellfree control (Fig. 1A).

2.6 Ex vivo studies

Adult mice C57/BL6 (n = 8) were housed in Plexiglas cages (55 cm × 33 cm × 19 cm) and maintained under standard laboratory conditions (21 \pm 2 °C; 55 \pm 5% humidity) on a 14/10 h light/dark cycle, with beverage and food ad libitum. Housing conditions and experimentation procedures were strictly in agreement with the European Community ethical regulations (EU Directive no 26/2014) on the care of animals for scientific research. Tissue collection was approved by local ethical committee ("G. d'Annunzio" University, Chieti, Italy) and Italian Health Ministry (Project no. F4738.N.5QP). For brain collection, rodents were sacrificed by CO2 inhalation (100% CO2 at a flow rate of 20% of the chamber volume per minute). Brain specimens were cut to obtain the prefrontal cortex (split into two, n = 16) and hypothalamus sections (n = 8). Thereafter, cerebral tissue samples were placed into 6-well plates in RPMI buffer, adding bacterial (E. coli) LPS (50 µg mL⁻¹) to each well except for the control (Ctrl). The prefrontal cortex specimens were treated with dandelion (MSE) hydroalcoholic extracts at 100 and 200 µg mL⁻¹ concentrations, whereas the hypothalamic sections were treated only at the highest concentrations, 200 μg mL⁻¹. After 4 h of incubation at 37 °C, the samples were collected and stored at -80 °C until the RNA extraction (Fig. 1B).

According to the manufacturer's protocol, total RNA was extracted from the brain tissues using TRI reagent, and 1 µg of total RNA from each sample in a 20 µL reaction volume was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Fig. 1C). Gene expression of TNF-α, BDNF, and NOS-2 was evaluated by quantitative real-time PCR using TaqMan probe-based chemistry, as previously reported.³⁶ GADPH (glyceraldehyde-3-phosphate dehydrogenase) was used as the housekeeping gene (Table S2). The real-time PCR was carried out in triplicate for each cDNA sample for each selected gene (Fig. S8). Data elaboration was performed with the Sequence Detection System (SDS) software version 2.3 (Thermo Fischer Scientific). Relative quantification of gene expression was performed by the comparative $2^{-\Delta\Delta Ct}$ method.³⁷

Results and discussion

3.1 HPLC-DAD analysis

HPLC-DAD identified polyphenolic compounds through a comparative profile of three dandelion ecotypes (LSE, OE, and MSE) for the Dan L and Dan R samples. Thirty phenolic constituents were detected by the chromatographic method applied, of which nineteen were quantified (Table 2, Fig. S1).

The analysis of phenolic profile indicated that all identified compounds were generally present in aerial part extracts across all ecotypes, except for vanillic acid, which was not detected in Dan L of the MSE ecotype. Consistent with findings reported in literature, 38,39 the hydroxycinnamic acid derivatives, particularly caffeic acid esters such as chlorogenic acid, dicaffeoyl tartaric (chicoric) acid, and monocaffeoyl tartaric (caftaric) acid were found throughout the plant (Fig. S2-S7). Other phenolic compounds were identified exclusively in the leaf extracts: 4-hydroxybenzoic acid, syringaldehyde, hyperoside, rutin, carvacrol, and thymol. Additionally, only for the MSE ecotype, gentisic acid was found in both plant parts (Fig. S2 and S3), 3-hydroxyflavone was not detected from the Dan R of the LSE (Fig. S5), hyperoside and rutin were not detected in the roots of both LSE and MSE ecotypes, and the MSE showed carvacrol presence only in the aerial part (Fig. S2).

According to the common trend reported in the literature, 40,41 the polyphenol content is higher in the aerial parts of the plant than in the roots (Fig. 2A).

Among these, chicoric acid, (-)-epicatechin, caftaric acid, 3-hydroxytyrosol, chlorogenic acid, and resveratrol were the most abundant at concentrations over 100 mg in 100 grams of Food & Function Paper

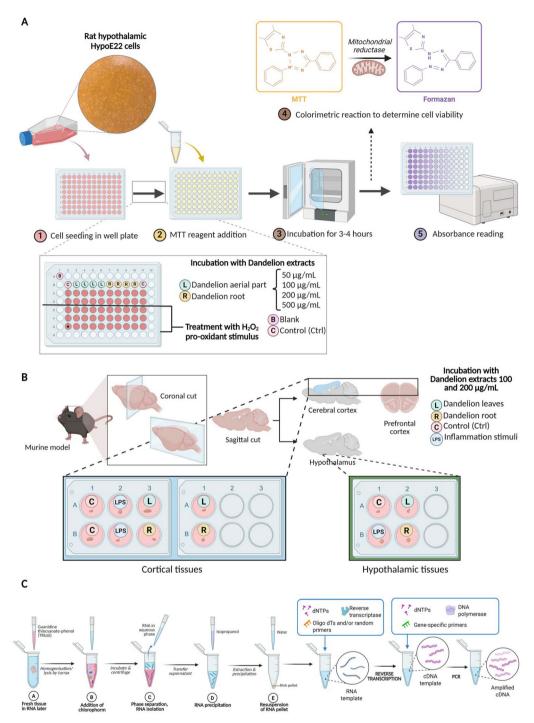


Fig. 1 Experimental design of (A) in vitro and (B) ex vivo studies on dandelion (MSE) hydroalcoholic extracts' biological activity. (C) RT-PCR steps from both cortical and hypothalamic tissues.

plant material. The MSE showed the highest content of chicoric and caftaric acids, confirmed by NMR-based untargeted metabolomic analysis previously performed. 14

The levels of (–)-epicatechin in Dan L of all ecotypes were higher than those of Dan R. In contrast, chlorogenic acid resulted to be reduced in the aerial part compared to the radical part.

Polyphenols, such as 3-hydroxyflavone, caffeic acid, hyperoside, gallic acid, vanillic acid, 4-hydroxybenzoic acid, rutin, and carvacrol, were present in lower concentrations, below 10 mg in 100 grams of matrix. 4-Hydroxybenzoic acid was not present in the roots of all ecotypes of dandelion.

At last, p-coumaric acid, t-ferulic acid, kaempferol, flavone, loganic acid, syringaldehyde (MSE), t-cinnamic acid (MSE),

Table 2 Phenolic compounds identified in the chromatographic analysis of hydroalcoholic Dan R and Dan L extracts

Compounds	tR	MSE		LSE		OE	
		Leaves	Root	Leaves	Root	Leaves	Root
Gallic acid	8.8	•	•	•	•	•	•
3-Hydroxytyrosol	11.7	•	•	•	•	•	•
Caftaric acid	12.9	•	•	•	•	•	•
(+)-Catechin	14.8	•	•	•	•	•	•
Gentisic acid	15.8	•	•	•		•	
4-Hydroxybenzoic acid	16.2	•		•		•	
Loganic acid*	16.6			•		•	
Chlorogenic acid	16.8	•	•	•	•	•	•
Vanillic acid	18.6		•	•	•	•	•
Caffeic acid	19.0	•	•	•	•	•	•
(–)-Epicatechin	19.4	•	•	•	•	•	•
Syringic acid	20.0	•	•	•		•	•
Syringaldehyde*	21.8		•				
Chicoric acid	22.2	•	•	•	•	•	•
<i>p</i> -Coumaric acid*	23.1	•	•	•	•	•	•
t-Ferulic acid*	24.0	•	•	•	•	•	•
Benzoic acid	26.4	•	•	•		•	•
Hyperoside	26.9	•		•		•	•
Rutin	27.1	•		•		•	•
Resveratrol	27.7	•	•	•	•	•	•
t-Cinnamic acid*	34.4		•				
Quercetin*	35.9		•				
Naringenin	36.8	•	•	•	•	•	•
Hesperidin*	39.4			•		•	
Kaempferol*	41.7		•			•	•
Carvacrol	44.7	•		•	•	•	•
Thymol*	44.9	•		•	•		
Flavone ^a	45.6	•					•
3-Hydroxyflavone	46.1	•	•	•		•	•
Emodin	47.7					•	

^a The asterisk indicates compounds which were detected but not quantified.

quercetin (MSE), hesperidin, thymol, and emodin were detected but not quantified.

In terms of total polyphenol levels, dandelion MSE exhibited the highest concentration of these compounds, while the other two ecotypes demonstrated significantly lower levels (Fig. 2B).

This observation aligns with the potential antioxidant and anti-inflammatory effects reported by Orlando *et al.*,⁴² thereby further reinforcing the need for pharmacological evaluation of the MSE ecotype extracts discussed below.

These results align with the literature, as the polyphenolic composition in different plant parts of dandelion is reported to be widely variable due to methodology (extraction solvent composition, type of extraction, analytical method), genetic background, and cultivation practices. 43–45

For instance, in our previous study, ¹⁴ a comparative metabolomics analysis was conducted on the three ecotypes (LSE, MSE, and OE) by applying NMR spectroscopy, showing a significantly higher content of phenolic acids (including chicoric and caftaric) in the MSE ecotype, particularly in the aerial parts.

Similarly, previous studies have reported that wild ecotypes of *Taraxacum officinale* from upland environments tend to accumulate higher levels of polyphenols than cultivated or lowland populations. For example, Kim *et al.* 46 observed sig-

nificantly higher levels of phenolic compounds in dandelion plants harvested in mountainous areas compared to cultivated ones.

Furthermore, autumnal environmental conditions and moderate altitude are known to favour the accumulation of defence-related secondary metabolites, including polyphenols, in different matrices. 47-49

3.2 Biological activity assays

3.2.1 Biocompatibility and antioxidant protection from H_2O_2 -induced cell damage. The cytotoxicity effects of dandelion MSE hydroalcoholic extracts in the 50–200 μg mL⁻¹ concentration range on the hypothalamic HypoE22 cell line viability under basal conditions were investigated. Cells were exposed to the Dan L and Dan R extracts in basal conditions, and both were tolerated up to the concentration of 500 μg mL⁻¹, demonstrating good biocompatibility.

However, only the highest concentration ($500 \mu g \text{ mL}^{-1}$) of Dan R extract showed a reduction of cell viability, but the extract could not be considered toxic because the relative percentage is above the biocompatibility limit (70% viability compared to the control group), as shown in Fig. 3.

Furthermore, the dandelion MSE hydroalcoholic extracts (50, 100, 200, and 500 $\mu g \text{ mL}^{-1}$) were tested on the HypoE22 cell line, also in the presence of hydrogen peroxide (H₂O₂),

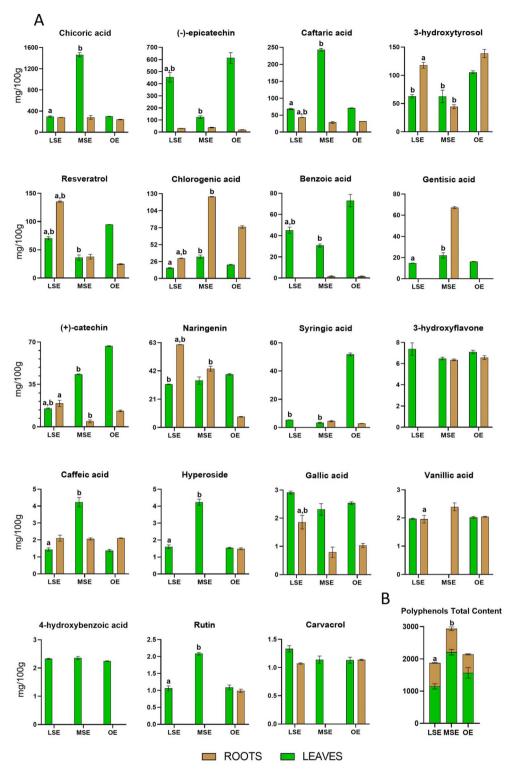


Fig. 2 (A) Histograms resulting from the quantitative HPLC-DAD analysis of quantified phenolic compounds (mg per 100 g) in the dandelion (LSE, MSE, OE) hydroalcoholic extracts. (B) A histogram resulting from the sum of polyphenols total content in the whole plant to highlight the ecotype containing the highest amount of this class of compounds. The difference between leaves (green) and roots (brown) is compared. Two-way ANOVA, followed by Tukey's multiple comparisons test, was applied to underline, among ecotypes, significant differences (ρ < 0.0001) for each metabolite according to the same plant section: (a) vs. MSE, (b) vs. OE.

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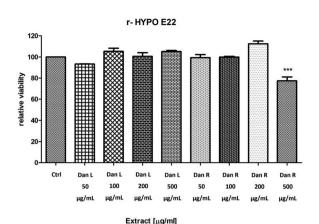


Fig. 3 Effects of Dan R and Dan L extracts $(50-200 \mu g \text{ mL}^{-1})$ on HypoE22 cell viability in basal conditions. ANOVA, p < 0.0001; ** p < 0.01, ***p < 0.001 vs. Ctrl (Control group). Data are presented as means + SD from three independent experiments, each performed in triplicate.

which is the reference pro-oxidant stimulus capable of reducing cell viability below the biocompatibility limit (70% viability compared to the control group). Dan L and Dan R extract effectively protected cells from cytotoxicity induced by 300 μ M of hydrogen peroxide from a phytochemical concentration of 200 μ g mL⁻¹, demonstrating good antioxidant activity. Notably, in the case of leaf extract, the highest concentration (500 μ g mL⁻¹) significantly counteracted the reduction of cell viability by H₂O₂, proving to contain a good antioxidant phytocomplex (Fig. 4). In contrast, incubation with the highest concentration of root extract (500 μ g mL⁻¹) only mitigated the pro-oxidant activity of H₂O₂ (Fig. 4). This effect may be due to the lower polyphenol content in dandelion root compared to its aerial parts, as illustrated in the histogram of total polyphenol levels (Fig. 2B).

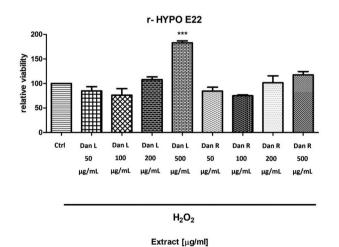


Fig. 4 Effects of Dan R and Dan L extracts ($50-200~\mu g~mL^{-1}$) on HypoE22 cell viability under hydrogen peroxide (H_2O_2) stimulus. ANOVA, p<0.0001; ** p<0.01, ***p<0.01 vs. Ctrl (Control group). Data are presented as means \pm SD from three independent experiments, each performed in triplicate.

3.2.2 *Ex vivo* studies. The dandelion extracts, at concentrations of 100 and 200 μg mL⁻¹, were then tested in isolated samples of mouse cortex and hypothalamus treated with *E. coli* LPS, a pro-inflammatory stimulus, to evaluate their potential protective activities on neuroinflammatory pathways.

In this context, the gene expression of TNF- α and NOS-2 was assessed, and the LPS (50 μg mL⁻¹) stimulus induced their upregulation in both the cortex and hypothalamus. All extracts effectively reverted the increased expression of these two genes, suggesting potential protective effects on neuroinflammation. Additionally, the TNF- α gene expression was even lower than that displayed by the control group after Dan L extract (200 μg mL⁻¹) was administered in the cortical specimen (Fig. 5A). Instead, the gene expression of NOS-2 after treatment with Dan R extract (200 μg mL⁻¹) was lower than that shown by the control in the hypothalamus (Fig. 5B).

Moreover, the effects of dandelion extracts on LPS-induced BDNF levels, a neurotrophic factor which is strongly reduced in proinflammatory states of brain tissue, were investigated. The extract's treatment was effective in both assayed tissues, in contrast to the LPS-induced down-regulation of the BDNF gene, demonstrating a possible neuroprotective effect. The higher concentration of Dan R extract (200 $\mu g\ mL^{-1}$) acted, reverting the decreased BDNF gene expression induced by LPS; after treatment, it was even higher than that shown by hypothalamic tissue incubated with vehicle (Fig. 5B).

It is noteworthy that the effects of root and leaf extracts vary according to the specific brain tissue under examination. At the cortical level, there is no clear trend regarding how the two extracts counteracted the LPS-induced activity on the expression of the selected genes (Fig. 5A). However, at the hypothalamic level, the root extract demonstrated greater effectiveness (Fig. 5B).

These effects could be attributed, albeit partially, to the polyphenolic content of the extracts and their scavenging/reducing properties.

3.2.3 Neuroprotective potential of MSE dandelion: role of chicoric acid and related polyphenols. The research revealed that the hydroalcoholic extract of MSE dandelion has neuroprotective effects against inflammation and oxidative stress pathways. The dandelion hydroalcoholic extracts contained polyphenols, which could be responsible for the observed effects. Phytochemical constituents in dandelion include hydroxycinnamic acid derivatives such as chicoric, caftaric, chlorogenic, and caffeic acids. Chicoric acid is the significant component of MSE dandelion, followed by caftaric, chlorogenic, and caffeic acids.

As reported in the literature, it is noteworthy that chicoric acid can cross the blood-brain barrier, achieving a concentration level of 85 ± 12 ng g⁻¹ in the brain tissue. In addition, it was found that chicoric acid, even at low concentration, remained at a stable level for a prolonged period, indicating the brain as a possible target organ for this phenylpropanoid compound.⁵⁰ Furthermore, the same phytochemicals, as well as the hydroalcoholic extract of *Taraxacum coreanum*, were able to interact with the expression of inflammatory mediators and

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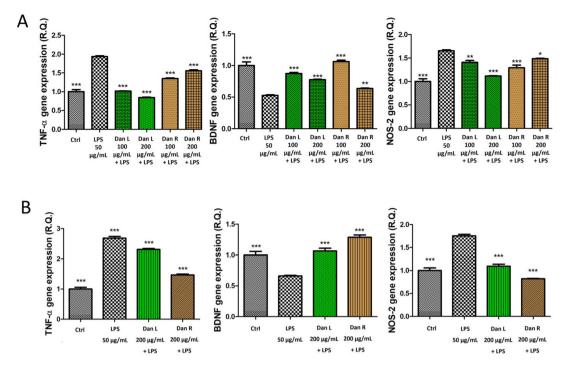


Fig. 5 Effects induced by the Dan L and Dan R extracts on basal and LPS-induced TNF-α, NOS-2, and BDNF gene expression, in cortical and hypothalamic tissues from mice. ANOVA, p < 0.0001; ***p < 0.001 vs. LPS. Data are presented as means + SEM from three independent experiments, each performed in triplicate.

cytokines such as iNOS, COX-2, IL-1β and TNF-α via suppression of NF-κB in mouse brain. 31-33,51

This could partially explain the observed inhibitory effects of MSE dandelion extract on selected pro-inflammatory biomarkers. Indeed, under LPS-induced oxidative stress, the extracts Dan L and Dan R decreased gene expression of TNF-α and NOS-2 in excised mice brain specimens. These pro-inflammatory factors contribute to the accumulation of ROS and oxidative stress dysregulation, thus leading to neurodegenerative processes. TNF- α is one of the most potent and early inflammatory mediators that can trigger a cascade response leading to apoptosis when overproduced, similar to NO.28,31,52 Thus, neurodegenerative and cognitive disorders could be ameliorated by acting upstream of inflammatory processes.

In parallel, the extracts Dan L and Dan R reversed LPSreduced BDNF levels and even returned them to normal levels. This neurotrophic factor is crucial for the survival of dopaminergic neurons and maintenance of memory, which are impaired in Parkinson's (PD) and Alzheimer's (AD) diseases, respectively. 18,53,54 This could be partially explained by the effect of chicoric acid in mouse models of PD and AD, as reported in the literature. 33,55 Chicoric acid protects neurons from inflammation by restoring BDNF levels (synaptic density increased), improving mitochondrial function, and regulating energy metabolism.32,56

Previous studies highlighted antioxidant effects exerted by some hydroxycinnamic acid derivatives. An in vivo study using C57BL/6J mice and BV-2 microglia showed that chicoric acid

reversed the reduction in cell viability and mitochondrial dysfunction, leading to a balancing of cellular redox status.⁵⁷ In vitro studies demonstrated the same scavenging activities for chicoric acid and its metabolites, such as chlorogenic and caffeic acids. 58,59 Consistent with these, the data showed that Dan L and Dan R extracts effectively scavenged activities against pro-oxidant stimulus H₂O₂ in the 50-200 μg mL⁻¹ concentration range. The Dan L at the highest concentration of 500 μg mL⁻¹ displayed significant counteractive effects against H₂O₂-induced reduction of cell viability, indicating its antioxidant properties due to the higher content of chicoric acid and other caffeoyl tartaric derivatives. 42 Intriguingly, the MSE dandelion extracts were well-tolerated by the hypothalamic HypoE22 cell line in the same concentration range, suggesting good biocompatibility.

While the ex vivo brain tissue model offers valuable insight into the effects of bioactive compounds on a complex neuroinflammatory environment, future investigations should include neuronal or glial cell lines (e.g., SH-SY5Y, BV2, PC12, or primary astrocytes) to further validate these observations in defined and controlled in vitro settings.

Conclusion

The composition of hydroalcoholic extracts from dandelion leaves and roots was investigated in the present study. It focused on the phytochemical content variation in ecotypes Paper

(LSE, MSE, OE) with respect to environmental factors. The HPLC-DAD analysis provided valuable insights regarding the polyphenols variation in the hydroalcoholic extracts of selected dandelion ecotypes. Targeted analysis allowed us to identify and quantify several secondary metabolites belonging to polyphenols, including hydroxycinnamic compounds such as chicoric, caftaric, chlorogenic, and caffeic acids, all of which were present in higher amounts in the MSE ecotype. Taraxacum officinale showed a chemical profile rich in healthy compounds, which makes the MSE extract a promising candidate for phytotherapeutic use. The present findings provide preclinical evidence demonstrating that MSE dandelion extract could prevent LPS-triggered neuroinflammation via TNF-α and NOS-2 gene expression reduction, along with the increased BDNF in the mice brain tissues. The HypoE22 cells well-tolerated the dandelion hydroalcoholic extracts, suggesting good biocompatibility. The Dan L and Dan R extracts were effective in protecting selected hypothalamic cell line from oxidative

According to the literature, hydroxycinnamic derivatives could partially account for such effects, particularly in ameliorating neuroinflammation and neurodegeneration.

stress in the concentration range $50-200 \,\mu \text{g mL}^{-1}$.

By integrating ecotype-specific phytochemical analysis with functional testing in relevant brain tissues, this study provides a translational bridge between plant biodiversity research and neuro-nutritional applications. This ecotype-to-tissue approach enables a more precise identification of botanical sources whose environmental adaptations yield bioactive profiles capable of modulating key neuroinflammatory pathways. This could thereby address the gap in nutritional neuroscience for workflow models that directly connect phytochemical diversity to neurological impact.

All the findings suggest a potential neuroprotective and anti-inflammatory effect of dandelion extract, supported by modulation of key gene expression in the inflammatory pathway. This evidence suggests that the bioactive components present in the extract, in particular polyphenols with anti-oxidant action, could help mitigate neuroinflammatory processes and promote neuronal survival and maintenance of synaptic function. This effect appears particularly relevant in oxidative stress, such as that experimentally induced by hydrogen peroxide, suggesting a possible use of dandelion as a complementary phytotherapeutic resource for prevention or support in neurodegenerative diseases characterised by chronic inflammation and neuronal damage.

This study establishes a theoretical foundation for intervention studies of natural functional food components in neuronutrition and provides new clues for developing healthy foods based on dandelion plant material. However, further studies are required to accurately evaluate its protective activity in a mouse model of cognitive and neurodegenerative impairment.

In conclusion, the present study demonstrated the potential application of the MSE dandelion hydroalcoholic extract and its considerable antioxidant potential in attenuating neuronal injury and cognitive impairment induced by oxidative stress. Indeed, dandelion could be considered a candidate on which

to base the development of innovative products, such as food supplements with protective effects on the brain.

Author contributions

Conceptualisation, F. M., and C. I.; data curation, F. M., C. I., and A. C.; formal analysis, F. M. and A. C.; funding acquisition, L. M.; investigation, F. M., D. A., M. L. L., A. A., and C. D. S.; methodology, F. M. and C. I.; project administration, C. I., and L. M.; resources, L. M., C. F., L. M., and G. O.; supervision, C. I., L. M., and C. F.; validation, C. I., A. C., and C. F.; visualisation, F. M., D. A. and C. I.; writing – original draft, F. M. and D. A.; writing – review and editing, C. I. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Supplementary information available: chromatographic profiles, heatmap of polyphenolic distribution, gene expression data, and experimental replicates are provided upon request to support transparency and reproducibility. Moreover, raw chromatographic output, quantitative analysis of phenolic compounds, and biological assay results (*in vitro* and *ex vivo*), are available on reasonable request. Requests for data access will be considered for non-commercial academic use and in accordance with institutional data sharing policies.

The data that support the findings of the study are available from the corresponding author upon reasonable request. For further information or to request access to the datasets, please contact annalisa.chiavaroli@unich.it. See DOI: https://doi.org/10.1039/d5f002646f.

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