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Research article

Evaluation of licorice (*Glycyrrhiza glabra* L.) as a novel microgreen from the anti-inflammatory potential of polyphenols

Ilaria Marotti*, Francesca Truzzi, Camilla Tibaldi, Lorenzo Negri and Giovanni Dinelli

Department of Agricultural and Food Sciences, University of Bologna, viale Fanin, 44-40127 Bologna, Italy

*** Correspondence:** Email: ilaria.marotti@unibo.it; Tel: +390512096673.

Abstract: Literature on microgreens, an emerging new functional food crop, remains limited. Further study on microgreens as a promising dietary component for potential use in diet-based disease prevention is, therefore, essential. Given that the anti-inflammatory and anti-oxidant properties of mature licorice root material are well-documented, the objective of the present, preliminary study was to present licorice (*Glycyrrhiza glabra* L.) as a novel, edible fresh-food microgreen candidate. The effect of leaf, stem and root polyphenol extracts of 20-day old licorice microgreen seedlings on cell proliferation and viability of Caco-2 cells (simulating the intestinal epithelium), after pro-inflammatory induction of lipopolysaccharide (LPS), was examined and then compared to the polyphenol, flavonoid and anti-radical activities of the respective tissue extracts. Root extracts contained a two-fold less polyphenol (including flavonoid) content compared to leaf extracts, but with a five-fold lower anti-radical scavenging activity. Only the root extracts provided functional protection in terms of preserving cell proliferation and viability of LPS-treated Caco-2 cells. Some protection was also afforded by the stems, but the young leaf material offered no anti-inflammatory protection. Results point to a differing composition of anti-inflammatory polyphenols between the root and leaf, suggesting that the protective efficacy of the root extracts (and to some degree, the stem extracts) resides in inhibiting the pro-inflammatory cascade and resultant cytotoxic effects as opposed to a direct anti-radical scavenging activity. Potential use of licorice as a microgreen is promising, but will necessitate further study.

Keywords: *Glycyrrhiza glabra* L.; licorice; microgreen; polyphenols; inflammation; Caco-2 cells; cell proliferation and viability

1. Introduction

Microgreens are an emerging new functional food crop for the 21st Century, with promise for sustainably, adaptation to urbanization and global climate change, and promoting human health [1,2]. Compelled by growing interest of consumers for diets that support health, microgreens are a new class of specialty crop, defined as tender immature greens produced from the seeds of vegetables, herbs, or grains, including wild species with delicate textures and distinctive flavors [3,4]. Recent reports demonstrated that microgreens contain higher amounts of phytonutrients and minerals than their mature leaf counterparts [2,4]. Representing an emerging food crop, literature on microgreens remains limited. Further study on microgreens as a promising dietary component for potential use in diet-based disease prevention is therefore, essential.

To date, the most commonly used microgreens do not include plants from the family Fabaceae. Renewed interest in under-utilized plant species for food, including the herbaceous perennials alfalfa and red clover, is based on well-documented functional attributes [5]. Aimed at addressing the scarce information on the nutritional, phytochemical and mineral profiles of seeds and microgreens, Butkutė et al. [5] demonstrated that microgreens of these species represented promising new sources of ingredients for the fortification of staple foods with bioactive compounds. Similarly, to the best of our knowledge no research has been conducted on the phytochemical profiles in microgreens of the herbaceous perennial, licorice (liquorice). Traditional medicinal licorice (*Glycyrrhiza glabra* L.) is obtained from the roots of *Glycyrrhiza uralensis* Fischer, *Glycyrrhiza glabra* L. or *Glycyrrhiza inflate* Batalin (Fabaceae). The pharmaceutical importance of licorice lies in the great variety of secondary metabolites, extracted from roots, with widely reported antitumor, antimicrobial, antiviral anti-inflammatory, antidiabetic, immunoregulatory hepatoprotective and neuro-protective activities [6–8].

Given that inflammation responses play an important role in the pathogenesis of a large number of acute and chronic diseases, a meta-analysis review of the literature on licorice was conducted, showing that 3 triterpenes and 13 flavonoids (including chalcones, isoflavans and isoflavonoids) exhibited evident anti-inflammatory properties [7]. The anti-inflammatory properties resided in the ability to reduce the activation of the transcription nuclear factor kappa, (NF-κB p65), responsible for regulating the expression of multiple NF-κB-dependent genes, including the cytokine, Tumour Necrosis Factor-α (TNF-α), Prostaglandins (PGE2) and inducible Nitric Oxide Synthase (iNOS) in various cell lines subjected to inflammatory stimuli [7,8]. The potential protective effect of licorice polyphenol extracts against Reactive Oxygen Species (ROS)-mediated injuries in intestinal cells was demonstrated for the first time by D'Angelo et al. [9], using H_2O_2-treated Caco-2 cells as the model system. Though various licorice compounds were shown to demonstrate potent anti-radical activities, and direct anti-oxidation appears to be a mechanism of *in vitro* protection, it has become increasingly evident that the anti-oxidant potential resides in the induction of the Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway [10–12]. Both licorice ethanol extracts and individual compounds, also responsible for reducing NF-κB p65 signaling [7], were similarly shown to stimulate Nrf2 though binding to anti-oxidant response elements (AREs) resulting in the expression of detoxifying enzymes and cytoprotective proteins, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and glutathione (GSH) [8,11,13,14].

The Caco-2 cell monolayer, a human colon intestinal carcinoma cell layer, is considered the "gold standard" of *in vitro* models of the human intestinal barrier in testing the transport,

metabolism and remedial (antioxidant, anticancer and anti-inflammatory) potential of functional food extracts [15]. Lipopolysaccharide (LPS) treatment of host cell lines induces inflammatory responses, more specifically by triggering NF-κB p65 upregulation of pro-inflammatory mediators and cytokines, including Cyclooxygenase-2 (COX-2), TNF-α, iNOS, and Interlukin-6 (IL-6) [16]. Both COX-2 and iNOS expression and enzymatic activity generate ROS and nitric oxide (NO), in turn leading to Tight Junction (TJ) permeability barrier through a number of mechanisms, including membrane peroxidation, disruption of mitochondrial function and apoptosis (Bose and Kim, 2013 [17,18]. Moreover, NO and ROS, in turn, further amplify inflammation, through the up-regulation NF-*κ*B dependent genes, constituting a vicious cycle [18].

Given the above-mentioned anti-inflammatory and anti-oxidant properties of mature licorice root material, the present study is aimed at investigating the potential functional benefits of licorice (*Glycyrrhiza glabra* L) as a microgreen. To the best of our knowledge, there are no published reports documenting the functional efficacy of immature roots as well as the stems and leaves, which are generally not eaten as food. To this end, the effect of leaf, stem and root polyphenol extracts of 20-day old microgreen seedlings on cell proliferation and viability of LPS-treated Caco-2 cells was examined and compared to the polyphenol, flavonoid and anti-radical activities of the respective tissue extracts.

2. Materials and methods

2.1. Materials

The reagent, 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolio (MTT assay) was from Life Technologies (Carlsbad, CA, USA). The Folin-Ciocalteau, 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and LPS were obtained from Sigma Chemical Company (St. Louis, MO). Reagents for cell cultures, including Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS) and Penicillin-Streptomycin, were purchased from GIBCO (Waltham, MA, USA). All other chemicals and solvents were of analytical grade.

2.2. Plant material

Glycyrrhiza glabra L. seeds were supplied by the "Peraga Garden Center" (Torino). Seeds were sown in alveolar containers filled with mix of peat and sand at a ratio of 2:1. Sprouted seedlings were grown for a period of 20 days under controlled temperature (22 ℃) and photoperiod (16 light hours/8 dark hours). At the end of the growth period (BBCH stage 12–18), the leaf, stem and root material, respectively, were sampled and used to extract polyphenol content.

2.3. Polyphenols, flavonoids and anti-radical scavenging activity

Free polyphenols were extracted according to Adom et al. [19]. The residue from the free phenolic extraction was subjected to alkaline and acid hydrolysis to recover the bound phenolic compounds as reported by Mattila et al. [20].

Total polyphenol content (free and bound fractions, respectively) was measured at 765 nm according to the Folin–Ciocalteu procedure based on the method of Singleton et al. (1999 [21]). The

results were expressed as mg Gallic Acid Equivalents (GAE) per 100 g fresh weight (FW). Total flavonoid content in the free and bound fractions was measure at 510 nm, according to the method of Adom et al. [19]. The results were expressed as mg Catechin Equivalents (CE) per 100 g fresh weight (FW).

The radical scavenging activity, in the free and bound polyphenol extracts, was determined with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to the spectrophotometric assay of Brand-Williams et al. [22]. Extract Aliquots (20 µl) of extracts were added to 3 mL of DPPH solution (6 × 10–5 mol/L) and incubated for 30 min. Since DPPH is reduced by accepting a hydrogen radical from the antioxidants to form a stable diamagnetic molecule DPPHH, the degree of DPPH not decolorized was measured at 517 nm. DPPH reduction was calculated with the following equation: Anti-Radical Capacity (%) − [(A0 − AS) / A0] × 100. Where A0 represented the absorption of control, AS the absorption of the sample tested. The values were expressed as Trolox Equivalents (µmol TE/g FW). Four replicates were conducted for analytical procedure.

2.4. Cell culture experiments

2.4.1. Growth conditions

The Caco-2 human epithelial cell line (ATCC HTB-37), obtained from Colorectal adenocarcinoma, was cultured with DMEM, supplemented with 10% FBS and 1% penicillin-streptomycin, as reported in Truzzi et al. [23]. Cultures were maintained at 37 ℃ in a humidified atmosphere containing 5% CO_2 in tissue culture flasks (75 cm^2; BD Biosciences, Italy), and the culture medium changed every two days.

Prior to experimentation, the Caco-2 cells were trypsinized and density evaluated microscopically using a Bürker counting chamber. The cells were plated into 96-well tissue culture plates (10^5 cells/well) in complete medium. After 24 h, cells were treated with two concentrations (1.25 and 2.5 µg GAE/ml in DMEM) of root, stem and leaf extract polyphenols, respectively. Those two concentrations were chosen on the basis of our previous results showing no significant reduction in Caco-2 cell proliferation and viability after gallic acid administration at concentrations lower than 5 µg/ml [23]. For the untreated controls, extract was not added to the cells. After 4 h, LPS (1 µg/ml) was added to the experimental wells containing tissue extracts, and the cells incubated for a further 24 h. LPS was also added to a portion of the control wells containing cells but without plant extract and similarly incubated for 24 h. Following the 24 h treatments, the medium was carefully aspirated and cell proliferation (MTT assay) and vitality measured. Six analytical replicas were shown for each sample and each experiment was repeated 3 times.

2.4.2. MTT assay

Proliferative cells were detected using the MTT assay, according to the ISO 10993-5 International Standard procedure (ISO 10993-5, 2009). The method is based on the reduction of MTT by mitochondrial dehydrogenase of intact cells to produce purple formazan. The MTT substrate was prepared in DMEM, then added to cells in culture to attain a final concentration of 1 mg/mL. Cells were then incubated for 2 h at 37 ℃ in a humidified atmosphere with 5% CO_2. After incubation, the medium-MTT solution was carefully removed by aspiration, and 100 µl isopropanol

added to solubilize the formazan crystals. The amount of formazan was determined by measuring the absorbance at 540 nm using a multi-well scanning spectrophotometer (Labsystems Multiskan MS Plate Reader, ThermoFisher Scientific). Results were expressed as percentage of viable cells with respect to untreated controls. The percentage of cell proliferation was calculated using the following formula: (absorbance value of treated sample/absorbance value of control) × 100 = % of cell viability. Six analytical replicas were sown for each sample and each experiment was repeated 3 times.

2.4.3. Cell viability

Cell viability was measured using Blue Trypan as reported in Truzzi et al. [23]. In brief, cells were carefully separated from the medium and resuspended in a 0.4% Trypan Blue (Gibco) solution. Vital cells were counted using Countess®II FL (ThermoFisher Scientific, Waltham, MA, USA) and results expressed as a percentage of the control.

2.4.4. Phase contrast microscopy

Phase contrast microscopy was performed on the LPS-treated Caco-2 cells in the 96-well plate (10^5 cells/well) after 24 h of incubation with root, stem and leaf extract polyphenols at 1.25 and 2.5 μg GAE/ml. Images from the inverted microscope (Eclipse Ts2, Nikon) were comparted with cells without both plant extract and LPS, as well as LPS-treated cells without extract.

2.5. Statistical analysis

Statistical analyses (polyphenols, anti-radical activity, MTT cell proliferation and vitality) were conducted using CoStat version 6.450 (2017) software (http://www.cohort.com). Significance was determined by one-way variance (ANOVA) and the Turkey-Kramer test to any significant differences between treatments at $p \leq 0.05$.

3. Results

3.1. Analysis of polyphenol and flavonoid content and antiradical scavenging activity

The polyphenol and flavonoid content, as well as the anti-radical scavenging activity was significantly higher in the free fractions compared to the matrix-bound fractions, respectively, for all tissue extracts (Figure 1). Total polyphenol content, predominately comprised of the free fraction, was significantly higher ($p < 0.05$) in the leaf material than in the stem and roots. (Figure 1A). Polyphenol content in the matrix bound fraction did not vary between the extracts (Figure 1A). Total flavonoid content amounted to *ca* 82% of the total polyphenol content in leaves and stems and *ca* 73% in the roots. Similar to the polyphenol content, total flavonoid content, as well the representative free and bound constituents, was significantly higher ($p < 0.05$) in the leaf material than in the stem and roots (Figure 1B). Total antiradical scavenging activity, primarily reflecting the contribution of the free fraction, was significantly higher ($p < 0.05$) in the leaves than in the stems, which in turn, was significantly higher than that in the roots (Figure 1C). Noteworthy, the polyphenol and flavonoid content in the leaves was double that of the roots, respectively, but with a five-fold higher anti-

radical activity than the roots. This indicates, not only a higher phytochemical content in the leaves but also a different composition of polyphenols (flavonoids).

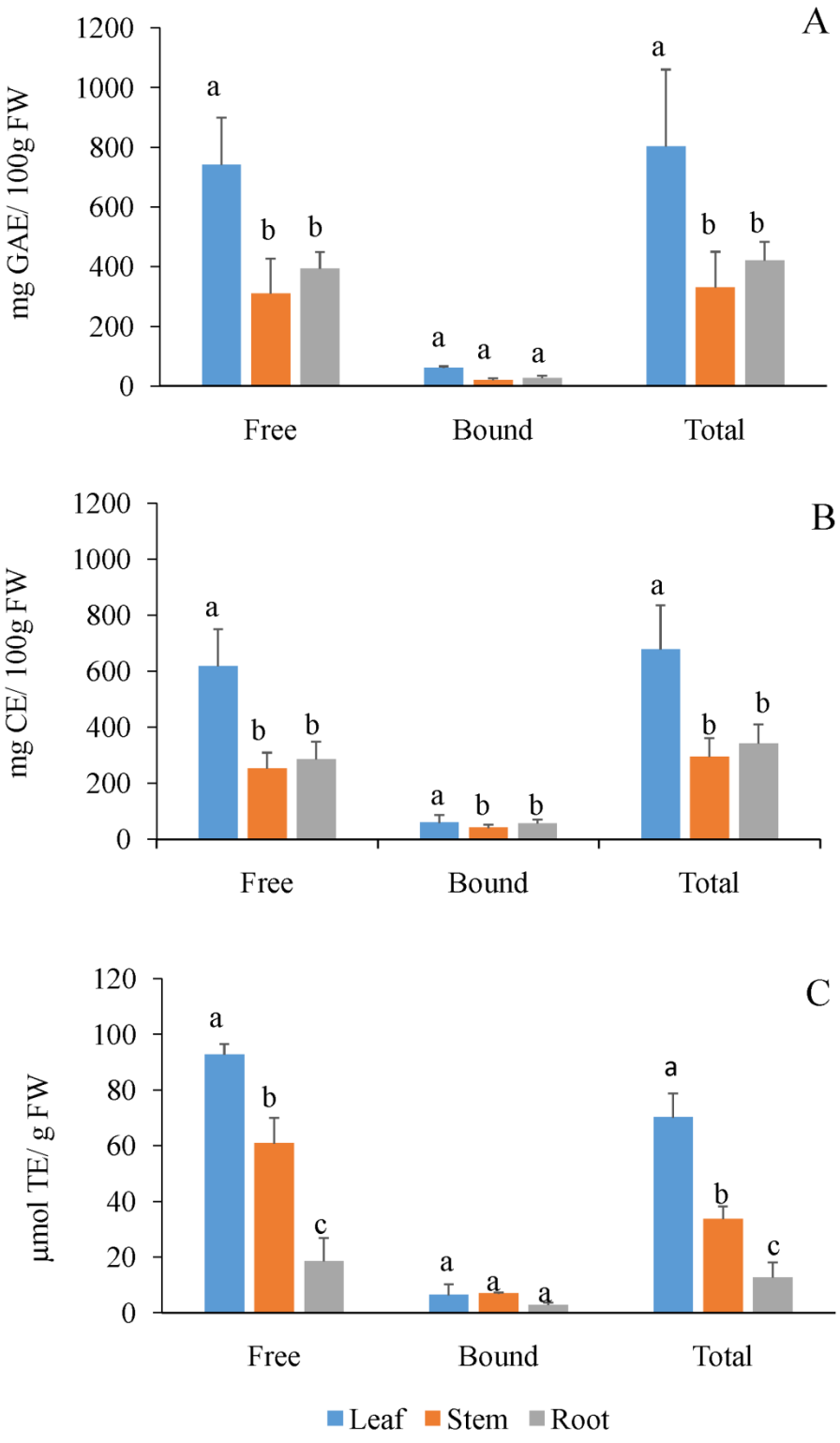

Figure 1. (A) Polyphenol, (B) flavonoid and (C) anti-radical activity (DPPH) expressed in the free bound and total fractions extracted from leaf, stem and root extracts. Different letters within each group represent significant differences at $p < 0.05$ probability level.

3.2. Effects of licorice tissue extracts on Caco-2 cells

The hatched line in Figure 2 is represented by the untreated control Caco-2 cells as a reference point to which neither extract nor LPS was added. The addition of LPS alone significantly reduced cell proliferation by more than 20% within the 24 h period. The root extracts preserved the cell proliferation, actually augmenting the latter when incubated with 1.25 μg GAE/ml despite the presence of LPS (Figure 2). The stem extracts similarly provided protection, only at 1.25 μg GAE/ml, whereas the leaf extracts at both concentrations exacerbated LPS damage (Figure 2).

Cell vitality was approximately 95% in the untreated control and significantly lower (*ca* 60%) in cells treated with LPS alone (Figure 3). The presence of root extracts at both concentrations preserved the viability of the cells to levels comparable to the control, despite the presence of LPS. The stem extract at both concentrations, as well as the leaf extract at 1.25 μg GAE/ml improved viability compared to the LPS treatment alone but cell viability was reduced to *ca* 70%. The leaf extract with 2.5 μg GAE/ml significantly worsened cell viability (Figure 3).

In the untreated control, Caco-2 cells were typically organized into colonies (Figure 4), whilst the LPS-treatment, reduced both the number and the typical colony aggregation of cells. Moreover, at the lowest polyphenol dose (1.25 μg GAE/ml), cells showed improved proliferation compared to the 2.5 μg GAE/ml dose. The images of the root cells are similar in appearance to the untreated control (Figure 4), thereby confirming the results of the aforementioned proliferation and cellular vitality tests.

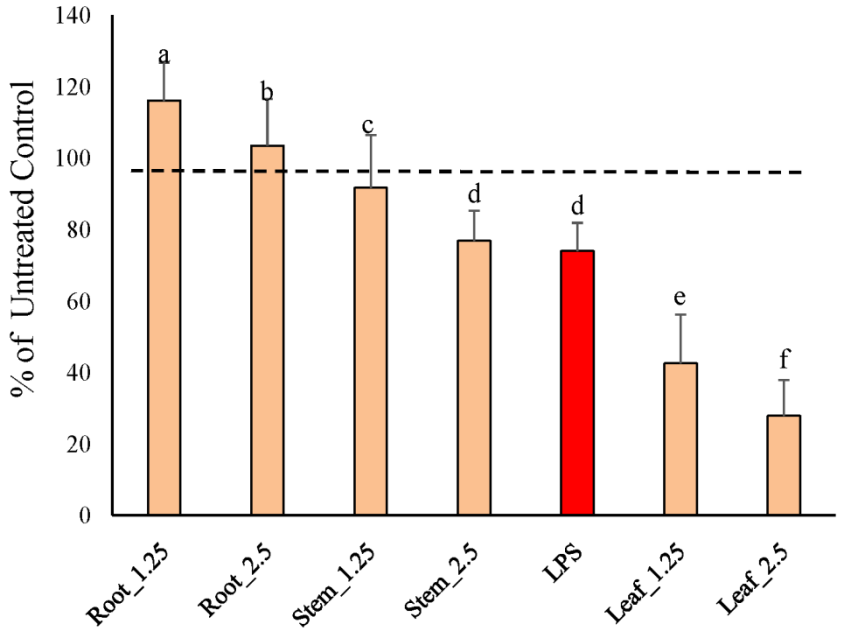

Figure 2. Cell proliferation of Caco-2 cells after 24 h lipopolysaccharide (LPS) treatment. Cells were pretreated with root, stem and leaf polyphenol extracts at concentrations of 1.25 and 2.50 μg/ml GAE for 4 hours prior to LPS (1μg/ml) treatment. Values are expressed as a percentage of untreated Caco-2 cells, represented by the hatched line. In red is the LPS treatment with no extract. Different letters represent significant (ANOVA) differences at $p < 0.05$.

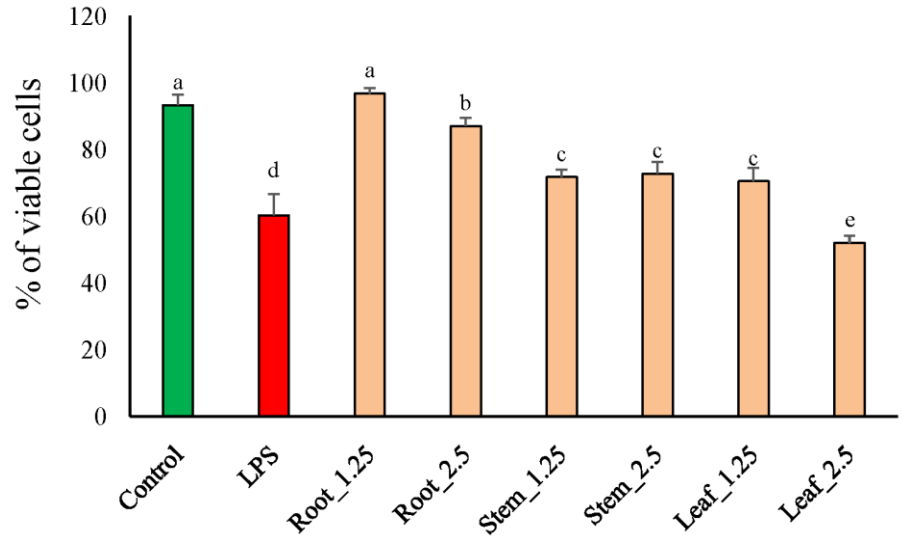

Figure 3. Cell viability of Caco-2 cells 24 h after lipopolysaccharide (LPS) treatment. Cells were pretreated with root, stem and leaf polyphenol extracts at concentrations of 1.25 and 2.50 μg/ml GAE for 4 hours prior to LPS (1μg/ml) treatment. In green is the untreated control and in red the LPS treatment with no extract. Different letters represent significant (ANOVA) differences at $p < 0.05$.

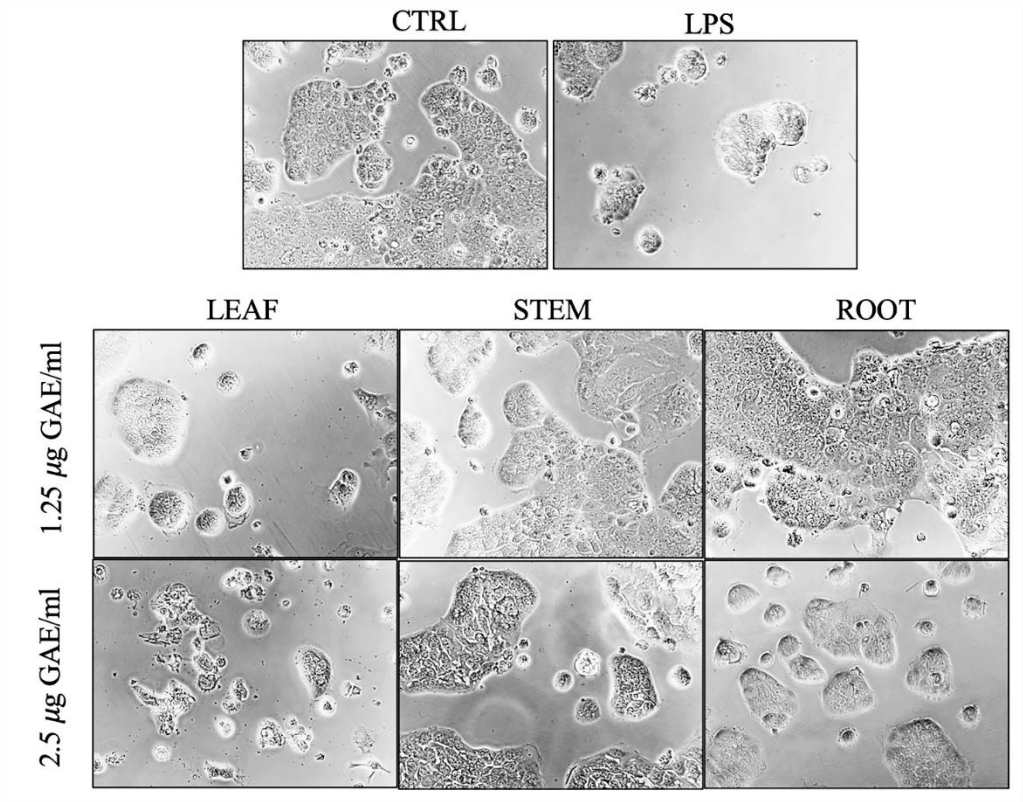

Figure 4. Phase contrast microscopy images (x 10 magnification) of the Caco-2 cells 24 h after lipopolysaccharide (LPS) treatment. Cells were pretreated with root, stem and leaf polyphenol extracts at concentrations of 1.25 and 2.50 μg/ml GAE for 4 hours prior LPS (1μg/ml) treatment.

4. Discussion

Combining the requisite to study microgreens as a favorable dietary component for potential use in diet-based disease prevention [1,2], with opportunities in promoting promising plant candidates as microgreens [5], the present study reports on the functional potential of *Glycyrrhiza glabra* microgreen seedlings. Licorice, with well-documented functional attributes due to a vast array of secondary metabolites [6–8], is a fitting choice. Given that total contents of phenols, flavonoids and tannins in licorice is shown to vary extensively at different harvest times [7], the use of licorice in the form of microgreens, which are generally harvested within 21 days of sowing [4], may offset environmental variations in the contents of these secondary metabolites. Moreover, the vast majority of information relating the health-benefits of licorice is based on studies of mature roots. Hence, information on the potential functional benefits of immature leaves, stems and roots, respectively, of licorice as novel, edible fresh foods is essential and is addressed for the first time in this preliminary study.

The present study reported that only young root extracts of the 20-day old microgreens provided functional protection (and to a lesser extent, the stems) in terms of preserving cell proliferation and viability, a protection that was not demonstrated by the leaf extracts. Interestingly, root extracts were shown to contain a two-fold less polyphenol (including flavonoid) content compared to that of leaf extracts, but with a five-fold lower anti-radical scavenging activity. This indicated a differing composition of polyphenol (flavonoid) molecules expressed in the immature root and leaves, respectively. Although, equivalent polyphenol contents, from each of the different tissue extracts, were administered to the Caco-2 cell lines to investigate potential *in vitro* protective effects, the anti-radical activity in the root extracts was over two-fold lower for the same overall polyphenol content. Notwithstanding the significantly lower anti-radical activity, the findings demonstrated that, root extracts exerted an *in vitro* protective effect on the proliferation and viability of LPS-treated Caco-2 cells. This suggests that the protective efficacy of the root extracts (and to some degree, the stem extracts) resides in inhibiting the pro-inflammatory cascade and resultant cytotoxic effects (NO- and ROS- induced damage) as opposed to a direct anti-radical scavenging activity. In contrast, the LPS-induced inflammatory cascade was not inhibited by the leaf extracts, and the resultant damage could evidently not be offset despite the significantly higher innate anti-radical scavenging activity. Possibility protection by leaf extracts was not afforded either because the resultant NO and ROS incurred by inflammation was extreme or cell viability (apoptosis) was compromised by alternative inflammation-induced mechanisms. This corroborates the notion that the stoichiometry for anti-oxidant quenching of pro-oxidative enzyme (or radicals) is one to one, and that bioavailability of polyphenol extracts is unlikely to be sufficient to afford direct anti-oxidant rescue from the extent of oxidation present [10].

Present results indicate that unlike leaf tissue extracts, root and to a lesser degree stem extracts may contain specific anti-inflammatory polyphenols, able to reduce the expression of multiple genes regulated by NF-κB p65. In a meta-analysis review article by Yang et al. [7], 13 root-based flavonoids (licochalcone A, licochalcone B, licochalcone C, licochalcone D, licochalcone E, isoangustone A, isoliquintigenin, licoricidin, glabridin, echinatin, licorisoflavan A, dehydroglyasperin C and dehydroglyasperin D) were identified and shown to demonstrate *in vitro* anti-inflammatory effects on LPS-induced cell lines. The mode of action resulted in reduced levels of TNF-α, IL-6, NO, thereby affording protection to the cell lines [7]. Moreover, the presence of

licochalcone A, licochalcone C, isoangustone A, isoliquiritigenin, dehydroglyasperin C and dehydroglyasperin D (and others) were also shown to upregulate the activities of SOD, CAT, GSH via Nrf2-ARE signaling [8,11]. Interestingly, all of the above anti-inflammatory (anti-oxidant) compounds were not identified amongst the vast array of flavonoid molecules extracted from the mature leaf tissue, as reported by Kim [6] and authors therein. Hence, it is possible that in young seedlings, anti-inflammatory flavonoids, as well as antioxidant signaling molecules, were similarly not expressed in leaf tissue. To verify a specific anti-inflammatory effect, or lack thereof, necessitates investigating the presence of standard specific inflammatory markers upstream of cellular proliferation and vitality, such as TNF-α, IL-6, IL-1, Il-8 and iNOS RNA and protein under LPS stimulation [7,12,15,16].

The present study did not examine the absorption or apparent permeability of the leaf, stem and root extracts into the Caco-2 cell lines to ascertain whether differences *in vitro* protective effects were associated with differences in bioavailability. Though this aspect requires verification, previous work demonstrated that Caco-2 cells exhibit an excellent absorption of some flavonoids from licorice [7,24,25]. Wang et al. [25] studied the absorption of various root licorice compounds using Caco-2 cell lines. Interestingly, among those compounds studied [25], 5 corresponded to the set of 13 identified as showing evident *in vitro* anti-inflammatory protection in other cell lines [7]. Of these 5 compounds, 4 were well-absorbed compounds and were transported predominantly through passive diffusion by the transcellular pathway, whereas the remaining compound was designated as moderately absorbed [25]. Results of the present study corroborate the absorption and subsequently anti-inflammatory protection in LPS-induced Caco-2 only in root and to some extent in stem extracts. Given that there is less research on leaf extracts, it is not possible to ascertain whether greater absorption difficulties were encountered among the array of leaf polyphenols. However, even if an equivalent absorption is assumed, as was reported previously [10], the amount of available molecules would be unlikely to be sufficient to afford direct anti-radical rescue, despite the higher anti-radical activities. Moreover, a higher dose of GAE equivalents would then be expected to improve scavenging and not worsen cell proliferation and viability, as was evident in extracts incubated with leaf extracts.

The present study also did not identify the specific polyphenol molecules responsible for the anti-inflammatory properties in licorice. The preliminary objective was to investigate whether functional potential in licorice micro-green tissues existed prior to identifying the responsible molecules. Given that there are no linear correlations between quantitative bioactive compound measurements and functional potential and that functional potential can only be effectively assessed from preliminary *in vitro* cell models (and subsequently verified with animal and human trials) [28], the approach taken was to investigate functional potential. Anti-inflammatory functions are generally evaluated *in vitro* by the quantification of several parameters such as chemokine and cytokine expression and release together with other factors including cell viability and proliferation. Regarding Caco-2 cells, it has been reported that LPS can affect cellular viability as well as the integrity of the intestinal epithelial barrier [29]. Although not a typical experiment, the effect of LPS and licorice extracts on cellular viability was performed as a preliminary analysis of the consequences of the inflammatory effects (LPS) and anti-inflammatory effects (licorice extracts) on Caco2 cells. More specific analysis regarding the inflammatory mechanism should be performed, as objective of a new and more specific work.

Dose-dependency of polyphenol extracts play an important role on cell proliferation and cell viability, suggesting signal transduction-mediated effects. Results show that the presence of leaf extracts (particularly at 2.5 μg GAE/ml) in LPS-treated Caco-2 cells exacerbated damage to level that was significantly worse than that induced by LPS damage alone. Previous results have shown the importance of dose dependency of individual flavonoid compounds. Whilst lower concentrations of well-known molecules such as quercetin and resveratrol exert beneficial effects at lower concentration ranges, higher ranges (5–10 fold higher than the cytoprotective contents) induce cytotoxicity resulting in apoptosis [26,27], a feature that is currently being favorably exploited in cancer treatments [27]. Additional research is warranting to further investigate the dose-dependent effects, specifically of leaf extracts. This aspect is important in ascertaining concentration range able to promote cytoprotective affects, and how this relates to the content potentially ingested in the form of microgreens.

5. Conclusions

Similar to mature licorice plants, the immature microgreen roots also demonstrate functional protection. Cell proliferation and vitality was protected in Caco2 cells under inflammatory LPS stimulus. The overall protection of the cells implicated the effective absorption of root polyphenols into the Caco-2 cells, as well as the efficacy in reducing inflammatory LPS stimulation of multiple NF-κB p65 regulated genes that induce NO and ROS damage affecting cell integrity. It is, therefore, recommended that the roots not be discarded at harvest, common practice in microgreen cultivation, but consumed fresh with the remainder of the seedling. Some protection is also afforded by the stems, but to a lesser extent to that afforded by the root tissue. Despite a higher overall anti-radical capacity, the young leaf material offered no anti-inflammatory protection to LPS-treated Caco-2 cells. This suggests that leaf does not have the same compliment of anti-inflammatory polyphenols, corroborating previous work [6,7,25]. Moreover, the leaf extracts worsened damage to cell proliferation and vitality over and beyond LPS alone, suggesting that the doses administered may have induced apoptosis, an aspect that warrants further investigation.

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Conflict of interest

All authors declare no conflicts of interest in this paper

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