Antioxidant properties and quantitative UPLC-MS/MS analysis of phenolic compounds in dandelion (*Taraxacum officinale*) root extracts

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ABSTRACT

Introduction: There is an increasing effort worldwide to investigate the antioxidant efficacy of traditional medicinal plants, which may be used to alleviate oxidative stress synonymous with the pathophysiology of many metabolic disorders as well as prevent oxidative deterioration of food products. In this study the antioxidant potential of dandelion (*Taraxacum officinale*) root was evaluated. **Methods:** The radical scavenging and antioxidant activities of solid-liquid extracts from freeze dried dandelion root was measuring *in vitro* using the modified DPPH and FRAP colorimetric assay. The phenolic content was also evaluated *in vitro* using the Folin-Ciocaltteu colorimetric assay. Subsequent identification and quantification of phenolic in active extracts was carried out using UPLC-MS/MS. **Results:** The ethyl acetate crude extract (E3) demonstrated the highest antioxidant activity for both the DPPH (227.728 ± 11.849 mg TE/g) and FRAP (463.066 ± 3.942 mg TE/g) assays. This extract also contained the highest phenolic content (228.723 ± 2.392 mg GAE/g). UPLC-MS/MS was used to identify and quantify phenolic compounds from E3 extract using an internal library of 36 phenolic standards. In total 18 phenolic compounds were quantified with chlorogenic acid, caffeic acid, syringic and vanillic being the most abundant compounds. The flavonoids apigenin-7-*O*-glycoside, luteolin-7-*O*-glycoside and naringenin-7-*O*-glycoside were detected for the first time in dandelion root. **Conclusion:** The results of this study suggest that dandelion root is a good source of natural antioxidants and could be used in foods with the potential to delay the onset of diseases linked to metabolic derived reactive oxygen species (ROS).

Keywords: *Taraxacum officinale*; Polyphenols; Radical scavenging; Antioxidant activity; UPLC-MS/MS.

1. INTRODUCTION

Prolonged exposure to metabolically derived reactive oxygen species (ROS) can cause an imbalance between radical species and *in vivo* physiological antioxidant defences. As a result, ROS have the potential to bind and irreversibly oxidise nucleic acids, proteins, lipids, carbohydrates and DNA.[1] Failure to repair or remove these oxidised molecules may lead to cellular dysfunction and mutation, which is associated with the pathophysiology of many age-related diseases.[2] Many dietary phytochemicals, particularly phenolic compounds, have demonstrated antioxidant characteristics in various disease states such as diabetes,[3] liver disease,[4] cardiovascular disease[5,6] and cancer.[7] Consequently, the commercial development of plants as sources of antioxidants for health and nutritional purposes is of great interest worldwide.

Dandelion (*Taraxacum officinale*) is a perennial herb found throughout Europe and N. America but is often regarded as a weed. However, dandelion plant tissues are often used in salads (leaf) and as a coffee substitute (root), while extracts of dandelion have also been used as flavour enhancers in soft drinks and baked goods.[8] In addition, dandelion is often marketed as a health food due to its history as a medicinal plant used to alleviate symptoms

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of inflammation, arthritis and liver disorders as well as having diuretic, choleretic and laxative properties.[9] Dandelion root has been reported to possess antioxidant activity that is linked to the presence of phenolic based compounds.[10–12] In spite of this, there is little evidence available that clearly identify the specific phenolic compounds responsible for the antioxidant activity within extracts of dandelion. In addition to having essential roles in the growth, reproduction and protection (phytoalexins) of plants themselves, phenols can play important roles as natural antioxidants in foods[13] and *in vivo*.[6]

Despite the limited number of recent studies into the antioxidant activities of extracts from dandelion root, there is reasonable evidence to support the use of dandelion tissues as natural sources of antioxidants, largely due to the presence of phenolic compounds. Detailed knowledge of the identity and quantity of the compounds responsible for the antioxidant activity of dandelion plants is essential if they are to be exploited as a source of compounds with the ability to alleviate the oxidative stress associated with some diseases. However, to date investigations into the antioxidant activity and phenolic content of dandelion have principally concentrated on the aerial parts and often only the evaluation of single crude extracts. For example, chromatographic analysis of a dandelion flower ethyl acetate fraction revealed the presence of luteolin, luteolin-7-O-glycoside, caffeic acid and chlorogenic acid,[14,15] while the luteolin, chicoric and total phenolic content leaf extracts has also been measured spectrophotometrically.[16] In the root itself, the presence of phenolic compounds such as chicoric acid, chlorogenic acid and monocaffeyltartaric acid have been reported from commercial root coffee and capsules.[17] In the present study we have adopted a sequential extraction approach in combination with molecular weight cut off (MWCO) dialysis in an attempt to reveal more detailed information about the antioxidant activity and phenolic content of multiple crude extracts. Following the selection of strongly active crude fractions for further evaluation of phenolic profiles by UPLC-MS we also aim to reveal more about the identity of phenolic compounds in antioxidant active extracts. Recently, we have demonstrated that this approach was beneficial in the identification and quantification of 18 phenolic compounds from fenugreek seeds (*Trigonella foenum-graecum*) and 13 compounds from bitter melon fruit (*Momordica charantia*).[18] In the present study, extracts with high antioxidant activity coupled to high phenolic content were analysed by UPLC-MS using an internal library of 36 known phenolic standards that was developed to deliver rapid and precise quantification of phenolic compounds present in dandelion root.

2. MATERIALS & METHODS

2.1 Materials and reagents

2,2 Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4, 6-tri(2-pyridyl)-1, 3, 5-triazine (TPTZ) ferrous sulphate heptahydrate, Iron(III) chloride hexahydrate, Folin-Ciocalteu (2N), sodium carbonate (Na_2CO_3), gallic acid, magnesium sulphate ($MgSO_4$), Dimethylsulfoxide (DMSO) (ACS spectrophotometric grade), formic acid (MS grade), the polyphenolic standards; quinic acid, ascorbic acid, protocatechuic acid, gallic acid, pyrocatechol, pyrogallol, procyanidin B_1, procyanidin B_2, epigallocatechin gallate, chlorogenic acid, epicatechin, catechin, syringic acid, caffeic acid, vanillic acid, verbascoside, rutin, narirutin, luteolin 7-O-glycoside, epicatechin gallate, ellagic acid, sinapic acid, ethyl gallate, ferulic acid, 3-coumaric acid, 4-coumaric acid, oleuropein, phlorizin, narigenin 7-O-glycoside, apigenin 7-O-glycoside, rosmarinic acid, myricetin, capsaicin, quercetin, kaempferol, and luteolin were obtained from Sigma-Aldrich Ltd. (Wicklow, Ireland). HPLC grade hexane, dichloromethane, acetone, ethyl acetate, methanol, acetonitrile and water along with BioDesignDialysis Tubing™ with 3.5 kDa cut off were purchased from Fisher Scientific Ltd. (Loughborough, Leicestershire, UK). Spectra/Por® Biotech cellulose ester dialysis tubing with 100 kDa cut- off was acquired from Apex Scientific (Co. Kildare, Ireland).

2.2 Plant material

Dandelion roots were collected and purchased from Irish Organic Herbs Ltd. (Drumshambo, Co. Leitrim, Ireland). A voucher specimen (DBN 27:2013) of dandelion was deposited in the Herbarium of the National Botanic Gardens (Dublin, Ireland). The sample was subsequently identified by a trained botanist as the microspecies *Taraxacum officinale* F.H. WIGG. The remaining roots were thinly sliced into disks (< 10 mm) and freeze dried in an A12/60 Freeze Dryer (Frozen In Time Ltd., York, England). The freeze dried roots were then blended using a Waring Commerical Blender (Christison Particle Technologies, Gateshead, UK) to a fine powder prior to extraction.

2.3 Extraction

700 grams of dandelion root powder was extracted exhaustively at room temperature using a sequential solid liquid extraction process with hexane (E1), dichloromethane (E2), methanol and water (E5) in that order. The extraction process involved mixing powder and solvent (1: 10) vigorously using a MaxQ6000 shaker

(Thermo Scientific, Iowa, USA) for 3 hours at 70 rpm. A Buchner funnel was used to filter the extract. The powder was washed three times using the same solvent followed by another 3 hour extraction, again using the same solvent (1: 10). After 3 hours the extract was filtered and washed as previously mentioned. Finally, the powder was extracted overnight using the same solvent (1: 20). The powder was then extracted by the next solvent in the sequential extraction process. Extracts of the same solvent were pooled together and dried using either a rotary evaporator (Rotavapor-R220, Bucchi Ltd., Switzerland) at 40°C, and/or a freeze dryer and stored at –80°C. Solvent partitioning of the methanol extract was carried out by first dissolving the extract in water and then washing repeatedly with ethyl acetate (methanol-hydrophobic extract) in a separating funnel. The methanol hydrophobic partition (E3) was filtered through magnesium sulphate ($MgSO_4$), under suction, to remove traces of water. The extract was then dried at 40°C using a rotary evaporator. The remaining water soluble extract (methanol-hydrophilic, E4) was freeze dried and reconstituted in a small volume of water. Fractionation of this extract based on molecular weight was performed using dialysis tubing with a molecular weight cut off (MWCO) of 3.5 kDa. The water crude extract (E5) was also subjected to fractionation by dialysis using tubing of 3.5 kDa and 100 kDa MWCO respectively. All dialysis extracts were freeze dried and stored at –80°C. In total, 11 extracts were generated from dandelion root. This included hexane (E1), DCM (E2), ethyl acetate (E3), methanol-hydrophilic (E4), methanol-hydrophilic <3.5 kDa (E4 <3.5 kDa), methanol hydrophilic >3.5 kDa (E4 >3.5 kDa), water (E5), water <3.5 kDa (E5 <3.5 kDa), water >3.5 kDa (E5 >3.5 kDa), water <100 kDa (E5 <100 kDa) and water >100 kDa (E5 >100 kDa) crude and dialysed extracts. Samples of these extracts were prepared for *in vitro* bioassay screening by dissolution in methanol containing 2% DMSO.

2.4 Diphenylpicrylhydrazyl (DPPH) assay

The free radical scavenging activity of each extract was measured using a method previously described method.[18] The assay was repeated in triplicate for all plant extracts and the results were expressed as both IC_{50} values (mg/ml) and also Trolox® equivalents (TE) per gram of extract (mg TE/g extract).

2.5 Ferric Reducing Antioxidant Power (FRAP) assay

This method was based on the assay proposed by Kenny et al.[18] The experiment was repeated in triplicate for all samples and results were expressed as Trolox® equivalents (TE) per gram of extract (mg TE/g extract) using the equation of the line for the Trolox® standard curve.

2.6 Total phenolic content

Dandelion extracts were evaluated for their total phenolic content using a modified version of the Folin-Ciocalteu assay.[18] The results were expressed in terms of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract), according to the equation of the line for the gallic acid standard curve.

2.7 Analysis of extracts by UPLC-MS

Ultra Performance Liquid Chromatography (Waters Acquity) managed by Acquity console software (Waters Corp., Milford, USA) coupled to triple quadrupole mass spectrometry (Waters Acquity TQD, Waters Corp., Milford, USA) (UPLC-MS) was carried out on the ethyl acetate dandelion extract based on its potent antioxidant properties and high phenolic content. The extract was dissolved in methanol, centrifuged at 14,000 rpm and syringe filtered using 0.22 μm polyvinylidene diflouride (PVDF) filters. In order to ensure that the detected concentrations of polyphenols were within the range of their respective standard curves for each standard, a series of high (20 mg ml^{-1}), medium (2 mg ml^{-1}) and low (0.2 mg ml^{-1}) concentration dilutions of the extract were prepared. Separation was achieved using a Waters Acquity UPLC HSS T3 (2.1 × 100 mm, 1.8 μm) column with a Waters C18 VanGuard (5 mm × 2.1 mm, 1.8 μm). The starting condition for each run was 98:2 mobile phase A (water + 0.5% formic acid): mobile phase B (ACN + 0.5% formic acid) held for 1 minute, with a ramp to 90:10 (A: B) by 2.5 minutes, then to 80:20 (A: B) by 3 minutes and held for 3 minutes. Further gradient increases to 65:35 (A: B) by 7.5 minutes with a final change to 10:90 (A: B) by 8.5 minutes and held for 1 minute and finally reconditioned to initial starting conditions. The mass spectrometry was operated in negative mode electrospray ionisation (ESI). The source was set 120°C and desolvation temperature to 350°C. Capillary voltage was set at 2.8 kV and the cone voltage was optimised for each of the compounds by using IntelliStart™ software (Waters Corp., Milford, USA). Nitrogen gas was used as both sheath gas and auxiliary gas (800 L hr^{-1} and 50 L hr^{-1} respectively). Detection of the 36 phenolics was conducted in multiple reaction monitoring (MRM) mode by analysing two transition ions per compound. The MRM conditions were determined and optimised by tuning the phenol standards using Waters integrated IntelliStart™ software (Waters Corp., Milford,USA). Detected

phenolic compounds were quantified against standard curves generated with commercial standards. Results were expressed as mg compound per 1 g of extract (mg compound g extract^{-1}).

3. RESULTS AND DISCUSSION

3.1 Antioxidant activity and phenolic content

The anti-radical scavenging and antioxidant activities of 11 crude and dialysed extracts from freeze dried dandelion root powder were evaluated using the DPPH and FRAP assays, while the Folin Ciocalteu assay was used to measure their total phenolic content. The results obtained from each assay are shown below in Table 1.

Results from the DPPH assay indicate that the ethyl acetate extract (E3) exhibited the highest anti-radical scavenging capacity, in terms of Trolox Equivalents (227.728 ± 11.849 mg TE g^{-1}), when compared to all other dandelion extracts analysed. The results also showed that at a concentration of 0.033 ± 0.002 mg ml^{-1}, the ethyl acetate extract was capable of reducing the absorbance (at 515 nm) of the DPPH radical by 50% (IC_{50}). This concentration is approximately 14-fold lower than that of the second most active extract, the water < 3.5 kDa extract (E5 < 3.5 kDa), which had an IC50 value of 0.461 ± 0.032 mg ml^{-1}. However, in comparison to the non-dialysed water extract (2.356 ± 0.069 mg ml^{-1}), E5 < 3.5 kDa demonstrated a higher radical scavenging capacity. Similarly, the dialysis >3.5 kDa methanol hydrophilic extract (E4 > 3.5 kDa) resulted in an increase in antioxidant activity when compared to the non-dialysed DRE4 extract. Therefore the use of dialysis in this case has proven beneficial for enriching the levels of antioxidant compounds in water soluble extracts. The hexane extract (E1) possessed the least activity (0.122 ± 0.010 mg TE g^{-1}), while the activities of the water >3.5 kDa (E5 > 3.5 kDa) and >100 kDa (E5 > 100 kDa) were also quite low respectively.

The results of the FRAP assay (Table 1), reported as Trolox Equivalents, are largely in agreement with those of the DPPH assay (Fig. 1). This is highlighted by the relatively high Pearson correlation value (R^2 = 0.998). The ethyl acetate extract again had the highest antioxidant capacity (463.066 ± 3.942 mg TE g^{-1}) in comparison to all other dandelion extracts screened. Meanwhile, fractionation of the water extract (2.250 ± 0.042 mg TE g^{-1}) by dialysis

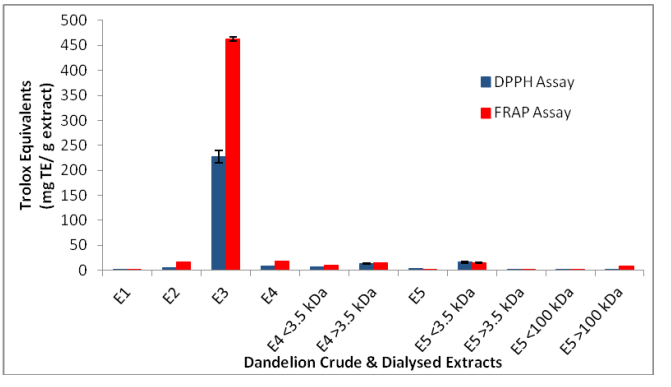

Figure 1. Antioxidant activities of dandelion extracts from DPPH and FRAP assays. Values are expressed in terms of Trolox Equivalents (TE). Values are means ± standard deviation (n = 3).

Table 1 Antioxidant activity and phenolic content of crude and dialysed extracts from dandelion root.

Extract	IC_{50} (DPPH) (mg ml^{-1})	Trolox Eqv. (DPPH) (mg TE g extract^{-1})	Trolox Eqv. (FRAP) (mg TE g extract^{-1})	Gallic Acid Eqv. (mg GAE g extract^{-1})
E1	61.946 ± 5.212	0.122 ± 0.010	0.040 ± 0.001	5.096 ± 0.023
E2	1.589 ± 0.136	4.743 ± 0.423	16.007 ± 0.367	17.606 ± 0.355
E3	0.033 ± 0.002	227.728 ± 11.849	463.066 ± 3.942	228.723 ± 2.392
E4	0.809 ± 0.007	9.272 ± 0.079	17.823 ± 0.462	10.210 ± 0.116
E4 <3.5 kDa	0.977 ± 0.006	7.674 ± 0.049	10.509 ± 0.240	13.243 ± 0.332
E4 >3.5 kDa	0.559 ± 0.027	13.436 ± 0.668	15.973 ± 0.181	17.58 ± 0.059
E5	2.356 ± 0.069	3.185 ± 0.092	2.736 ± 0.198	9.491 ± 0.169
E5 <3.5 kDa	0.461 ± 0.032	16.314 ± 1.146	15.308 ± 0.672	15.25 ± 0.076
E5 >3.5 kDa	9.532 ± 1.900	0.811 ± 0.018	1.503 ± 0.198	5.059 ± 0.033
E5 <100 kDa	5.588 ± 0.046	1.342 ± 0.011	2.250 ± 0.042	15.386 ± 0.024
E5 >100 kDa	9.063 ± 1.078	0.836 ± 0.104	7.995 ± 0.232	9.825 ± 0.037

Values are expressed in; Trolox Equivalents (TE) and IC50 values for the DPPH assay, Trolox Equivalents for the FRAP assay and Gallic acid Equivalents (GAE) for the total phenolics assay. Values are means ± standard deviation (n = 3)

resulted in an increase in the antioxidant activity of the E5 < 3.5 kDa fraction (15.308 ± 0.672 mg TE g^{-1}). As with the DPPH assay, the hexane extract (0.040 ± 0.001 mg TE g^{-1}) and large molecular weight dialysed water fractions demonstrated low antioxidant activity.

The results of the total phenolic assay were expressed as Gallic Acid Equivalents (GAE) (Table 1). The highest phenolic content was observed in the ethyl acetate (E3) extract (228.723 ± 2.392 mg GAE g^{-1}). This suggests that E3 is a rich source of phenolic based compounds, which may also be responsible for its relatively high antioxidant activity. An increase in phenolic content in the <3.5 kDa dialysed water extract (15.25 ± 0.076 mg GAE g^{-1}) was detected when compared to the non-dialysed water extract (9.491 ± 0.169 mg GAE g^{-1}). The additional amount of phenolic based compounds within the E5 < 3.5 kDa extract may be responsible for the increase in antioxidant activity observed between this fraction and the non-dialysed crude. A high Pearson correlation can be seen between the phenolic content of these extracts and their antioxidant activities in both the DPPH ($R^2 = 0.995$) and FRAP ($R^2 = 0.996$) assays. This further suggests that the presence of phenolics plays an important role in the antioxidant activity of extracts from dandelion root.

At present, little is known about the antioxidant activity of root extracts from *Taraxacum officinale*. A study by Wojdylo et al[19] measured the antioxidant activity and phenolic content of crude methanol extracts (80% v/v) from 32 plants species, including dandelion roots. They reported that, in comparison with the other plants under investigation, the results of the DPPH (53.312 ± 1.191 mg TE g^{-1}) and FRAP (3.979 ± 0.776 mg TE g^{-1}) assays for dandelion root did not rank highly[24] as having antioxidant activity. Similarly the dandelion extract was found to have low phenolic content (0.126 ± 0.03 mg GAE g^{-1}). In contrast, the findings of the present study have shown the hydrophobic ethyl acetate fraction of the methanol extract to have considerably higher antioxidant activity (DPPH, 227.728 ± 11.849 mg TE g^{-1}. FRAP, 463.066 ± 3.942 mg TE g^{-1}) and phenolic content (228.723 ± 2.392 mg GAE g^{-1}) than the crude methanol extract of Wojdylo et al.[19] This difference is most probably due to the amount of water soluble material present in the methanol extract. In our study fractionation of the methanol extract into an ethyl acetate fraction (E3) and a water soluble fraction (E4) revealed that over 97% of the methanol extract was water soluble. The amount of polysaccharide material coupled with the low level of antioxidant activity seen in E4 (DPPH 9.272 ± 0.079 mg TE g^{-1}. FRAP 17.823 ± 0.462 mg TE g^{-1}) may explain the variation between these studies.

3.2 UPLC-MS/MS analysis

In a previous study we have shown UPLC-MS to be a useful and rapid tool in the quantification of phenolics from natural sources.[18] In the present study UPLC-MS analysis of E3 was carried against an internal library of phenolic standards in order to quantify known phenolic compounds, which may be responsible for its high antioxidant activity and phenolic content. The results of this analysis are shown in Table 2. A total of 18 phenolic compounds were detected and quantified from E3 extract. Chlorogenic acid (31.059 mg g^{-1}), caffeic acid (3.932 mg g^{-1}), syringic acid (2.1812 mg g^{-1}) and vanillic acid (1.694 mg g^{-1}) were present in the highest concentrations. HPLC-DAD phenolic quantification analysis by Wojdylo et al[19] showed a methanol dandelion root extract to possess both caffeic acid (0.726 ± 0.001 mg g^{-1}) and *p*-coumaric acid (0.021 ± 0.001 mg g^{-1}). Though the present study has detected smaller amounts of *p*-coumaric acid (0.006 mg g^{-1}) than reported by Wojdylo et al[19] large amounts of *m*-coumaric acid were found (0.224 mg g^{-1}). Williams et al[17] identified three caffeic acid esters from a methanolic (80% v/v) dandelion root extract including chlorogenic acid, chicoric acid and caftaric acid. However, no antioxidant evaluation of this extract was reported by Williams et al.[17] The study also highlighted the absence of flavonoid glycosides in root extracts in comparison to similar leaf, bract and flower

Table 2 Levels of phenolic compounds in an ethyl acetate extract (E3) of dandelion root as identified and quantified by UPLC-MS/MS.

Compound	Quantity (mg g^{-1})
Apigenin-7-*O*-glycoside	0.012
Caffeic acid	3.932
Chlorogenic acid	31.059
3-Coumaric acid	0.224
4-Coumaric acid	0.006
Epicatechin gallate	0.372
Ferulic acid	0.317
Gallic acid	0.334
Luteolin	0.301
Luteolin-7-*O*-glycoside	0.503
Naringenin-7-*O*-glycoside	0.031
Narirutin	0.002
Protocatechuic acid	0.535
Pyrocatechol	0.642
Pyrogallol	0.008
Quinic acid	0.022
Syringic acid	2.182
Vanillic acid	1.694
Total	**42.174**

Values are expressed as mg compound g $extract^{-1}$

extracts. However, the present study has identified small amounts of the flavonoid glycosides; apigenin-7-*O*-glycoside (0.012 mg g^{-1}), luteolin-7-*O*-glycoside (0.503 mg g^{-1}), and narirutin-7-*O*-glycoside (0.031 mg g^{-1}) in E3. Similarly, Wojdylo et al[19] was unable to detect either luteolin or ferulic acid in the methanol root extract, which were both detected and quantified in the present study (0.301 mg/g and 0.317 mg/g) respectively. This may be due to the increased detection sensitivity obtained in our study using MS/MS monitoring. Schütz et al[20] have used HPLC-MS to identify 43 phenolic compounds in 10% aqueous methanol (fraction 1) and pure methanol (fraction 2) extractions of a freshly harvested juice from dandelion root and herb. Fraction 1 consisted of a number of mono and di-caffeoylquinic acids, tartaric acid derivatives and hydroxycinnamates, where chicoric acid was found to be the most predominant compound. Fraction 2 contained a variety of chrysoeriol, quercetin and luteolin glycosides, including two luteolin-7-*O*-glycosides. However, 7 of the compounds detected in fraction 2 remain unidentified with a number of the quercetin glycoside compounds also requiring identification of the attached glycoside unit. In addition, the lack of standard phenolic compounds available in the Schütz et al[20] study means that a number of these compounds can only be considered to be tentatively identified. The limited number of standards used in the study also means that quantification of most of these reported compounds is unclear. Furthermore, the fact that there is no distinction between the root and herb juices means that it is unclear as to what number of these compounds are from the root alone. In contrast, the present study has quantified compounds in dandelion root using a library of known standards. This has facilitated the confident confirmation of the presence and quantity of these compounds in our extract.

The structure of phenols plays a pivotal role in their radical scavenging capacities. The antioxidant activity of hydroxybenzoic acids such as syringic acid a vanillic acid is determined by the positioning of the hydroxyl groups in relation to the carboxyl functional group, where the activity increases with the presence of additional hydroxyl groups.[21,22] However, the presence of methoxy groups in the 3 and 5 positions of vanillic and syringic acids decreases their antioxidant activity in comparison to trihydroxybenzoic acids such as gallic acid.[21] Therefore, vanillic acid and syringic acid can be described as mild antioxidant phenolic compounds. However, the high concentrations of these compounds in E3 suggest that they may still play important roles as antioxidants in dandelion roots. The antioxidant capacity of hydroxycinnamates is believed to be greater than that of hydroxybenzoic acids due to the insertion of an ethylenic group between the hydroxyl phenyl ring and carboxyl group in their basic structures.[21] This offers hydroxycinnamtes greater potential for hydrogen donation and radical stabilisation.[21] The SAR and relative concentrations of the hydroxycinnamate caffeic acid and its ester, chlorogenic acid, in E3 suggest that these phenols could be responsible for the majority of the overall antioxidant activity and phenolic content of this extract.

4. CONCLUSION

The present study evaluated the antioxidant activity and phenolic content of crude and dialysis enriched dandelion extracts. The dandelion ethyl acetate extract demonstrated the highest antioxidant activity and total phenolic content. Dialysis proved beneficial in increasing the antioxidant activity between the non dialysed water and <3.5 kDa water dandelion extracts. However, the increase in activity was not deemed sufficient to warrant further investigation especially when compared to the activity of E3 extract. Quantification of 18 phenols in E3 by UPLC-MS/MS analysis suggested that this extract was a good source of natural antioxidants, particularly chlorogenic acid, caffeic acid, syringic acid and vanillic acid. Though present in small amounts, apigenin-7-*O*-glycoside (0.012 mg g^{-1}), luteolin-7-*O*-glycoside (0.503 mg g^{-1}) and naringenin-7-*O*-glycoside (0.031 mg g^{-1}) are reported here for the first time in extracts of dandelion root. This study represents a comprehensive evaluation of the antioxidant capacity of dandelion root, where the high correlation between antioxidant activity and phenolic content suggests that phenolic compounds form an intrinsic part of the overall antioxidant activity of dandelion root. The fact dandelion root is already available as a commercial crop and food additive would suggest that the results of study may prove influential if dandelion root is to be considered as a source of natural antioxidants for inclusion in foods with the potential to delay the onset of diseases linked to metabolically derived ROS.

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