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Extraction and characterization of polyphenols from non-conventional edible plants and their antioxidant activities

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Abstract: Narrow leaf plantain, white clover, perennial ryegrass, and tall fescue are non-conventional edible plants having widespread existence in many temperate and Mediterranean regions worldwide. These edible plants represent valuable food resources, and genetic and genomic approaches to improving and utilising these plants for human consumption are ongoing. These plants have characteristic high antioxidant capacities attributed to their polyphenol composition. This study performed the screening of polyphenols by applying a high-throughput LC-ESI-QTOF/MS technique and determined their antioxidant potential. Seventy different polyphenols were detected with 25 compounds in narrow leaf plantain, 27 in white clover, 15 in perennial ryegrass and 14 in tall fescue. Total phenolic content (TPC) was highest in white clover (1.81 ± 0.16 mg GAE/g fresh weight (FW)), while total tannin content (TTC) was highest in perennial ryegrass (0.89 ± 0.04 mg GAE/g FW) compared with their three counterparts, respectively. Narrow leaf plantain and white clover had greater total flavonoid content (TFC) than perennial ryegrass and tall fescue. The results of this investigation provided valuable information about the unique phenolic composition and antioxidant potential of the studied non-conventional edible plants that could be used to promote their utilization in human foods, nutraceutical preparations and functional foods besides being used as a valuable source of polyphenols for different industrial sectors. Besides, the results of the study can also be used as a baseline information for the planned and targeted delivery of bioactive compounds like polyphenols to the animals by devising an appropriate feeding strategy based on the phenolic composition of these plants.

Keywords: Bioactives; extraction; polyphenols; characterization; LC-ESI-QTOF/MS; antioxidants; nutraceuticals

1. Introduction

Plants have been an integral source of important nutrients and bioactive compounds in human food since the prehistoric era (Pawera et al., 2020). Human dietary needs and cultural backgrounds have mainly dictated their use by humans. There are several thousand species of plants growing on our planet. Out of these, only 103 species contribute to 90% of the world's food supplies (Prescott-Allen & Prescott-Allen, 1990) and include plants consumed directly and those used to produce oils, spices and condiments for cooking. They are called food plants owing to the usage of their one or more parts as human food (Leal, Alves & Hanazaki, 2018). However, many thousand edible plants have remained underutilised despite having food value for humans. These plants are referred to as "unconventional food plants" (Prescott-Allen & Prescott-Allen, 1990) and include under-commercialized species having no market value due to requiring use of unique processing techniques for their utilisation (Lorenzi & Kinupp, 2014). According to an estimate, 27 thousand species of plants with the potential to be exploited for human food fall under this category (Irani, Khaled & Dutta, 2018) that include various species of native and exotic plants and cultivated and wild varieties. Out of these, 57% of

plant species are perceived to contribute significantly to food security, particularly during famine and pandemic affected areas (Cruz-Garcia, 2017).

Recently, these non-conventional plants are gaining importance particularly from the perspective of enormously increasing demand of food supply for the overwhelming human population that is estimated to exceed nine billion by 2050 (FAO, 2017) owing to their use as vital components of food and nutrition in the prehistoric era (Borelli et al., 2020). Therefore, a movement is building to intensify crop production sustainably through the domestication of non-conventional edible plant species (Crews et al., 2016), including narrow leaf plantain (Yang et al., 2020), white clover (Jakubczyk et al., 2021), perennial ryegrass (Halling, 2012) and tall fescue. These non-conventional edible plants are widely distributed in natural grasslands of the world and are usually used for animal feeding. However, these plant species can be exploited for human food using suitable processing techniques.

These plants are referred to as non-convention plants because vegetative parts of these plants (leaves, shoots, flowers and seeds) have been employed in human diets (Ray, Ray & Sreevidya, 2020). Aerial parts of narrow leaf plantain (*Plantago lanceolata*) have greater potential for human consumption and are considered healthy foods as ingredients of various meals and drinks because of their high nutritional value and aesthetic properties (Eldesoky et al., 2018). Extracts of *Plantago lanceolata* are also known for wound healing properties (Kovač et al., 2015). Dried flowers of white clover (*Tifolium repens*) could be used to make tea substitutes and the tender leaves can be used in sauces, soups and salads or can be cooked like spinach (Parente & Frame, 1993). Aerial parts of white clover have been applied as antirheumatic and depurative remedies (Kolodziejczyk-Czepas, 2016) and are known to have anti-diarrheal property (Kicel & Wolbiś, 2012). At the same time, perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*) are grass species that were consumed in some parts of the world, including Africa in times of famine and real emergency (Irvine, 1952). They were referred to as emergency food and should be consumed only when other options fail to meet the dietary needs.

A characteristic of the selected non-conventional edible plants is their high antioxidant capacities attributed to their polyphenol composition (Bahadori et al., 2020; Jakubczyk et al., 2021), minerals and vitamins. While vitamin and mineral composition of these plants have been well documented in various scientific reports (Dodd et al., 2017; Minneé, McCready & Woodward, 2017), knowledge of their polyphenolic profiles and their related antioxidant activity is limited. Moreover, minerals and vitamins did not explain the health-promoting effects of plants and their extracts, and research has been directed to search for the health-promoting polyphenols from the wild edible plants (Åhlberg, 2021). Due to their promising antioxidant activities and health benefitting properties, polyphenols are being utilised in food manufacturing and processing industries as food additives in various drinks including black tea, coffee and beer (Shahidi & Ambigaipalan, 2015) as flavour enhancers and foods like meat and meat products to prevent the oxidation of food components (lipids and proteins) to enhance their shelf life (Papuc et al., 2017). Therefore, polyphenols are considered very important functional components in human nutrition (Bayir, Aksoy & Koçyiğit, 2019). Given the importance of polyphenols in the food system, phenolic profile of narrow leaf plantain, white clover, perennial ryegrass and tall fescue, as well as their antioxidant potential were investigated in this study. These plants produce some unique polyphenols as their secondary metabolites. For example, narrow leaf plantain produces a phenolic acid plantamajoside that is well known for its wound healing properties (Ashkani-Esfahani et al., 2019; Zubair et al., 2016).White clover contains isoflavones that represent the main phytoestrogens of current interest as nutraceuticals and dietary supplements (Cornwell, Cohick & Raskin, 2004). Further, no scientific report is available about the phenolic composition of perennial ryegrass and tall fescue as per our understanding. These facts were the main impetus for the selection of plants. This investigation can provide important baseline information for the optimum application and utilization of the selected plants in human foods, nutraceuticals and functional foods. Further, the results of the study can be used to devise an appropriate feeding strategy through feed supplementation for targeted delivery of bioactive compounds to animals through feed.

2. Materials and Methods

2.1. Collection and processing of plant materials

Aerial parts (stems and leaves) of narrow leaf plantain, white clover, perennial ryegrass and tall fescue were collected from the Hamilton Research Station, Agriculture Victoria Research, Department of Jobs, Precincts and Regions, Hamilton, Victoria, 3300, Australia. The details of cultivars, growing season, and harvesting stage of non-conventional edible plants used for screening and quantification of polyphenols are given in Table 1. Upon collection, approximately 1000 g individual sample was put into separate plastic bags as entire plants and transported to the University of Melbourne, Parkville 3030 in a coolbox with ice and processed immediately for extraction of polyphenols.

[Table 1 about here]

2.2. Chemicals and reagents

Analytical grade ethanol, methanol, hydrochloric acid, sodium acetate, and glacial acetic acid were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). H_2SO_4 (98%) was procured from RCI Labscan (Rongmuang, Thailand). Na_2CO_3 was procured from Chem-Supply Pty Ltd. (Adelaide, Australia). Folin-2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Ciocalteu reagent, 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, quercetin, vanillin, gallic acid, $AlCl_3.6H_2O$, $FeCl_2$, Ferrous sulphate, hydrogen peroxide (H_2O_2), 3-hydroxybenzoic acid, catechin, ferrozine, trichloroacetic acid, potassium ferricyanide, and $FeCl_3$ were acquired from Sigma-Aldrich (Castle Hill, New South Wales, Australia). 2-hydroxybenzoic acid, catechin, coumaric acid, gallic acid, sinapic acid, syringic acid, quercetin-3-rhamnoside, *p*-hydroxybenzoic acid, epicatechin gallate and phloretic acid were used as standards in HPLC-PDA analysis.

2.3. Samples preparation

Individual samples (narrow leaf plantain, white clover, perennial ryegrass and tall fescue) were cleaned and 50g of the cleaned samples were made into a paste-like consistency by crushing in a mortar and pestle and stored at –20 ^{0}C until extraction and analysis of polyphenols.

2.4. Extraction of polyphenols

Ethanol (80%) was used for extraction of polyphenols from the individual samples. The ethanol-sample mixtures (~10g sample in 25mL of 80% ethanol) were homogenized in 50 mL tubes and incubated in a shaking incubator (ZWYR-240, Labwit, Ashwood, VIC, Australia) set at 120 rpm for 12 hr at 4 ^{0}C in the dark. Following incubation, samples were centrifuged at 5000 rpm for 20 min in Hettich Rotina 380R centrifuge machine (Tuttlingen, Germany) at 4 ^{0}C and supernatants collected into separate tubes. Prior to storage at –20 ^{0}C, the supernatants were filtered using 0.22 μm syringe filter and transferred to HPLC vials for characterization and further analysis.

2.5. Phenolics estimation and antioxidant potential

Estimation of phenolics was performed by measuring total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) and antioxidant potential was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing antioxidant power (FRAP) assay, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay, hydroxyl (OH^-) radical scavenging activity assay, chelating ability of ferrous ion (Fe^{+2}) (FICA) and reducing power assay (RPA) according to the method of (Iqbal et al., 2021) explained in the supplementary material. Multiskan® Go microplate photometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to read the absorbance. Quantification was performed by constructing standard curves with $r^2 \geq 0.99$ by making standard solutions of various concentrations and the results are reported on a fresh weight basis.

2.6. Screening of polyphenols by LC-ESI/QTOF-MS

Polyphenols were characterised by our previously published method (Iqbal et al., 2021) on HPLC system (Agilent 1200 series) connected to Agilent 6520 Accurate-Mass Q-TOF LC/MS (Agilent Technologies, CA, USA) with an electrospray ionization (ESI) source. RP-80A Column (250 mm × 4.6 nm, 4 μm) was used for the analysis. Mobile phase A was composed of water/acetic acid solution (98:2 v/v) and mobile phase B was composed of water/acetonitrile/acetic acid (50:49.5:0.5, v/v/v). The gradient used was: 90% A, 0 min; 75% A, 20 min; 65% A, 30 min; 60% A, 40 min; 45% A, 70 min; 20% A, 75 min; 0% A, 77–79 min; 90% A, 82–85 min with a steady flow rate of 0.8 mL/min. Injection volume for each sample was set as 6 μL. Analysis of data was achieved on Agilent LC/MS/QTOF Mass Hunter Data Acquisition Software (Version B.03.01).

2.7. Quantification of polyphenols

Polyphenols were quantified by our previously published method (Iqbal et al., 2021) on HPLC system (Waters Alliance 2690, Chromatograph Separation Module) equipped with a PDA detector (Model 2998, Waters). RP-80A Column (250 mm × 4.6 nm, 4 μm) was used for the analysis. Mobile phases and gradient program were kept same as LC/MS analysis and 20 μL of each sample was injected for the analysis. Peaks were identified at 280 nm, 320 nm and 370 nm, with 1.25 scans/s. Empower Software (2010) (Shimadzu Scientific Instruments, Sydney, NSW, Australia) was used for the purpose of data analysis and quantification was performed by constructing standard curves with different concentrations of external standards.

2.8. Statistics analysis

Results for polyphenol contents and antioxidant activities were reported as means ± standard deviation of the values of 3 independent analyses and one-way analysis of variance (ANOVA) was performed to test for difference in means among the non-conventional edible plant species used, followed by the Tukey's honestly significant differences (HSD) multiple rank test ($p \leq 0.05$) by Minitab Program for Windows version 19.0 (Minitab, LLC, State College, PA, USA). For correlation between polyphenol contents and antioxidant activities, XLSTAT 2021.2.1 (Addinsoft Inc., NY, USA) was used.

3. Results and Discussion

3.1. Phenolics estimation from non-conventional edible plants

The polyphenol content of sample extracts was determined as total phenolic content (TPC), (total flavonoids content (TFC) and total tannins content (TTC) and results are reported on fresh weight basis. Polyphenol content varied significantly among plant species used in this study (Table 2). White clover showed the highest value (1.81 ± 0.16 mg GAE/g fresh weight) and narrow leaf plantain showed the lowest value for TPC (0.64 ± 0.07 mg GAE/g fresh weight). Whereas, TPC values for perennial ryegrass and tall fescue were moderate and the concentrations were 0.68 ± 0.07 mg GAE/g fresh weight and 0.89 ± 0.04 mg GAE/g fresh weight, respectively. TFC value was highest for narrow leaf plantain (0.12 ± 0.01 mg QE/g fresh weight), lowest for perennial ryegrass (0.01 ± 0.01 mg QE/g fresh weight) and intermediate for clover and tall fescue. No significant difference was observed in TFC values of narrow leaf plantain (0.12 ± 0.01 mg QE/g fresh weight) and white clover (0.10 ± 0.01 mg QE/g fresh weight). A similar trend was also observed for TFC values of perennial ryegrass (0.01 ± 0.01 mg QE/g fresh weight) and tall fescue (0.02 ± 0.01 mg QE/g fresh weight). Total tannins content varied significantly among the plant materials used in this study. TTC value was highest for perennial ryegrass (2.11 ± 0.06 mg CE/g fresh weight) as compared to lowest for tall fescue (0.66 ± 0.03 mg CE/g fresh weight) while the other two plant had moderate concentrations and values for narrow leaf plantain and white clover were 0.86 ± 0.01 mg CE/g fresh weight and 1.17 ± 0.05 mg CE/g fresh weight, respectively. The extreme variations in TPC, PFC and TTC among these plant species imply that their impact on alleviating parasite burden, methane emission or nutrient loss in the rumen might be different.

[Table 2 about here]

3.2. Antioxidant activities of non-conventional edible plants

Phenolic acids and flavonoids were detected in all plant materials used in this study and are well known for their strong antioxidant capacity (Dorta et al., 2014). The antioxidant potential of sample extracts was determined by DPPH, FRAP, ABTS, OH^- radical scavenging ability, chelating ability of Fe^{+2} and reducing power assays and results were reported on fresh weight basis (Table 3). These assays are extensively used for the determination of the antioxidant activities of plant extracts. Narrow leaf plantain showed the highest value (0.52 ± 0.01 mg AAE/g) for DPPH assay and perennial ryegrass showed the lowest value (0.05 ± 0.01 mg AAE/g). The DPPH values for white clover and tall fescue were 0.26 ± 0.01 mg AAE/g and 0.34 ± 0.01 mg AAE/g respectively. Narrow leaf plantain also showed the highest value (0.74 ± 0.01 mg AAE/g) for ABTS assay and lowest value of ABTS was observed for tall fescue (0.16 ± 0.01 mg AAE/g). The ABTS value for white clover and perennial ryegrass were 0.63 ± 0.02 mg AAE/g and 0.43 ± 0.01 mg AAE/g respectively. The FRAP values for the plants used in this study remained the lowest among all the antioxidant assays performed in this study. Narrow leaf plantain showed the highest value (0.05 ± 0.01 mg AAE/g) for FRAP, white clover showed moderate value (0.04 ± 0.01 mg AAE/g) for FRAP and perennial ryegrass and tall fescue had the lowest, and FRAP value for both these plants was 0.01 ± 0.01 mg AAE/g.

All the plant extracts showed very high hydroxyl (OH^-) scavenging ability. Tall fescue showed the highest value (15.41 ± 0.43 mg AAE/g) for hydroxyl (OH^-) scavenging ability. Hydroxyl (OH^-) scavenging ability of perennial ryegrass was comparable to tall fescue (14.90 ± 0.32 mg AAE/g). Hydroxyl (OH^-) scavenging ability of narrow leaf plantain and white clover were 7.30 ± 0.11 mg AAE/g and 6.58 ± 0.14 mg AAE/g, respectively. Values for chelating ability of Fe^{+2} were 0.02 ± 0.01 mg EDTAE/g, 0.13 ± 0.02 mg EDTAE/g, 0.18 ± 0.01 mg EDTAE/g and 0.20 ± 0.02 mg EDTAE/g for narrow leaf plantain, white clover, perennial ryegrass and tall fescue respectively. Among the plants analyzed in this study, narrow leaf plantain showed the highest value for reducing power assay (1.04 ± 0.03 mg AAE/g). Reducing power of white clover (0.94 ± .03 mg AAE/g) was also comparable to narrow leaf plantain. Whereas, tall fescue (0.35 ± 0.01 mg AAE/g) and perennial ryegrass (0.25 ± 0.01 mg AAE/g) showed lower values for reducing power assay as compared to narrow leaf plantain and white clover. Among the plants studied in this study, narrow leaf plantain showed a superior antioxidant properties followed by white clover. Tall fescue presented the lowest antioxidant properties.

[Table 3 about here]

3.3. Screening of polyphenols by LC-ESI-QTOF/MS

Based on *m*/*z* values from mass spectra, polyphenols were putatively identified in negative ionization ($[M-H]^-$) and positive ionization ($[M+H]^+$) modes on an Agilent LC-ESI-QTOF/MS using the Mass Hunter Qualitative Software and Personal Compound Database and Library (PCDL). Compounds detected with mass error <±10 ppm were selected for *m*/*z* verification and characterization. Seventy (70) polyphenol compounds belonging to different subgroups were detected in the selected non-conventional edible plants and presented in Table 4 and the LC-ESI/QTOF-MS profiles are shown in Figure 1.

[Figure 1 about here]

Twenty seven compounds were detected in white clover (Tables S1 Supplementary materials) whereas twenty five, fifteen and fourteen compounds were identified in narrow leaf plantain, perennial ryegrass and tall fescue (Tables S2-S4 Supplementary materials) respectively. Most of the compounds identified in the studied non-conventional edible plants belong to the flavonoids and phenolic acids subgroups (Table 4). Lignans were identified in white clover and ryegrass whereas stilbenes were detected only in white clover.

[Table 4 about here]

3.3.1 Phenolic acids

Polyphenols exert strong effects on sensory attributes, and nutritional quality of foods (Maga & Katz, 1978). Sixteen phenolic acids were identified in the selected plants in this investigation. They represented members of various distinct subclasses including hydroxycinnamic acids (10), hydroxybenzoic acids (2), hydroxyphenyl propanoic acids (2), and hydroxyphenyl pentanoic acids (2). Majority of the phenolic acids extracted in this study were hydroxycinnamic acids. Sinapic acid was characterized in perennial ryegrass. It has also been identified in various rye varieities (Andreasen et al., 2000). Sinapic acid is considered safe and non toxic even at high concentration (Hameed, Aydin & Başaran, 2016) and is frequently found in human diets (Mustafa, 2019).

Caffeic acid 3-*O*-glucuronide, 1-feruloyl-5-caffeoylquinic acid, dihydroferulic acid 4-*O*-glucuronide and 5-(3'-methoxy-4'-hydroxyphenyl)-ɤ-valerolactone were characterized in tall fescue. Caffeic acid 3-*O*-glucuronide could be used in food supplements for boosting athletic performance and reducing exercise-related fatigue due to its higher antioxidant capacity as compared to other caffeic acid derivatives (Sova & Saso 2020). Cinnamoyl glucose, 3-caffeoylquinic acid, ferulic acid 4-*O*-glucoside, 1,2-disinapoylgentiobiose, 4-hydroxybenzoic acid 4-*O*-glucoside and 3-hydroxyphenylpropionic acid were characterized in narrow leaf plantain. 2,5-di-S-glutathionyl caftaric acid, cinnamic acid, m-coumaric acid, 2-hydroxyhippuric acid and 5-(3',5'-dihydroxyphenyl)-ɤ-valerolactone 3-*O*-glucuronide were identified in white clover. Cinnamic acid is considered safe for human consumption (Yilmaz, Sova & Ergün, 2018). It is a common food supplement in the food processing industry (Zhang et al., 2015) as a natural flavouring agent (Cháfer et al., 2009).

3.3.2 Flavonoids

Fourty one flavonoids belonging to seven different subclasses (isoflavonoids, flavonols, flavones, flavanones, flavanols, dihydrochalcones and anthocyanins) were detected in this study in the selected non-conventional edible plants.

Several studies emphasized on the health benefits of polyphenols particularly isoflavonoids in animals and humans (Khalighi-Sigaroodi et al., 2012; Moravčíková et al., 2012; Iqbal et al., 2020). The isoflavonoids represent a special group of polyphenols termed as phytoestrogens and can be applied for correction of hormonal disbalances as hormone replacement therapy due to their hormone like properties (Toiu et al., 2016). Nine isoflavonoids (compounds 17, 18, 19, 20, 21, 22, 23, 24 & 25) were charaterised in the selected non-conventional edible plants. 3'-hydroxygenistein was identified in all the selected plants. 3',4',7-trihydroxyisoflavanone was identified in white clover and narrow leaf plantain. Puerarin was identified in narrow leaf plantain. 5,6,7,3',4'-pentahydroxyisoflavone, pseudobaptigenin and dalbergin were identified in white clover. Dihydrobiochanin A, glycitin and 6''-*O*-acetylglycitin were characterized in perennial ryegrass. Dihydrobiochanin A exhibited potent activity against cariogenic bacteria (Ferrazzano et al., 2011).

Seven flavonol compounds (compounds 26, 27, 28, 29, 30, 31, 32 & 33) were charaterised. Isorhamnetin and 3-methoxysinensetin were characterised in white clover and narrow leaf plantain. Quercetin 3-*O*-glucosyl-xyloside was identied in white clover. Kaempferol 3,7,4'-*O*-triglucoside and patuletin 3-*O*-(2''-feruloylglucosyl)(1->6)-[apiosyl(1->2)]-glucoside were characterised in narrow leaf plantain. Patuletin 3-*O*-glucosyl-(1->6)-[apiosyl(1->2)]-glucoside and spinacetin 3-*O*-(2 were characterised in tall fescue. Isorhamnetin exhibited strong antibacterial activity (Bhattacharya et al., 2018). Isorhamnetin can also inhibit lipid peroxidation (Xiao et al., 2012). These properties make isorhamnetin an important agent in food preservation and extension of shelf life of meat products.

Four compounds with flavone backbone (compounds 33, 34, 35 & 36) were characterised from the selected non-conventional edible plants. Out of the four flavones, three compounds (luteolin 7-*O*-glucuronide, apigenin 6,8-di-C-glucoside and apigenin 7-*O*-(6''-malonyl-apiosylglucoside)) were characterised from narrow leaf plantain. Whereas, one compound (luteolin 7-O-diglucuronide) was characterised from perennial ryegrass. Luteolin-7-*O*-glucuronide has also been characterised from *Perilla frutescens*, *Remirea*

maritima, *Codariocalyx motorius*, and *Ixeris dentata* (Jeon et al., 2014; Karki et al., 2015). Apigenin is a flavonoid of low toxicity and multiple beneficial bioactivities (Wang et al., 2019). Apigenin has also been identified in *Scutellaria barbata* (Sato et al., 2000), *Castanea sativa* (Basile et al., 2000), *Portulaca oleracea L.* (Nayaka et al., 2014), *Marrubium globosum ssp. Libanoticum* (Rigano et al., 2007), *Combretum erythrophyllum* (Martini, Katerere & Eloff, 2004), *Aquilegia oxysepala* (Yu, Yi & Liang, 2007), and propolis (Koru et al., 2007). Apigenin can reduce dental caries in humans by inhabiting the growth of *Streptococcus mutans* that is the main causitive organism for dental caries (Wang et al., 2019).

Two flavanones (compounds 37 & 38) were characterised in this study. Geranylnaringenin was characterised from perennial ryegrass and neoeriocitrin was identified in narrow leaf plantain. Four flavanols (compounds 39, 40, 41 & 42) were characterised from the selected plants. (+)-catechin 3-*O*-gallate and (+)-gallocatechin 3-*O*-gallate were characterised from white clover. Whereas, (-)-epigallocatechin 3'-*O*-glucuronide and prodelphinidin dimer B3 were characterised from perennial ryegrass. Prodelphinidin dimer B3 has also been identified in barley (Klausen et al., 2010), beer and pomegranate peels (Plumb et al., 2002). Only one compound (43) belonging to the subfamily dihydrochalcones was identified in narrow leaf plantain as dihydromyricetin 3-*O*-rhamnoside.

The anthocyanins are well known for their cardioprotective effect because they provide protection to arteries, endothelial tissues, and inhibit aggregation of platelets (Carazzone et al., 2013). Fourteen anthocyanins (compounds 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 & 57) were characterised from the selected non-conventional edible plants. Cyanidin, cyanidin 3-*O*-xyloside and delphinidin 3-*O*-sambubioside were characterised only in white clover. Delphinidin 3-*O*-(6''-acetyl-galactoside), pelargonidin 3-*O*-rutinoside, malvidin 3,5-*O*-diglucoside and cyanidin 3-*O*-diglucoside-5-*O*-glucoside were characterised only in narrow leaf plantain. Delphinidin 3-*O*-glucosyl-glucoside, cyanidin 3-*O*-(6''-*p*-coumaroyl-glucoside), cyanidin 3-*O*-(6''-malonyl-3''-glucosylglucoside) and malvidin 3-*O*-(6''-caffeoyl-glucoside) were characterised in tall fescue only. Delphinidin 3-*O*-(6''-p-coumaroyl-glucoside) was identified in tall fescue, white clover and perennial ryegrass. Petunidin 3-*O*-(6''-*p*-coumaroyl-glucoside) was identified in tall fescue and perennial ryegrass. Whereas, delphinidin 3-*O*-feruloyl-glucoside was identified in perennial ryegrass and white clover.

Three lignans (compounds 58, 59 & 60) and two stilbenes (compound 61 & 62) were characterised in this work. Two lignans (dimethylmatairesinol and schisandrin) were characterised in perennial ryegrass. Whereas, one lignan (sesaminol 2-*O*-triglucoside) was characterised in clover. Two stilbenes (pinosylvin and d-viniferin) were identified in white clover. Two hydroxybenzoketones (compounds 63 & 64), one hydroxybenzaldehyde (compound 65), one curcuminoid (compound 66) and four other polyphenols (compounds 67, 68, 69 & 70) were characterised were also characterised. Hydroxybenzoketones (2,3-dihydroxy-1-guaiacylpropanone and 3-methoxyacetophenone) and hydroxybenzaldehyde (*p*-anisaldehyde) were characterised from narrow leaf plantain. The curcuminoid (demethoxycurcumin) was characterised from white clover. Out of the four other polyphenols, three compounds (arbutin, salvianolic acid G and pyrogallol) were were characterised from white clover. Whereas, salvianolic acid C was identified in perennial ryegrass and tall fescue. Arbutin is used as whitening agent in cosmetic products (Cho et al., 2011). Pyrogallol is a strong antioxidant and applied as oxygen scavenger in boilers (Ozturk Sarikaya, 2015).

3.4. Quantification of Polyphenols by HPLC-PDA

Ten polyphenol compounds were quantified from the plant samples using HPLC-PDA and data of the quantified polyphenols was presented in Table 5.

[Table 5 about here]

Overall, tall fescue and perennial ryegrass showed higher concentrations of sinapic acid as compared to clover and narrow leaf plantain. 2-hydroxybenzoic acid was also higher in perennial ryegrass as compared to narrow leaf plantain. Coumaric acid was highest in perennial ryegrass among the plants studied. Results of our study showed that coumaric acid concentration in narrow leaf plantain is 15.34 ± 0.3 μg/g in comparison to a previous

study that reported a higher concentration (24 ± 1 μg/g) of coumaric acid in narrow leaf plantain (Bahadori et. al., 2020). Gallic acid was more abundant in white clover as compared to narrow leaf plantain. Our results reported higher concentration (27.68 ± 0.6 μg/g) of gallic acid in narrow leaf plantain as compared to a previous study that reported gallic acid in concentration of 18 ± 1 μg/g in narrow leaf plantain (Bahadori et. al., 2020). Syringic acid was quantified in tall fescue (86.07 ± 1.6 μg/g) and narrow leaf plantain (30.93 ± 1.2 μg/g). Previously, syringic acid was quantified in narrow leaf plantain in concentration of 31 ± 1 μg/g by Bahadori et. al., (2020). Catechin was higher in concentration in narrow leaf plantain (34.73 ± 2 μg/g) in comparison to clover (18.77 ± 1.2 μg/g). Catechin was also quantified lower concentration in narrow leaf plantain (23 ± 1 μg/g) in a recent study (Bahadori et. al., 2020). The variation in concentrations of the phenolic compounds with previous report could be explained by the fact that plants grown under varying conditions in different climates produce varying amounts of these bioactive compounds (Kabtni et al., 2020). Quercetin-3-rhamnoside was more abundant in tall fescue than narrow leaf plantain, white clover and perennial ryegrass.

3.5. Correlation of polyphenol contents and antioxidant activities

Polyphenol compounds found in plants have strong antioxidant properties and contribute significantly to the antioxidant potential of plant extracts. Therefore, we determined the polyphenol contents (TPC, TFC and TTC) and antioxidant activities (DPPH, ABTS, FRAP, OH^- RSA, FICA and RPA) to measure the antioxidant potential of the selected plants. Considering the contribution of polyphenol towards antioxidant capacity of the plants studied, regression analysis was performed to evaluate the correlation between antioxidant activities and polyphenol composition of the selected plants (Table 6).

[Table 6 about here.]

Polyphenolic cotents (TPC, TFC and TTC) and the antioxidant activities (RPA, FRAP, ABTS, OH^- RSA) showed highly significant correlation. FICA also showed positive correlation with phenolic acids. This suggests that TFC and phenolic acids significantly contribute to the antioxidant potential of plant extracts (Gu et al. 2019). Interestingly, OH^- RSA and FICA showed highly significant negative correlation with TFC and DPPH, ABTS, FRAP and RPA were negatively correlated with phenolic acids. DPPH was also negatively correlated with TTC. ABTS showed highly positive correlation with FRAP and RPA but highly negatively correlated with OH^- RSA and FICA. FRAP showed highly significant positive correlation with RPA but highly significant negative correlation with OH^- RSA and FICA. OH^- RSA was highly positively correlated with FICA and phenolic acids. FICA showed positive correlation with phenolic acids but negatively correlated with RPA. From the results, it could be established that several factors affect the correlation of polyphenols with antioxidant activities that include concentration and diversity of polyphenol compounds, their synergistic effect, types of samples, compounds quantified, and assays applied. Overall, TFC showed strong correlation with antioxidant activities of the plants studied in this study.

Principal component analysis further showed that FICA and OH^- RSA were strongly correlated with phenolic acids and ABTS, FRAP, RPA and DPPH were more correlated with TPC and TFC (Figure 2). Moreover, ABTS, FRAP and RPA were negatively correlated with phenolic acids and OH^- RSA. TPC and TFC also showed negative correlation with OH^- RSA.

[Figure 2 about here]

Results also depicted that antioxidant potential of these plants varied significantly. This could be explained by the diversity of polyphenol compounds present in these plants, their concentration, synergistic and antagonistic action with other compounds in the plant matrix. It is possible that antioxidant activities could partially be attributed to non-polyphenol moieties present in these plants. This could also be explained by the effect of synergistic actions between vitamins and polyphenols or essential fatty acids and polyphenols.

4. Conclusions

Narrow leaf plantain, white clover, perennial ryegrass and tall fescue are valuable non-conventional edible plants having strong antioxidant potential attributed to their polyphenol composition that varied considerably among these plants. Present work provided the first report on the polyphenol composition of perennial ryegrass and tall fescue. The study results provided valuable insights about the polyphenolic profiles of these non-conventional edible plants that can serve as potential sources of natural antioxidants for utilisation in human foods, nutraceuticals and functional food formulations. In addition, the findings of the study can improve the utilisation of these plant species in animal production by taking into consideration the health benefits associated with the identified phenolic compounds. This study mainly focused on the identification of phenolic metabolites and antioxidant potential of the selected non-conventional edible plants. Therefore, it is recommended that further research studies involving parameters like safety concernes and the optimum concentrations of the identified metabolites should be conducted to assess the safety limits of the identified metabolites before their utilization in human foods and nutraceutical formulations.

Abbreviations: LC-ESI-QTOF/MS (Liquid chromatography-Electrospray ionization-Quadrapole time of flight/Mass spectrometry); TPC (total phenolic content); TFC (total flaovonoids content); TTC (total tannin content); GAE/g (gallic acid equalent per gram); FW (fresh weight); HPLC-PDA (high performance liquid chromatography-photodiode array detection); DPPH (2,2-diphenyl-1-picrylhydrazyl); FRAP (ferric reducing antioxidant power); ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid); RPA (reducing power assay): OH^- RSA (hydroxyl radical scavenging activity); FICA (chelating ability of ferrous ion)

CRediT authorship contribution statement

Yasir Iqbal: Conceptualization, Methodology, Investigation, Formal analysis, Software, Data curation, Validation, Writing – original draft. **Eric N. Ponnampalam:** Writing – review & editing. **Jeremy James Cottrell:** Writing – review & editing. **Hafiz Ansar Rasul Suleria:** Methodology & Software. **Frank Rowland Dunshea:** Writing – review & editing, Supervision & Resources.

Declaration of Competing 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.

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Supplementary materials

Supplementary data for this article is available online at xxxxxxxxx.

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Figure Captions

Figure 1. Figure 1. LC-ESI/QTOF/MS profiles of selected non-conventional edible plants in negative and positive ionization modes: (a) narrow leaf plantain in negative ionization mode; (b) white clover in negative ionization mode; (c) perennial ryegrass in negative ionization mode; (d) tall fescue in negative ionization mode; (e) narrow leaf plantain in positive ionization mode; (f) white clover in positive ionization mode; (g) perennial ryegrass in positive ionization mode; (h) tall fescue in positive ionization mode.

Figure 2. Principal component analysis of phenolic contents and antioxidant activities of narrow leaf plantain, white clover, perennial ryegrass, and tall fescue

Table 1. Details of cultivars, growing season and harvesting stage of non-conventional edible plants used for screening and quantification of polyphenols

Non-conventional edible plant	**Cultivar**	**Season of growth**	**Harvesting stage**
Narrow leaf plantain	Tonic	Spring	prebloom
White clover	Mink	Spring	prebloom to flowring
Perrenial ryegrass	Bealey	Spring	prebloom
Tall fescue	Quantum Max P	Spring	prebloom

Table 2. Total phenolic content, total flavonoid content and total tannin content of clover, narrow leaf plantain, perennial ryegrass and tall fescue

Phenolic contents	**Narrow leaf plantain**	**White clover**	**Perennial ryegrass**	**Tall fescue**
TPC (mg GAE/g FW)	0.64 ± 0.07^{c}	1.81 ± 0.16^{a}	0.68 ± 0.07^{c}	0.89 ± 0.04^{b}
TFC (mg QE/g FW)	0.12 ± 0.01^{a}	0.10 ± 0.01^{a}	0.01 ± 0.01^{b}	0.02 ± 0.01^{b}
TTC (mg CE/g FW)	0.86 ± 0.01^{c}	1.17 ± 0.05^{b}	2.11 ± 0.06^{a}	0.66 ± 0.03^{d}

Results are reported on fresh weight basis; n = 3 replicates for one sample. TPC (total phenolic contents); TFC (total flavonoid contents); TTC (total tannin contents) GAE (gallic acid equivalents); QE (quercetin equivalents); CE (catechin equivalents); AAE (ascorbic acid equivalents); FW (fresh weight). Superscript letters (a–d) indicate significant difference in rows ($p \leq 0.05$).

Table 3. Antioxidant activities of narrow leaf plantain white clover, perennial ryegrass and tall fescue

Antioxidant Assays	Narrow leaf plantain	White clover	Perennial ryegrass	Tall fescue
DPPH (mg AAE/g FW)	0.52 ± 0.01^{a}	0.26 ± 0.01^{c}	0.05 ± 0.01^{d}	0.34 ± 0.01^{b}
ABTS (mg AAE/g FW)	0.74 ± 0.01^{a}	0.63 ± 0.02^{b}	0.43 ± 0.01^{c}	0.16 ± 0.01^{d}
FRAP (mg AAE/g FW)	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
OH− RSA (mg AAE/g FW)	7.30 ± 0.11^{c}	6.58 ± 0.14^{d}	14.90 ± 0.32^{b}	15.41 ± 0.43^{a}
FICA (mg EDTAE/g FW)	0.02 ± 0.01^{b}	0.13 ± 0.02^{a}	0.18 ± 0.01^{a}	0.20 ± 0.02^{a}
RPA (mg AAE/g FW)	1.04 ± 0.03^{a}	0.94 ± 0.03^{b}	0.25 ± 0.01^{d}	0.35 ± 0.01^{c}

Results are reported on fresh weight basis; n = 3 replicates for each sample. DPPH (2,20 -diphenyl-1-picrylhydrazyl assay); ABTS (2,20-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assay); FRAP (ferric reducing antioxidant power assay); OH− RSA (hydroxyl-radical scavenging activity); FICA (ferrous ion chelating activity); RPA (reducing power assay); EDTA (ethylenediaminetetraacetic acid). Superscript letters (a–d) indicate significant difference in rows ($p \leq 0.05$).

1 Table 4. Polyphenols detected and identified in narrow leaf plantain, white clover, perennial ryegrass and tall fescue

Sr. No.	Compounds	Molecular Formula	RT (min)	Mode of Ionization	Molecular Weight	Theoretical (*m*/*z*)	Observed (*m*/*z*)	Mass Error (ppm)	Samples
	Phenolic acids								
	Hydroxycinnamic acids								
1	Sinapic acid	$C_{11}H_{12}O_5$	9.791	$[M+H]^+$	224.0685	225.0758	225.0759	0.44	Perennial ryegrass
2	Caffeic acid 3-*O*-glucuronide	$C_{15}H_{16}O_{10}$	48.197	$[M-H]^-$	356.0743	355.0670	355.0682	3.38	Tall fescue
3	Cinnamoyl glucose	$C_{15}H_{18}O_7$	9.986	$[M+H]^+$	310.1053	311.1126	311.1118	-2.57	Narrow leaf plantain
4	3-Caffeoylquinic acid	$C_{16}H_{18}O_9$	24.545	$[M+H]^+$	354.0951	355.1024	355.0995	-8.17	Narrow leaf plantain
5	Ferulic acid 4-*O*-glucoside	$C_{16}H_{20}O_9$	14.176	$[M+H]^+$	356.1107	357.1180	357.1166	-3.92	Narrow leaf plantain
6	1-Feruloyl-5-caffeoylquinic acid	$C_{26}H_{26}O_{12}$	14.798	$[M-H]^-$	530.1424	529.1351	529.1388	6.99	Tall fescue
7	2,5-di-S-Glutathionyl caftaric acid	$C_{33}H_{42}N_6O_{21}S_2$	32.667	$[M+H]^+$	922.1844	923.1917	923.1895	-2.38	White clover
8	1,2-Disinapoylgentiobiose	$C_{34}H_{42}O_{19}$	81.727	$[M-H]^-$	754.232	753.2247	753.2249	0.27	Narrow leaf plantain
9	Cinnamic acid	$C_9H_8O_2$	12.476	$[M+H]^+$	148.0524	149.0597	149.0583	-9.39	White clover
10	*m*-Coumaric acid	$C_9H_8O_3$	8.401	$[M+H]^+$	164.0473	165.0546	165.0531	-9.09	White clover
	Hydroxybenzoic acids								
11	4-Hydroxybenzoic acid 4-*O*-glucoside	$C_{13}H_{16}O_8$	79.11	$[M-H]^-$	300.0845	299.0772	299.0791	6.35	Narrow leaf plantain
12	2-Hydroxyhippuric acid	$C_9H_9NO_4$	43.291	$[M-H]^-$	195.0532	194.0459	194.0445	-7.21	White clover
	Hydroxyphenylpropanoic acids								
13	Dihydroferulic acid 4-*O*-glucuronide	$C_{16}H_{20}O_{10}$	76.111	$[M-H]^-$	372.1056	371.0983	371.0983	0.00	Tall fescue
14	3-Hydroxyphenylpropionic acid	$C_9H_{10}O_3$	9.986	$[M+H]^+$	166.063	167.0703	167.0697	-3.59	Narrow leaf plantain
	Hydroxyphenylpentanoic acids								
15	5-(3'-Methoxy-4'-hydroxyphenyl) -ɤ-valerolactone	$C_{12}H_{14}O_4$	18.741	$[M-H]^-$	222.0892	221.0819	221.0811	-3.62	Tall fescue
16	5-(3',5'-dihydroxyphenyl) -ɤ-valerolactone 3-*O*-glucuronide	$C_{17}H_{20}O_{10}$	75.232	$[M-H]^-$	384.1056	383.0983	383.0975	-2.09	White clover
	Flavonoids								
	Isoflavonoids								
17	3'-Hydroxygenistein	$C_{15}H_{10}O_6$	44.736	$[M+H]^{+*}$ / $[M-H]^-$	286.0477	287.0550	287.0523	-9.41	Narrow leaf plantain *, White clover, Perennial ryegrass & Tall fescue

18	5,6,7,3',4'-Pentahydroxyisoflavone	$C_{15}H_{10}O_7$	85.354	$[M+H]^+$	302.0427	303.0500	303.0478	-7.26	White clover
19	3',4',7-Trihydroxyisoflavanone	$C_{15}H_{12}O_5$	82.572	$[M+H]^{+*}$ / $[M-H]^-$	272.0685	273.0758	273.0732	-9.52	White clover* & Narrow leaf plantain
20	Pseudobaptigenin	$C_{16}H_{10}O_5$	86.829	$[M+H]^+$	282.0528	283.0601	283.0573	-9.89	White clover
21	Dalbergin	$C_{16}H_{12}O_4$	87.45	$[M+H]^+$	268.0736	269.0809	269.0792	-6.32	White clover
22	Dihydrobiochanin A	$C_{16}H_{14}O_5$	20.295	$[M-H]^-$	286.0841	285.0768	285.0753	-5.26	Perennial ryegrass
23	Puerarin	$C_{21}H_{20}O_9$	33.286	$[M-H]^-$	416.1107	415.1034	415.1055	5.06	Narrow leaf plantain
24	Glycitin	$C_{22}H_{22}O_{10}$	45.973	$[M-H]^-$	446.1213	445.1140	445.1102	-8.54	Perennial ryegrass
25	6''-*O*-Acetylglycitin	$C_{24}H_{24}O_{11}$	7.306	$[M+H]^+$	488.1319	489.1392	489.1405	2.66	Perennial ryegrass
	Flavonols								
26	Isorhamnetin	$C_{16}H_{12}O_7$	84.576	$[M+H]^+$	316.0583	317.0656	317.0627	-9.15	White clover* & Narrow leaf plantain
27	3-Methoxysinensetin	$C_{21}H_{22}O_8$	26.608	$[M-H]^-$	402.1315	401.1242	401.1247	1.25	White clover* & Narrow leaf plantain
28	Quercetin 3-*O*-glucosyl-xyloside	$C_{26}H_{28}O_{16}$	67.363	$[M-H]^-$	596.1377	595.1304	595.1311	1.18	White clover
29	Kaempferol 3,7,4'-*O*-triglucoside	$C_{33}H_{40}O_{21}$	70.163	$[M-H]^-$	772.2062	771.1989	771.1957	-4.15	Narrow leaf plantain
30	Patuletin 3-*O*-glucosyl-(1->6)-[apiosyl(1->2)]-glucoside	$C_{33}H_{40}O_{22}$	37.577	$[M-H]^-$	788.2011	787.1938	787.1971	4.19	Tall fescue
31	Spinacetin 3-*O*-(2	$C_{43}H_{48}O_{24}$	23.689	$[M+H]^+$	948.2536	949.2609	949.2657	5.06	Tall fescue
32	Patuletin 3-*O*-(2''-feruloylglucosyl)(1->6)-[apiosyl(1->2)]-glucoside	$C_{43}H_{48}O_{25}$	22.783	$[M-H]^-$	964.2485	963.2412	963.2448	3.74	Narrow leaf plantain
	Flavones								
33	Luteolin 7-*O*-glucuronide	$C_{21}H_{18}O_{12}$	44.736	$[M+H]^+$	462.0798	463.0871	463.0834	-7.99	Narrow leaf plantain
34	Luteolin 7-*O*-diglucuronide	$C_{27}H_{26}O_{18}$	73.226	$[M-H]^-$	638.1119	637.1046	637.107	3.77	Perennial ryegrass
35	Apigenin 6,8-di-C-glucoside	$C_{27}H_{30}O_{15}$	9.621	$[M+H]^+$	594.1585	595.1658	595.1641	-2.86	Narrow leaf plantain
36	Apigenin 7-*O*-(6''-malonyl-apiosyl-glucoside)	$C_{29}H_{30}O_{17}$	52.884	$[M-H]^-$	650.1483	649.1410	649.1424	2.16	Narrow leaf plantain
	Flavanones								
37	6-Geranylnaringenin	$C_{25}H_{28}O_5$	33.642	$[M+H]^+$	408.1937	409.2010	409.2009	-0.24	Perennial ryegrass
38	Neoeriocitrin	$C_{27}H_{32}O_{15}$	24.87	$[M-H]^-$	596.1741	595.1668	595.1701	5.54	Narrow leaf plantain
	Flavanols								

39	(-)-Epigallocatechin 3'-*O*-glucuronide	$C_{21}H_{22}O_{13}$	67.576	$[M-H]^-$	482.106	481.0987	481.1004	3.53	Perennial ryegrass
40	(+)-Catechin 3-*O*-gallate	$C_{22}H_{18}O_{10}$	23.395	$[M-H]^-$	442.09	441.0827	441.0836	2.04	White clover
41	(+)-Gallocatechin 3-*O*-gallate	$C_{22}H_{18}O_{11}$	23.428	$[M-H]^-$	458.0849	457.0776	457.0786	2.19	White clover
42	Prodelphinidin dimer B3	$C_{30}H_{26}O_{14}$	42.66	$[M-H]^-$	610.1323	609.1250	609.1277	4.43	Perennial ryegrass
	Dihydrochalcones								
43	Dihydromyricetin 3-*O*-rhamnoside	$C_{21}H_{22}O_{12}$	6.862	$[M-H]^-$	466.1111	465.1038	465.1047	1.94	Narrow leaf plantain
	Anthocyanins								
44	Cyanidin	$C_{15}H_{11}O_6$	22.715	$[M-H]^-$	287.0556	286.0483	286.0501	6.29	White clover
45	Cyanidin 3-*O*-xyloside	$C_{20}H_{19}O_{10}$	22.649	$[M-H]^-$	419.0978	418.0905	418.0896	-2.15	White clover
46	Delphinidin 3-*O*-(6''-acetyl-galactoside)	$C_{23}H_{23}O_{13}$	33.286	$[M-H]^-$	507.1139	506.1066	506.109	4.74	Narrow leaf plantain
47	Delphinidin 3-*O*-sambubioside	$C_{26}H_{29}O_{16}$	26.658	$[M-H]^-$	597.1456	596.1383	596.1406	3.86	White clover
48	Pelargonidin 3-*O*-rutinoside	$C_{27}H_{31}O_{14}$	34.893	$[M-H]^-$	579.1714	578.1641	578.1646	0.86	Narrow leaf plantain
49	Delphinidin 3-*O*-glucosyl-glucoside	$C_{27}H_{31}O_{17}$	49.29	$[M-H]^-$	627.1561	626.1488	626.1506	2.87	Tall fescue
50	Malvidin 3,5-*O*-diglucoside	$C_{29}H_{35}O_{17}$	34.876	$[M-H]^-$	655.1874	654.1801	654.1779	-3.36	Narrow leaf plantain
51	Cyanidin 3-*O*-(6''-p-coumaroyl-glucoside)	$C_{30}H_{27}O_{13}$	48.163	$[M-H]^-$	595.1452	594.1379	594.1357	-3.70	Tall fescue
52	Delphinidin 3-*O*-(6''-p-coumaroyl-glucoside)	$C_{30}H_{27}O_{14}$	42.315	$[M-H]^-$	611.1401	610.1328	610.1316	-1.97	Tall fescue*, White clover & Perennial ryegrass
53	Cyanidin 3-*O*-(6''-malonyl-3''-glucosyl-glucoside)	$C_{30}H_{33}O_{19}$	26.892	$[M-H]^-$	697.1616	696.1543	696.1525	-2.59	Tall fescue
54	Petunidin 3-*O*-(6''-p-coumaroyl-glucoside)	$C_{31}H_{29}O_{14}$	49.29	$[M-H]^-$	625.1557	624.1484	624.1465	-3.04	Tall fescue* & Perennial ryegrass
55	Delphinidin 3-*O*-feruloyl-glucoside	$C_{31}H_{29}O_{15}$	31.991	$[M-H]^-$	641.1506	640.1433	640.1422	-1.72	Perennial ryegrass* & White clover
56	Malvidin 3-*O*-(6''-caffeoyl-glucoside)	$C_{32}H_{31}O_{15}$	44.834	$[M-H]^-$	655.1663	654.1590	654.1559	-4.74	Tall fescue
57	Cyanidin 3-*O*-diglucoside-5-*O*-glucoside	$C_{33}H_{41}O_{21}$	24.953	$[M-H]^-$	773.214	772.2067	772.2086	2.46	Narrow leaf plantain
	Lignans								
58	Dimethylmatairesinol	$C_{22}H_{26}O_6$	84.375	$[M+H]^+$	386.1729	387.1802	387.1789	-3.36	Perennial ryegrass
59	Schisandrin	$C_{24}H_{32}O_7$	18.105	$[M+H]^+$	432.2148	433.2221	433.2234	3.00	Perennial ryegrass
60	Sesaminol 2-*O*-triglucoside	$C_{36}H_{46}O_{22}$	52.254	$[M-H]^-$	830.2481	829.2408	829.2394	-1.69	White clover
	Stilbenes								

61	Pinosylvin	$C_{14}H_{12}O_2$	87.425	$[M+H]^+$	212.0837	213.0910	213.0897	-6.10	White clover
62	*d*-Viniferin	$C_{28}H_{22}O_6$	54.911	$[M+H]^+$	454.1416	455.1489	455.1481	-1.76	White clover
	Other polyphenols								
	Hydroxybenzoketones								
63	2,3-Dihydroxy-1-guaiacylpropanone	$C_{10}H_{12}O_5$	11.311	$[M+H]^+$	212.0685	213.0758	213.075	-3.75	Narrow leaf plantain
64	3-Methoxyacetophenone	$C_9H_{10}O_2$	9.125	$[M+H]^+$	150.0681	151.0754	151.0748	-3.97	Narrow leaf plantain
	Hydroxybenzaldehydes								
65	*p*-Anisaldehyde	$C_8H_8O_2$	16.661	$[M+H]^+$	136.0524	137.0597	137.059	-5.11	Narrow leaf plantain
	Curcuminoids								
66	Demethoxycurcumin	$C_{20}H_{18}O_5$	11.698	$[M-H]^-$	338.1154	337.1081	337.1079	-0.59	White clover
	Other polyphenols								
67	Arbutin	$C_{12}H_{16}O_7$	86.795	$[M-H]^-$	272.0896	271.0823	271.0845	8.12	White clover
68	Salvianolic acid G	$C_{20}H_{18}O_{10}$	22.632	$[M-H]^-$	418.09	417.0827	417.0861	8.15	White clover
69	Salvianolic acid C	$C_{26}H_{20}O_{10}$	45.413	$[M-H]^-$	492.1056	491.0983	491.1024	8.35	Tall fescue* & Perennial ryegrass
70	Pyrogallol	$C_6H_6O_3$	11.532	$[M+H]^+$	126.0317	127.0390	127.0391	0.79	White clover

2 RT = Retention time; *m*/*z* = mass to charge ratio; * = compound detected in more than one pasture samples, data reported only with asterisk.

Table 5. Quantification of targeted polyphenols from narrow leaf plantain, white clover, perennial ryegrass and tall fescue

Phenolic compounds	Narrow leaf plantain	Clovers	Perennial ryegrass	Tall fescue	Polyphenol Class
2-hydroxybenzoic acid	84.11 ± 1.6 ^{b}	-	99.38 ± 1.3 ^{a}	-	Phenolic acid
Coumaric acid	15.34 ± 0.3 ^{c}	25.56 ± 1.0 ^{b}	33.47 ± 1.1 ^{a}	30.46 ± 1.2 ^{a}	Phenolic acid
Gallic acid	27.68 ± 0.6 ^{b}	35.96 ± 0.8 ^{a}	-	-	Phenolic acid
Sinapic acid	13.74 ± 0.4 ^{c}	65.04 ± 1.4 ^{b}	136.4 ± 1.8 ^{a}	135.6 ± 1.4 ^{a}	Phenolic acid
Syringic acid	30.93 ± 1.2 ^{b}	-	-	86.07 ± 1.6 ^{a}	Phenolic acid
Phloretic acid	3.13 ± 0.4 ^{c}	3.94 ± 0.6 ^{c}	80.28 ± 1.3 ^{a}	11.06 ± 0.7 ^{b}	Phenolic acid
p-hydroxybenzoic acid	9.38 ± 0.5	-	-	-	Phenolic acid
Epicatechin gallate	29.45 ± 1.1 ^{b}	28.21 ± 1.5 ^{b}	54.04 ± 1.4 ^{a}	-	Flavonoid
Catechin	34.73 ± 2.0 ^{a}	18.77 ± 1.2 ^{b}	-	-	Flavonoid
Quercetin-3-rhamnoside	4.45 ± 0.4 ^{d}	8.58 ± 0.6 ^{c}	12.81 ± 0.8 ^{b}	44.64 ± 1.2 ^{a}	Flavonoid

All values are expressed as μg/g FW, n = 3 replicates for each sample. Superscript letters (a–d) indicate significant difference in rows ($p \leq 0.05$).

Table 6. Pearson's correlation between antioxidant activities and phenolic contents.

Variables	TPC	TFC	TTC	DPPH	ABTS	FRAP	OH^- RSA	FICA	RPA
TFC	0.341								
TTC	-0.127	-0.425							
DPPH	-0.112	0.695*	-0.860**						
ABTS	0.189	0.866**	0.070	0.344					
FRAP	0.282	0.996**	-0.365	0.674*	0.901**				
OH^- RSA	-0.509	-0.971**	0.279	-0.516	-0.890**	-0.965**			
FICA	0.120	-0.887**	0.291	-0.718**	-0.871**	-0.918**	0.792**		
RPA	0.386	0.999**	-0.443	0.689*	0.849**	0.990**	-0.976**	-0.862**	
Phenolic acids	0.676*	-0.882**	0.595*	-0.619*	-0.596*	-0.837**	0.896**	0.576*	-0.905**

* Significant correlation at $p \leq 0.05$; ** Significant correlation at $p \leq 0.01$

Figure 1

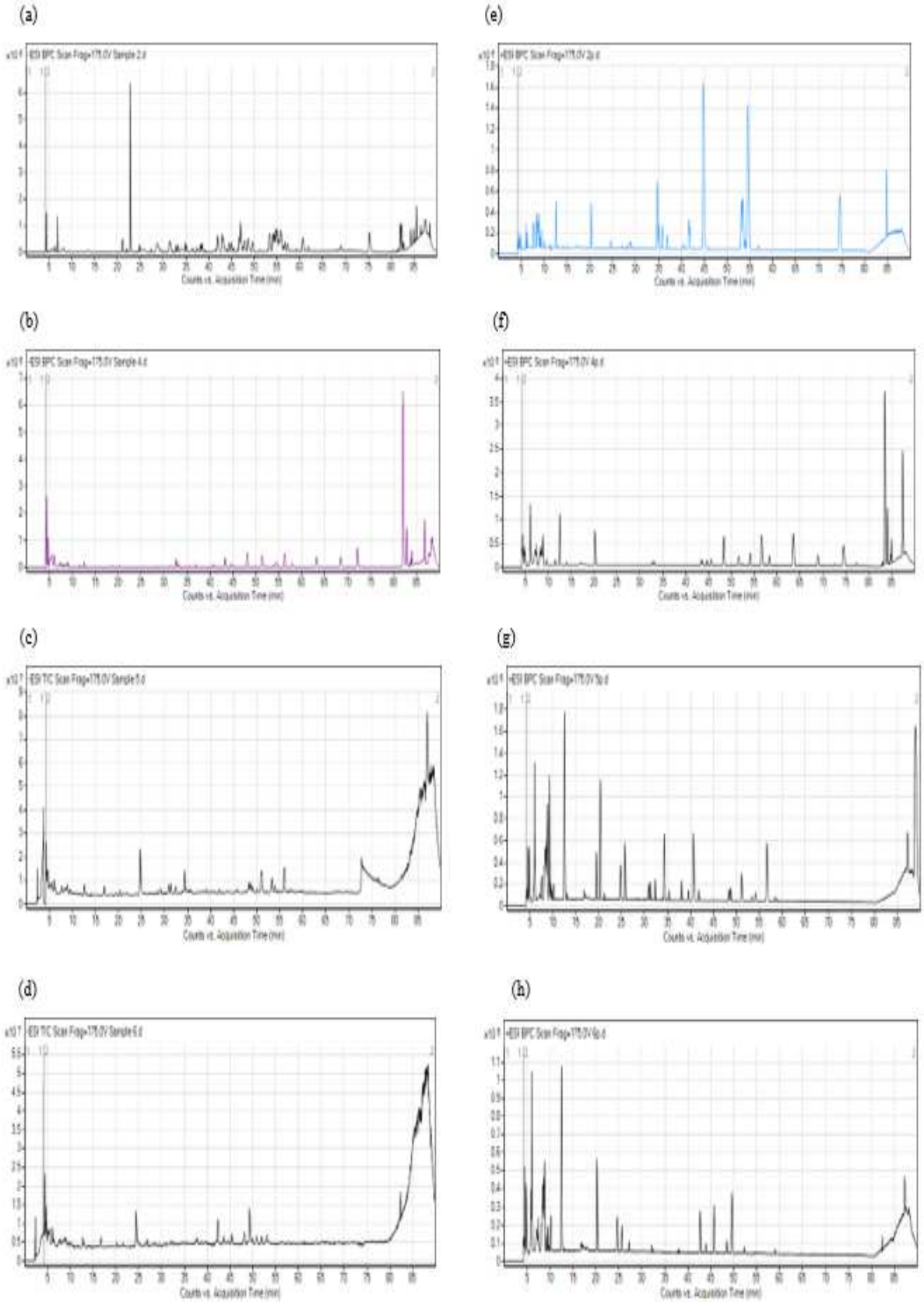
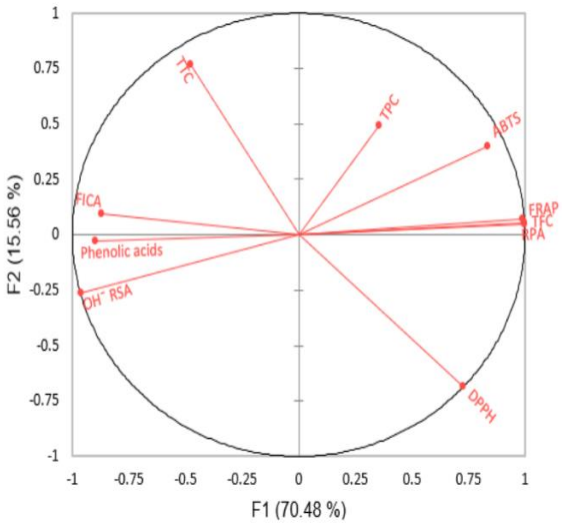


Figure 2


TFC
TPC
ABTS
FICA
FRAP
TFC
RPA
Phenolic acids
DH RSA
DPPH
F2 (15.56 %)
F1 (70.48 %)
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1