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Identification and characterization of anthocyanins and non-anthocyanin phenolics from Australian native fruits and their antioxidant, antidiabetic, and anti-Alzheimer potential

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ABSTRACT

Polyphenols are vital bioactive constituents that have beneficial effects on human health. The aim of this study was to characterize the biologically active phenolic metabolites in Australian native commercial fruits (Kakadu plum, Davidson's plum, quandong peach, and muntries) and their antioxidant, α-glucosidase, and acetylcholinesterase inhibition activities. Polyphenols were measured through total phenolic content (TPC), total flavonoid content (TFC), total condensed tannin (TCT), and total monomeric anthocyanin content (TMAC). Moreover, different in-vitro biological assays (DPPH, ABTS, FICA, OH-RSA, α-glucosidase, and acetylcholinesterase inhibition activities) were conducted to measure the antioxidant, anti-diabetic, and anti-Alzheimer's potential of these selected fruits. LC-ESI-QTOF-MS/MS was implied for identification and quantification purposes. In this study, a total of 307 bioactive metabolites (51 phenolic acids, 194 flavonoids, 15 tannins, 23 other polyphenols, 5 stilbenes, 12 lignans, and 7 terpenoids) were putatively identified. A total of 41 phenolic compounds were quantified/semi-quantified. Kakadu plum was identified with a higher concentration of polyphenols and biological activities compared to Davidson plum, quandong peach, and muntries. Molecular docking was also conducted to discover the actual role of the most abundant phenolic metabolites in the α-glucosidase and acetylcholinesterase inhibition activities.

1. Introduction

Plants have formed the basis of traditional medicine systems that have been in existence for thousands of years. In recent decades, the interest has increased in discovering the nutritional, nutraceutical, and therapeutic constituents of more than 300 Australian native fruits and medicinal plants (Sakulnarmrat & Konczak, 2012). Phytochemicals, including polyphenols, are the secondary plant metabolites abundantly found in fruits, vegetables, fruits, spices, and medicinal plants. They show a positive impact on human health when interacting with living tissues. Phenolic compounds are a diverse class of bioactive metabolites, including phenolic acids, flavonoids, lignans, stilbenes, coumarins, phenolic terpenes, tyrosols, and other polyphenols (Ali, Bashmil, et al., 2021). In polyphenols, flavonoids (anthocyanins, flavanols, flavonols, flavanones, flavones, chalcones, and dihydroxy chalcones) are the most abundant phenolic compounds in nature (Tsao, 2010). Nutritionists suggest that polyphenols-rich fruits and vegetables can inhibit or lower the risk of cardiovascular diseases, chronic inflammation, degenerative diseases, and various cancers in the human body (Tsao, 2010). Polyphenols are the main antioxidant compounds that can neutralize the free radicals in the body. The 3-hydroxy groups and highly conjugated system of polyphenols make them more potent antioxidant constituents in the biological system. They have strong radical scavenging capacity and metal chelation ability. Anthocyanins are the colored pigments that contribute mainly to the blue, purple, and red colors in fruits and vegetables. They have antioxidant activity (Kalt et al., 2020), anti-inflammatory activity (Rossi et al., 2003), antidiabetic activity (Jankowski et al., 2000), anti-cancer activity (Hou, 2003), neuro- and cardioprotective activities (Youdim et al., 2000). The structure and electron deficiency make them more reactive towards reactive oxygen species (ROS) which is the base for potent health benefits (Martín et al., 2017). Anthocyanins are water-soluble and stable in highly acidic conditions (Dangles & Fenger, 2018). There are several factors that are required to improve the extraction efficiency of anthocyanins. For example, solvent

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to sample ratio, solvent type, time, and temperature for extraction are critical factors for the extraction of these pigments. The reaction activity of anthocyanins decreased with long storage, at high temperature, and with repeated freeze–thaw cycles over the time period.

Excessive production of ROS and reactive nitrogen species (RNS) in the body ultimately causes oxidative stress which is also the leading cause of many different pathological conditions including Alzheimer's disease and diabetes. Alzheimer's disease is one of the leading causes of death among old people around the world (Mahmudov et al., 2022). Therefore, the inclusion of antioxidants can minimize or neutralize the free radicals in the biological system. The use of natural phytochemicals, mainly phenolic compounds, as antioxidants in traditional and modern therapy has gained much interest as an alternative to synthetic antioxidants. For many decades, it has been generally proven that fruits and medicinal plants inhibit or minimize the prevalence of chronic diseases in the human body. These plants contain bioactive metabolites that have a positive impact on human health. The bioactive metabolites, including carotenoids, terpenoids, vitamins, and phenolic compounds have the potential to lessen the risk of cardiovascular diseases, multiple types of cancers and neurodegenerative disorders.

The emerging interest in the food supply required detailed analytical identification, characterization, and quantification of these pigments to collectively understand their role in food and human health. Previously, a few studies were conducted for the characterization and identification of phenolic compounds from selected Australian native fruits by (Tan et al., 2011a, 2011b) and Konczak et al. (2010b), but comprehensive profiling of these fruits was still lacking due to the complex nature of phenolic and non-phenolic metabolites, and expense and non-availability of commercial standards. The main objective of this study was an in-depth screening of Australian native commercial Kakadu plums, Davidson plum, quandong peach and muntries for phenolic compounds and their biological activities. To do this, the phenolic contents in Kakadu plum (family; combretaceae), Davidson plum (family; cunoniaceae), quandong peach (family; santalaceae) and muntries (family; myrtaceae) were measured through total phenolic content (TPC), total flavonoid content (TFC), total condensed tannins (TCT) and total monomeric anthocyanin content (TMAC) while the antioxidant potential was measured through 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric ion chelating assay (FICA) and hydroxyl radical scavenging assay ($^{\bullet}$OH-RSA). Anti-diabetic and anti-Alzheimer potential was measured through the α-glucosidase and acetylcholinesterase (AChE) inhibition activities. Moreover, liquid chromatography – electrospray ionization – quadrupole time of flight – mass spectrometry (LC-ESI-QTOF-MS/MS) was used to identify and quantify the anthocyanins and non-anthocyanin phenolic compounds. The study will further explore the utilization of these Australian native fruits as a potential source of polyphenols in different industries including functional foods, pharmaceuticals, nutraceuticals and cosmetics due to their antioxidant, anti-diabetic and anti-Alzheimer potential.

2. Materials and methods

2.1. Chemicals and reagents

Analytical, HPLC, and LC-MS grade chemicals were used in this study. Folin-Ciocalteu reagent, gallic acid, sodium carbonate, aluminium chloride, hydrated sodium acetate, quercetin, vanillin, sulfuric acid, *L*-ascorbic acid, iron(III) chloride hexahydrate (Fe[III] $Cl_3{\cdot}6H_2O$), ABTS, potassium persulfate, potassium ferrocyanide (III), trichloroacetic acid (TCA), ferric chloride, sodium phosphate dibasic heptahydrate ($Na_2HPO_4{\cdot}7H_2O$), sodium phosphate monobasic monohydrate ($Na_2HPO_4{\cdot}H_2O$), hydrochloric acid (HCl), trisodium phosphate, iron (II) sulfate heptahydrate, ferrozine, ethylenediaminetetraacetic (EDTA), 3-hydroxybenzoic acid, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), acetylcholine (ATCl), tacrine, rat intestinal powder, phosphate buffer saline (pH 7.4), acarbose and *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) were purchased from Sigma Aldrich. Sodium carbonate anhydrous and hydrogen peroxide (H_2O_2) 30% were purchased from Chem-Supply Pty Ltd. (Adelaide, Australia). LC-MS grade formic acid (98–100%) were used for LC-MS analysis.

2.2. Samples collection and preparation

The freeze dried Australian native commercial fruits were purchased (at two different times) from the Australian Superfood Co (https://austsuperfoods.com.au). The method of Solomakou et al. (2022) was used to extract bioactive metabolites from the Australian native fruits with modifications. The bioactive compounds were extracted from fruits using a 1/20 (*w/v*) sample to solvent ratio with 80% methanol in 1% formic acid in triplicate. Briefly, samples were extracted at 150 rpm and 4 °C for 2 h in an orbital shaker (ZWYR-240) and centrifuged at 8000 × g for 20 min and the supernatant was collected. Same extraction process was repeated for 2nd and 3rd time in the orbital shaker and centrifuged. All the pooled supernatants were mixed and concentrated using a rotary evaporator at 40–50 °C under vacuum. The concentrated extracts were centrifuged again, filtered through a 0.45 μm syringe filter, and stored at − 20 °C for further LC-MS and antioxidants analysis within a week. Moreover, the left-over extract was freeze dried for 72 h and stored at − 20 °C for α-glucosidase and AChE inhibition activities.

2.3. Measurement of phenolic contents in Australian native fruits

2.3.1. Total phenolic content (TPC)

The estimation of total phenolic content was carried out by following the method of Ali, Wu, et al. (2021) while all tests were performed in triplicate. A 25 μL of each phenolic extract was mixed with 25 μL Folin-Ciocalteu (F-C) reagent (25%, *v/v*) and 200 μL water in a 96-well plate and incubated for 5 min before the mixing of 25 μL 10% sodium carbonate. The mixture was incubated for 1 h at room temperature in the dark and absorbance was recorded at 765 nm. Results were expressed as milligram of gallic acid (0–200 μg/g) equivalents per gram sample (mg GAE/g).

2.3.2. Total flavonoid content (TFC)

TFC was quantified by following the method of (Suleria et al., 2020). Briefly, using phenolic extract of 80 μL in a 96-well plate followed by shaking after adding 80 μL amount of aluminium chloride solution (2 %) and sodium acetate of 120 μL (50 g/L). The reaction mixture was then retained in dark for 2.5 h at 25 °C. The absorbance was measured at 440 nm and all samples were analyzed in triplicate. The standard curve (0–50 μg/mL of quercetin was generated ($R^2 = 0.999$) and results were presented as mg QE/g.

2.3.3. Total condensed tannins (TCT)

The TCT was performed by following the method of Ali, Wu, et al. (2021) with modifications. A sample solution of 25 μL was used and mixed with 150 μL of vanillin solution (4%). After that, 25 μL of 32% H_2SO_4 was added into the mixture. It was then incubated for 15 min at 25 °C. The absorbance was read at 500 and the standard curve of catechin (0–1000 μg/mL) was generated while results were presented as mg CE/g.

2.3.4. Measurement of total monomeric anthocyanin content (TMAC)

Total monomeric anthocyanin content was measured by following the method of Rafi et al. (2018) and Inácio et al. (2013) after modifications into 96-well plate method. Two buffers (0.025 M potassium chloride of pH 1.0 and 0.4 M sodium acetate of pH 4.5) were prepared. A 50 μL of the extract was added in 150 μL of each buffer and placed at room temperature in dark for 30 min. After the said period, absorbance was recorded at 520 nm and 700 nm through a spectrophotometer.

TMAC was measured as cyanidin 3-glucoside (mg/g) equivalents by following the given equation.

$$\mathrm{TMAC(mg/g)} = \mathrm{A} \times \mathrm{MW} \times \mathrm{DF}/\varepsilon \times \mathrm{l}$$

Where A = (A510 − A700 nm) pH 1 − (A510 nm − A700nm) pH 4.5; MW = 449.2 g/mol; DF = dilution factor; ε = extinction coefficient of cyanidin-3-glucoside (26,900 L/cm mol).

2.4. Biological activities

2.4.1. Measurement of antioxidant potential

The antioxidant activity was quantified by the free radical-scavenging effect on the DPPH radical with few modifications, against a standard curve of 0–50 μg/mL ascorbic acid ($C_6H_8O_6$). The methods of Peng et al. (2019) and Sharifi-Rad et al. (2021) were used for DPPH and ABTS assays. A 20 μL extract was added in 275 μL of 0.1 M methanolic DPPH dye and incubated for 30 min in dark and absorbance was measured at 517 nm while a 10 μL of the phenolic extract was mixed with 290 μL ABTS dye and absorbance was measured at 734 nm after the incubation of 6 min in dark. The FICA and $^{\bullet}$OH-RSA assays were conducted by following the methods of Bashmil et al. (2021) and Chou et al. (2021). For FICA, a 15 μL of the phenolic extract was mixed with 85 μL water, 50 μL of 2 mM ferrous chloride (diluted with water 1:15 *v/v*) and 50 μL of 5 mM ferrozine (diluted with water 1:6 *v/v*) and incubated for 10 min at room temperature in dark before the measurement of absorbance at 562 nm. EDTA was used as a reference standard. For $^{\bullet}$OH-RSA assay, a 50 μL of each phenolic extract, 6 mM $FeSO_4{\cdot}H_2O$ and 6 mM H_2O_2 mixed and incubated at room temperature for 10 min. Then, 50 μL of 6 mM 3-hydroxybenzoic acid was added and absorbance was measured at 510 nm. Ascorbic acid was used as a reference standard.

2.4.2. Measurement of acetylcholinesterase (AChE) inhibition activity

The acetylcholinesterase inhibition activity of Australian native fruits was measured by following the method of Leonard et al. (2021) with some modifications. 100 μL of 50 mM Tris-HCl buffer (pH 7.8), 20 μL extract solution, and 20 μL of AChE solution (from Electrophorus electricus, 5 units/mL) were added together in 96-well plates and incubated for 30 min at room temperature in dark. Then, 40 μL of 3 mM 5,5′ -Dithiobis-2-nitrobenzoic acid (DTNB) and 20 μL of 15 mM acetylcholine iodide (ATCl) were successively mixed in each well. The absorbance of the reaction mixture was monitored through Varioskan LUX at 405 nm.

Acetylcholinesterase inhibitory activity was expressed as % inhibition at 1 mg/ml using the formula.

$$\mathrm{Inhibition\%} = \left[1 - \frac{\mathrm{Asample} - \mathrm{Asamplebackground}}{\mathrm{Acontrol} - \mathrm{Acontrolbackground}}\right] \times 100$$

Where Asample was the absorbance of the extract, Asamplebackground was the absorbance with buffer replaced by enzyme, Acontrol was the absorbance of blank sample with methanol, and Acontrolbackground was the absorbance of methanol and buffer instead enzyme. Tacrine at 1 mg/mL was used as a reference drug.

2.4.3. Measurement of α-Glucosidase inhibition activity

Alpha-glucosidase enzyme solution was prepared from the intestinal acetone powder by following the method of Xiong et al. (2020). Briefly, one gram of intestinal acetone powder was added in 25 mL potassium phosphate buffer (0.12 M with 1% NaCl of pH 6.8). Mixture was sonicated for 3 min at 50 Hz by a Q55 sonicator (Qsonica, CT, USA). After that the mixture was centrifuged at 4000x g at 4 °C for 30 min. The supernatant was collected and further centrifuged at 14000x g for 20 min. The collected supernatant was stored in a freezer at − 20 °C and the assay was completed within two days. The protein content of the α-glucosidase (4.99 ± 0.27 mg/mL) was measured using bovine serum albumin as protein standard through the Bradford assay. Alpha glucosidase inhibition assay was conducted by following the method of Xiong et al. (2020) and Elwekeel et al. (2022) after modifications. To do this, 90 μL potassium phosphate buffer (0.12 M, pH 6.8) and 20 μL of phenolic extract were mixed in a 96 well plate. Then, 20 μL of alpha glucosidase solution was added and incubated at 37 °C for 25 min. After the incubation period, 20 μL of 25 mM *p*NPG solution was mixed and incubated for 30 min at 37 °C. Then 70 μL dimethyl sulfoxide (DMSO) was added to dissolve the precipitates and absorbance was measured at 405 nm. The inhibition of α-glucosidase was determined using the same formula used of AChE inhibition activity in triplicate. Acarbose at a rate of 1 mg/mL was used as a reference drug.

2.5. LC-MS/MS identification and quantification of individual phenolic metabolites

LC-ESI-Q-TOF-MS/MS (Accurate-Mass Q-TOF LC/MS Agilent 6520) equipped with Agilent HPLC 1200 series was used for the analysis of the untargeted phenolic metabolites from Australian native Kakadu plum, Davidson plum, quandong peach and muntries by following the method of Ali, Bashmil, et al. (2021). The screening of the phenolic extracts was conducted using a Synergi 4 μm Hydro-RP 80 Å LC column (250 × 4.6 mm) protected with C18 ODS (4.0 × 2.0 mm) guard column (Phenomenex, Torrance, California, United States). An aliquot of 20 μL from each phenolic extract was injected while the flow rate of mobile phase A (0.1% formic acid in Milli-Q water) and mobile phase B (0.1% formic acid in acetonitrile) was 600 μL/min with the following gradient; 0–10 min (10–20 % B), 10–20 min (20–25% B), 20–30 min, (25–30% B), 30–40 min (30–45% B), 40–50 min (45–60% B), 50–65 min (60–90% B), 65–67 min (90–100% B), 67–68 min (100–10% B) and 68–70 min (10% B). The following LC conditions: scan mode 50–1300 amu, capillary voltage (3500 V), nitrogen gas flow rate (9 L/min) at 325 °C, nebulization 45 psi and collision energies (10, 20 and 40) were used in auto MS/MS mode. Agilent MassHunter Workstation Software Quality Analysis (version B.06.00) was used for the identification and characterization of phenolic metabolites with the help of Personal Compounds Database and Library (PCDL) for metabolites, PubChem (https://pubchem.ncbi.nlm.nih.gov), Human Metabolome Database (https://hmdb.ca) and FooDB (https://foodb.ca). All the samples were repeated in duplicate and a total of 41 phenolic compounds were semi-quantified in this experiment. MS/MS spectra of 40 commercial standards were also acquired in this experiment. A mixture of 26 commercial standards was used to generate equations through LC-MS/MS.

2.6. Molecular docking

Molecular docking was conducted to investigate the actual role of abundant individual metabolites of Australian native fruits towards α-glucosidase and acetylcholinesterase inhibition activities by following the methods of Anil et al. (2022) and (Ali et al., 2022). All the structures of the selected compounds were prepared in ChemDraw (version 12.0.2) while the X-ray crystallographic structure of human acetylcholinesterase in complex with tacrine (PDB ID: 7E3I) and structure of human lysosomal acid alpha-glucosidase in complex with acarbose (PDB ID: 5NN8) were directly obtained from the Protein Data Bank (https://www.rcsb.org). Molecular docking was conducted in Schrodinger (https://www.schrodinger.com) based Maestro software (version 11.2). The optimization process was completed through the addition of hydrogen atoms, deletion of water molecules, completion of bond orders, assignment of hydrogen bonds and fixed the potential of receptor atoms and energy minimization using the OPLS_2005 force field. Grid box dimensions were centroid and 20 Å from each side. Scaling factor (1.0) and partial charge cutoff (0.25) were used as preset in the software. Other default parameters were used for receptor grid preparation and molecular docking. All compounds were docked into the active site of proteins 7E3I and 5NN8 using the extra precision (XP) docking mode of the 'glide' program.

2.7. Statistical analysis

XLSTST-2019.1.3 was used for biplot analysis while Minitab Program for Windows version for One-Way Analysis of Variance (ANOVA) followed by Tukey's Honestly Significance test.

3. Results and discussion

3.1. Measurement of total phenolic content, total flavonoid content, total condensed tannins, and total monomeric anthocyanin content

The interest in the measurement of polyphenols has increased due to their health benefits. We studied Kakadu plum, Davidson plum, Muntries and quandong peach for their total phenolic content (TPC), total flavonoid content (TFC), total condensed tannins (TCT), and total monomeric anthocyanin content (TAC) Table 1.

The level of phenolic content varied from 23.04 to 74.84 mg GAE/g in selected Australian native fruits (Table 1). The TPC of Kakadu plum (74.84 ± 4.28 mg GAE/g, $p < 0.05$) was significantly higher than the other fruits, followed by quandong peach (42.85 ± 0.39 mg GAE/g), Davidson plum (23.04 ± 1.19 mg GAE/g) and muntries (23.04 ± 1.19 mg GAE/g), respectively. The higher TPC of Kakadu plum was also reported by Tan et al. (2011a) and Konczak et al. (2010b) compared to other native fruits. Furthermore, Sommano et al. (2013) also quantified TPC 3.08 mg GAE/g in Kakadu plum which is very low compared to the present TPC . The TPC of quandong peach is also comparable to Australian-grown thyme (43.16 mg/g), basil (39.91 mg/g) and sage (47.62 mg/g) reported by Ali, Bashmil, et al. (2021). The TPC of these Australian native fruits was found higher than Australian native finger lime, tamarind, and mountain pepper reported by (Cáceres-Vélez et al., 2022). Interestingly, the highest total flavonoid content (20.63 ± 0.30 mg QE/g) was quantified in quandong peach followed by Kakadu plum (16.07 ± 0.74 mg QE/g) while the least total flavonoid content was measured in Davidson plum (3.12 ± 0.76 mg QE/g) and muntries (3.52 ± 0.04 mg QE/g). Previously, the higher TFC value was measured in purified Davidson plum extract compared to quandong peach (Sakulnarmrat et al., 2014). The TFC value of Davidson plum and quandong peach was comparable to rosemary and mint (Ali, Bashmil, et al., 2021) which have been reported to have highly abundant phenolic contents.

The highest TCT value was measured in Davidson plum (5.34 ± 0.91 mg CE/g) while the least TCT was quantified in muntries (1.25 ± 1.99 mg CE/g). Previously, no study was conducted to quantify the condensed tannins in these Australian fruits. The TCT value was comparable to pears (Wang et al., 2021), raspberries and strawberries (Subbiah et al., 2020) while the TCT of Kakadu plum, Davidson plum and quandong peach are comparable to Australian-grown thyme, oregano, rosemary, sage, bay, parsley and fenugreek (Ali, Bashmil, et al., 2021). The TMAC value of Davidson plum (2.80 ± 0.33 mg/g) was significantly higher compared to quandong peach (2.07 ± 0.20) and muntries (0.78 ± 0.09 mg/g), respectively while no TMAC was measured in Kakadu plum (Tan et al., 2011a). Blueberries and blackberries were used as a positive control to measure the total monomeric anthocyanin content from Australian native fruits. Davidson plum and quandong peach were measured with higher TMAC than blueberries (1.38 ± 0.06 mg/g) and blackberries (1.18 ± 0.15 mg/g).

Overall, various studies reported that some Australian native fruits contain higher total phenolic content compared to commercially grown blueberries (Konczak et al., 2010b; Sakulnarmrat et al., 2015). Among them, Kakadu plum is traditionally identified as a 'medicinal plum' and chemo-preventive agent due to its vital bioactive compounds especially higher phenolic compounds (Sakulnarmrat et al., 2015).

Table 1
Measurement of phenolic contents in Australian native fruits.

Variables	TPC (mg GAE/g)	TFC (mg QE/g)	TCT (mg CE/g)	TMAC (mg C3G/g)
Kakadu Plum	74.84 ± 4.28 ^{a}	16.07 ± 0.74^{b}	5.44 ± 1.76^{b}	0.00 ± 0.00 ^{d}
Quandong Peach	42.85 ± 0.39^{b}	20.63 ± 0.30 ^{a}	3.90 ± 0.93^{c}	2.07 ± 0.20^{b}
Davidson Plum	32.49 ± 0.79^{c}	10.56 ± 0.13^{c}	6.31 ± 1.75 ^{a}	2.80 ± 0.33 ^{a}
Muntries	23.04 ± 1.19 ^{d}	8.31 ± 2.04 ^{d}	2.14 ± 0.67 ^{d}	0.78 ± 0.09^{c}

TPC; total phenolic content, TFC; total flavonoid content, TCT; total condensed tannins, TMAC; total monomeric anthocyanin content, GAE; gallic acid equivalent, QE; quercetin equivalent, CE; catechin equivalent, C3G; cyanidin 3-glucoside. Values are mean ± standard deviation in triplicate (n = 3). Values in each column with superscript letters (a-d) are significantly different from each other ($p < 0.05$).

3.2. Antioxidant potential of Australian native fruits

Oxidative stress is an abnormal condition of the body which occurs due to excessive production of reactive oxygen and nitrogen species (ROS, RNS) which is the leading cause of different pathological conditions including Alzheimer's disease (AD) and diabetes. It has been reported that oxidative damage is a detrimental event in AD that kills the neuronal cells (Dhakal et al., 2019). Antioxidant polyphenols have the ability to neutralize the free radicals in the body. Therefore, the identification of antioxidant polyphenols is important. The results of the biological activities of Australian native fruits are given in Table S1 and Fig. 1.

In this study, the highest value of DPPH was found in Kakadu plum (30.98 ± 0.54 mg AAE/g) while the least value of DPPH (12.42 ± 0.45 mg AAE/g) was measured in muntries (Table S1, Fig. 1) Previously, Sommano et al. (2013) and Tan et al. (2011a) also reported a higher DPPH for Kakadu plum than other selected fruits in our study. The ABTS is also a widely used assay for estimating the free radical scavenging capacity of plant extracts, including hydrophilic and lipophilic constituents, based on the polyphenols' hydrogen ion donating ability. ABTS value of Kakadu plum (80.95 ± 0.49 mg AAE/g) was higher than the other selected fruits. Some other studies also reported higher ABTS values for Kakadu plum (Sommano et al., 2013; Tan et al., 2011a). The FICA value of Kakadu plum (5.88 ± 0.62 mg AAE/g) and Davidson plum (3.60 ± 0.21 mg AAE/g) was found higher compared to quandong peach (3.26 ± 0.10 mg AAE/g) and muntries (3.02 ± 0.09 mg AAE/g). Furthermore, the $^{\bullet}$OH-RSA value of Kakadu plum (47.25 ± 0.95 mg AAE/g) and quandong peach (43.25 ± 0.42 mg AAE/g) was higher compared to Davidson plum (19.66 ± 0.31 mg AAE/g) and muntries (23.32 ± 0.55 mg AAE/g). Previously, Konczak et al. (2010b) reported higher antioxidant activity of Kakadu plum, quandong peach and Davidson plum compared to blueberries.

It is reported that antioxidant activities vary in selected Australian native fruits due to their complex mixture of bioactive compounds and mainly depend on the method used for extraction. To determine the antioxidant potential of fruits, there is a list of methods with their benefits and limitations (Bashmil et al., 2021; Sharifi-Rad et al., 2021). Due to the complex nature of phenolic compounds and multiple mechanisms of reactions in the biological system, no defined method truly reflects the same antioxidant potential of these bioactives as reported by Granato et al. (2018). Different studies have been conducted for the measurement of different antioxidant activities but a detailed study on Australian native fruits is limited due to the complex nature of bioactive metabolites.

3.3. Alpha-Glucosidase and acetylcholinesterase inhibition activities

Diabetes is one of the leading causes of death in the world, an average of 1.4 million people died in 2019 while 422 million people were affected in 2014 (Taslimi et al., 2020). Alpha-glucosidase involved in hydroxylation and absorption of carbohydrates in the gut, thus the

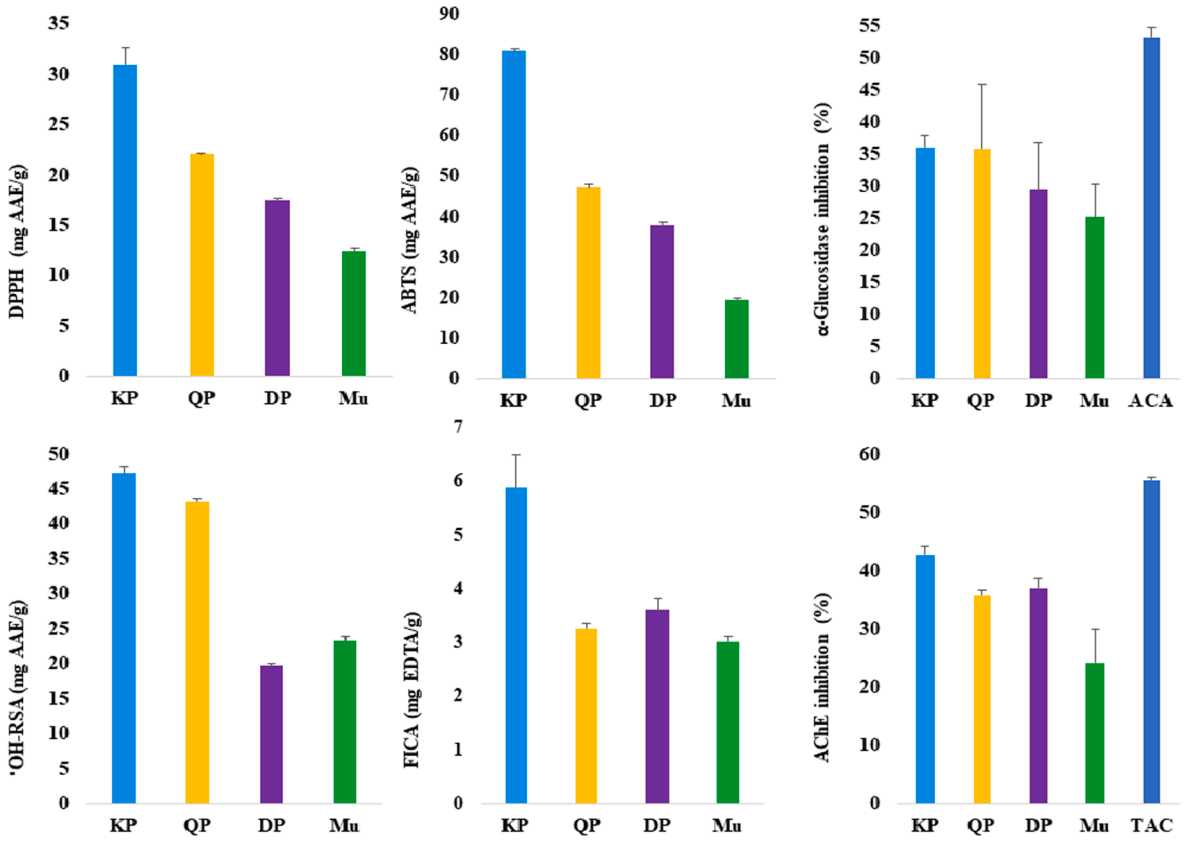

Fig. 1. Biological activities of Australian Kakadu plum (KP), quandong peach (QP), Davidson plum (DP) and Muntries (Mu).

inhibition of α-glucosidase is desirable in pre-diabetic and diabetic conditions (Leonard et al., 2021). The plant extracts are widely used to inhibit diabetes through the inhibition of α-glucosidase. The identification of polyphenols in fruit extracts as α-glucosidase inhibitors is one of the therapeutic approaches to suppress or minimize the onset of diabetic conditions. Kakadu plum at the rate of 10 mg/mL was identified with the highest α-glucosidase inhibition activity (36.06 ± 1.89 %) while muntries with the same concentration were measured the least α-glucosidase inhibition activity (25.22 ± 5.10 %) compared to other plant extracts (Table S1, Fig. 1). Previously, Sakulnarmrat et al. (2014) reported higher α-glucosidase inhibition activity of purified fractions of Davidson plum and quandong peach compared to the values reported in this study. Polyphenols mainly proanthocyanidins have been reported for α-glucosidase inhibition properties and inhibition activity depends on the degree of polymerization of these metabolites (Sakulnarmrat et al., 2014). The results of this study were in accordance with the previously published studies. Previously, Wang et al. (2019) reported that total phenolics and flavonoids in fruit extracts significantly inhibit the α-glucosidase activity.

Alzheimer's disease is one of the most common neurodegenerative disorders which results due to alterations of energy metabolism, oxidative stress, loss of neurotropic support, alterations of protein functioning and dysfunction of the neuro-vascular system (Maher, 2019). About 131.5 million people will be affected by Alzheimer's disease by 2050 (Emir et al., 2022). AChE is a serine hydrolase (enzyme) that is involved in blocking impulse transmission through the hydrolysis of acetylcholine (a neurotransmitter) (Colović et al., 2013). Thus, the inhibition of AChE is required for the normal functioning of the brain. In this study, Kakadu plum and Davidson plum were measured with higher AChE inhibition activity (42.70 ± 1.50 % and 36.96 ± 1.62 %, respectively) compared to quandong peach and muntries (Table S1, Fig. 1). The variation in AChE inhibition activity could be due to the different individual metabolites, concentration, and their bioavailability during the biological reaction. AChE inhibition activities of these Australian native fruits were still less compared to the standard drug (positive control) of tacrine (55.54 ± 0.44 %) at the rate of 1 mg/mL. Flavonoids have the potential to block the age-related toxicity and oxidative stress pathways associated with the progression of Alzheimer's disease (Maher, 2019; Simunkova et al., 2019). Thus, the daily consumption of these selected fruits still has the potential to slow down the risk of Alzheimer's disease.

3.4. Correlation analysis

The health benefits of polyphenols are due to their antioxidant properties and depend upon their amount and bioavailability [23]. Previously, we established a strong positive correlation between total phenolic content and antioxidant activities of 10 widely used Australian-grown herbs (Ali, Bashmil, et al., 2021). Here, we reported the results of anthocyanins and non– anthocyanins in Kakadu plum, Davidson plum, quandong peach, and muntries and their antioxidant, α-glucosidase, and AChE inhibition activities.

Correlation analysis was carried out between phenolic contents (TPC, TFC, TCT, and TMAC) of the Australian native fruits and their biological activities obtained by the different assays (Table 2).

It was observed that TPC showed highly significant positive correlation with ABTS, DPPH ($r = 0.99$, $p < 0.05$), and FICA (0.94, $p < 0.01$). Interestingly, TFC showed a positive correlation with α-glucosidase inhibition activity (0.92, $p < 0.01$) and •OH-RSA ($r = 0.86$). Moreover, TCT and TMAC had lower correlations with biological activities compared to TPC and TFC. Noteworthy, DPPH and ABTS are perfectly correlated with each other ($r = 1.00$, $p < 0.05$) (Miliauskas et al., 2004). These correlation results indicate that total phenolic compounds in these Australian native fruits had a direct relationship with free radical scavenging ability, ferric chelation ability, the AChE inhibition capacity while total flavonoids were more closely related to peroxyl inhibition

Table 2
Pearson correlation between phenolic contents and their biological activities.

Variables	TPC	TFC	TCT	TMAC	ABTS	DPPH	FICA	•OH-RSA	α-Glu
TFC	0.57								
TCT	0.47	0.17							
TMAC	−0.55	0.02	0.33						
ABTS	** 0.99	0.61	0.57	−0.44					
DPPH	** 0.99	0.67	0.52	−0.43	** 1.00				
FICA	* 0.94	0.27	0.53	−0.62	* 0.93	0.89			
•OH-RSA	0.84	0.86	0.08	−0.49	0.81	0.86	0.62		
α-Glu	0.82	* 0.92	0.45	−0.12	0.85	0.89	0.60	* 0.90	
AChE	0.84	0.58	0.84	−0.03	* 0.90	0.89	0.78	0.61	0.84

*Significant correlation at $p < 0.01$, **Highly significant correlation at $p < 0.05$.

and α-glucosidase inhibition ability of these fruits (Wang et al., 2019). A positive correlation of phenolic compounds and flavonoids with biological activities indicates that these compounds are the main antioxidant constituents in these Australian native fruits. These results are in accordance with the previously published studies (Ali, Bashmil, et al., 2021). Previously, Zheng and Wang (2001) reported that the position and number of hydroxyl groups on flavonoids on the B-ring and their ability to donate hydrogen to a free radical, affect the anti-radical capacity of these metabolites in plant extracts. Furthermore, the activity of these phenolic compounds depends on the synergistic/antagonistic reactions of these compounds in these extracts and the mechanism of antioxidant reactions in the biological system (Ali, Bashmil, et al., 2021).

A biplot (Fig. 2) also indicates that Kakadu plum has more potential compared to other fruits while the muntries were identified with the least effective biological potential from selected fruits. Davidson plum was observed to have the highest anthocyanin contents, which were not closely associated with the conducted assays. Moreover, the biplot indicates that TFC in quandong peach contributes highly to the α-glucosidase and AChE despite their lower concentration compared to TPC. There is an association between the structures of flavonoids and their antioxidant activity, for example, more hydroxyls among flavonoids are favourable for antioxidant reactions and the antioxidant capacity of flavonoids will increase when C3-C4 position in B-ring is replaced with hydroxyls. The ortho position of hydroxyls with electron donor group (methoxyl) will also improve the antioxidant activity of these metabolites while phenolic hydroxyls on substitutional positions have more contribution in antioxidant activity than the number of hydroxyls (Lin et al., 2014).

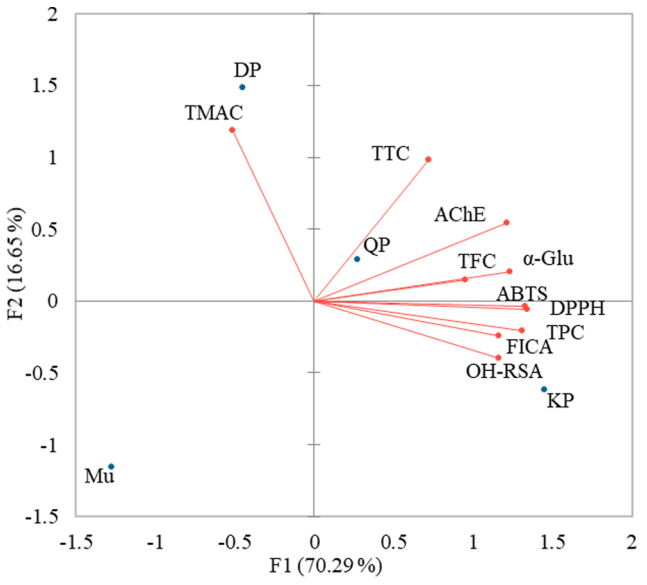

Fig. 2. Biplot analysis of phenolic contents in Australian native fruits and their activities.

3.5. LC-MS/MS characterization of native fruits

3.5.1. Anthocyanins

LC-ESI-QTOF-MS/MS was employed for the untargeted identification and quantification of anthocyanins from Australian native Kakadu plum, Davidson plum, muntries, and quandong peach. The MS/MS fragmentation pattern can provide detailed structural information of each metabolite to separate anthocyanins from other phenolic and non-phenolic compounds. In this study, a total of 77 anthocyanins were tentatively identified while five were identified with pure standards (Table S2). Anthocyanins are unique naturally occurring positively charged colored pigments in plants that can easily donate protons to free radicals. It is widely reported that MS/MS product ions in anthocyanins produced after the removal of sugar units (132 Da for pentose, 150 Da for xylose and arabinose, 162 Da for hexose, 308 Da for rutinoside moiety from the basic aglycone of corresponding anthocyanins (331 Da for malvidin, 317 Da for petunidin, 303 Da for delphinidin, 301 Da for peonidin and 287 Da for cyanidin) in positive mode.

Compound 61 at *m/z* 449 ($C_{21}H_{21}O_{11}$) produced a fragment ion at *m/z* 287 which showed cyanidin aglycone after the removal of the glucoside moiety (162 Da) from the precursor ion in a positive mode. Therefore, compound 61 was tentatively identified as cyanidin-3-*O*-glucoside in Davidson plum. Previously, Oh et al. (2008) also quantified cyanidin-3-*O*-glucoside in different grape cultivars from 2.7 to 51.7 μg/mL. The mass spectra of compound 71 ($C_{27}H_{31}O_{14}$) produced a fragment ion at *m/z* 271 (pelargonidin) after the removal of rutinoside moiety [M − 308 Da] from the precursor ion. Thus, compound 71 was tentatively identified as pelargonidin 3-*O*-rutinoside. In Fig. S2, compound 79 showed the chromatogram and spectrum of delphinidin 3-glucoside ($C_{21}H_{21}O_{12}$ – *m/z* 465) found in Davidson plum obtained through Personal Compound Database and Library (PCDL) while MS/MS further confirmed the removal of glucoside from the basic aglycon structure (delphinidin) which showed product spectra at *m/z* 303. Delphinidin 3-*O*-glucoside had been quantified in grapes from 1.4 to 250.2 μg/mL by Oh et al. (2008). Compounds 63 and 110 at ESI^+ *m/z* 597.1456 and *m/z* 581.1521 showed fragment ions at *m/z* 303 and *m/z* 287, respectively after the loss of sambubioside (294 Da) moiety from the precursor ions. Compounds 62 and 110 were tentatively identified as delphinidin 3-sambubioside and cyanidin 3-sambubioside, respectively.

Pelargonidin3-*O*-glucoside (compound 94) was identified at ESI^+ *m/z* 433 which produced a characteristic fragment at *m/z* 271 (aglycone of pelargonidin) after the loss of glucoside moiety (162 Da) from the precursor ion. Compounds 62 and 77 at ESI^+ *m/z* 595.1653 and *m/z* 611.1614 generated product ions at *m/z* 287 and *m/z* 303 after the neutral loss of rutinoside moiety (308 Da) from the precursor ions. Compounds 62 and 77 were tentatively identified as cyanidin 3-rutinoside and delphinidin 3-rutinoside found in quandong peach and muntries. Overall, Australian native fruits are a rich source of anthocyanins and have considerable health remedies.

3.5.2. Non-Anthocyanin flavonoids

Flavonoids are also important secondary metabolites and indispensable components of nutraceutical, functional, medicinal, and pharmaceutical applications (Ali, Wu, et al., 2021). They have potent antioxidant, anti-mutagenic, anti-carcinogenic, and anti-inflammatory properties (Panche et al., 2016). In this quest, a total of 122 non-anthocyanin flavonoids (41 flavones, 28 flavonols and dihydroflavonols, 24 isoflavonoids, 17 flavanones, 4 flavans, 8 chalcones and dihydrochalcones) were tentatively identified in selected fruits (Table S2). Compound 123 at ESI^- produced a fragment ion at *m/z* 245 after the loss of CO_2 (44 Da) from the precursor ion. Compound 123 was identified as epicatechin which was further confirmed through pure standard. Epicatechin was identified in muntries and Kakadu plum. Compound 137 (apigenin 6-C-glucoside) was produced at product at *m/z* 271 (apigenin) after the removal of hexoxide (162 Da) from the precursor ion in positive mode. Apigenin 6–8-di-C-glucoside (compound 144 – $C_{27}H_{30}O_{15}$ – *m/z* 593.1515) was tentatively identified in Kakadu plum, muntries and quandong peach in a negative mode which generated daughter ions at *m/z* 503 and 473 (Makita et al., 2016). Compound 147 at ESI^- *m/z* 505.0980 produced a product ion at *m/z* 329 after the removal of glucuronide moiety from the aglycone. Compound 147 was putatively identified as tricin 7-*O*-glucuronide which was identified in Kakadu plum. Quercetin 3′-*O*-glucuronide (compound 185 – 477.0676) produced a product ion at 301 after the loss of glucuronide from the parent ion. Compound 185 was putatively identified in Kakadu plum and Davidson plum. Compound 189 (myricetin 3-*O*-rhamnoside) produced a product ion at *m/z* 319 after the loss of rhamnoside moiety from the precursor ion in positive mode. Previously, these compounds were reported in mint and lemon with strong antioxidant potential (Chou et al., 2021). Compounds 123, 149, 156, 160, 164, 176, 180 and 196 were identified with pure standards. Previously, quercetin, rutin and myricetin were identified in Davidson plum (Sakulnarmrat et al., 2014) while kaempferol and rutin were identified in quandong peach Konczak et al. (2010a). Quercetin has been reported for antioxidant (Boots et al., 2008; Kadam et al., 2018) anti-inflammatory (Saeedi-Boroujeni & Mahmoudian-Sani, 2021), anti-cancer activities (Ay et al., 2016, Chap. 4). Therapeutic role of quercetin against cardiovascular diseases, cancers and neurodegenerative disorders is well known (Ay et al., 2016, Chap. 4). Previously, vitexin (compound 130) and luteolin (compound 142) were also identified in Kakadu plum (Courtney et al., 2015).

3.5.3. Phenolic acids

They are widespread aromatic secondary plant metabolites and have promising health effects. A total of 51 phenolic acids (10 hydroxybenzoic acids, 34 hydroxycinnamic acids and 7 other phenolic acids were tentatively identified and characterized through their MS/MS spectra (Table S2). Mainly, phenolic acids show the fragmentation pattern through the removal of CO_2 and hexosyl moiety from the parent ions (Ali, Wu, et al., 2021). Compound 1 (gallic acid 4-*O*-glucoside exhibited the fragment ions at *m/z* 169 (gallic acid) and *m/z* 125 via the loss of hexosyl moiety (162 Da) and CO_2 (44 Da) from the parent and fragment ions (Singh et al., 2016). Compounds 4 (protocatechuic acid), 7 (gallic acid), 9 (*p*-hydroxybenzoic acid), 11 (caffeic acid), 32 (*p*-coumaric acid) and 36 (cinnamic acid) exhibited the product ions at *m/z* 109, *m/z* 125, *m/z* 95, *m/z* 135, *m/z* 119 and *m/z* 103 after the neutral loss of CO_2 (44 Da) from the parent ions, respectively. Ferulic acid (compound 29) at ESI^- *m/z* 193.0509 generated product ions at *m/z* 178, 149 and 143 after the loss of [M − H − CH_3], [M − H − CO_2] and [M − H − CH_3 + CO_2], respectively from the precursor ion. Compound 44 (3-feruloylquinic acid) was tentatively identified in Kakadu plum and Davidson plum which produced daughter ions at *m/z* 351, 325 and 163 in positive mode. Compounds 4, 5, 7, 9, 10, 11, 16, 29, 31, 32, 36 and 48 were compared and identified through pure standards. Previously, chlorogenic acid (compound 31) was also identified in quandong peach (Sakulnarmrat et al., 2014).

3.5.4. Tannins

Tannins are structurally complex phenolic compounds and classified into hydrolyzable, condensed and complex tannins. A total of 15 compounds were tentatively identified as tannins (Table S2). Ellagic acid, procyanidin B2, 2,3-dimethylellagic acid and prodelphinidin B3 has been identified due to their health potential (Cuadrado-Silva et al., 2016; Gupta et al., 2021). Previously, corilagin (compound 246) and punicalin (compound 247) were identified in Kakadu plum (Courtney et al., 2015). Previously, ellagic acid (compound 247) was identified in Davidson plum (Sakulnarmrat et al., 2014). Previously, Sharifi-Rad et al. (2022) were reported antioxidant, anti-inflammatory, anti-proliferative and anti-mutagenic properties of ellagic acid.

3.5.5. Lignans and stilbenes

A total of 12 lignans and 5 stilbenes were putatively identified in selected Australian native fruits (Table S2). Lignans are well-known bioactive compounds for antioxidant, anti-cancer and anti-inflammatory properties (Ali, Bashmil, et al., 2021). Compound 261 at ESI^- *m/z* 383.1495 was generated product ions at *m/z* 355 and 341 and was identified as schisandrin C while compound 269 at ESI^+ *m/z* 401.1962 produced a fragment ion at *m/z* 386 after the loss of [M + H − CH3] and was tentatively identified as schisandrin B. Previously, antioxidant, anti-inflammatory, anti-cancer and anti-neurogenerative properties of schisandrin C has been reported (Takanche et al., 2018; Yang & Yuan, 2021). These compounds were also identified in *Schisandra chinensis* which is well-known for its medicinal potential (Subbiah et al., 2020). Compound 267 at ESI^+ *m/z* 299.1259 was identified in Kakadu plum which produced fragment ions at *m/z* 281, 187 and 165 after the loss of [M − H − H_2O], [M − H − $C_6H_8O_2$] and [M − H − $C_9H_8O_2$], respectively from the precursor ion (Ali, Wu, et al., 2021). Compound 267 was putatively identified as enterolactone and has antioxidant, anticancer, antimetastatic and anti-neurodegenerative properties (Mali et al., 2019; Polat Kose & Gulcin, 2021; Reddy et al., 2020). Compound 271 (4-hydroxy-3,5,4′-trimethoxystilbene), compound 272 (polydatin) and compound 274 (resveratrol) are well-known stilbenes for their reported health benefits (Ali, Bashmil, et al., 2021; Galiniak et al., 2019; Luo et al., 2022), were tentatively identified in Kakadu plum.

3.5.6. Other polyphenols

Two tyrosols, two curcuminoids, nine coumarins and derivatives, one phenylpropene, two nitrophenols and naphthoquinones, one hydroxybenzoketones, one cyclitol, two alkylphenols and three other polyphenols were also putatively identified in Australian native fruits (Table S2). Compound 282 at ESI^- *m/z* 177.0210 was generated product ions at *m/z* 149, 133, and 89 after the loss of [M − H − CO], [M − H − CO_2], and [M − H − 2CO_2], respectively from the precursor ion (Ali, Wu, et al., 2021). Compound 282 was tentatively identified as esculetin in Kakadu plum. Previously, Yun et al. (2012) also identified esculetin in their study with the same product ions through MS/MS. Compound 285 (6-methylcoumarin), compound 286 (7-methoxycoumarin), compound 287 (scopoletin), compound 288 (umbelliferone), compound 289 (mellein) and compound 290 (coumarin) were generated product ions at *m/z* 115, 133, 147, 119, 135 and 103 after the loss of CO_2 (44 Da), respectively from the precursor ion.

The application of LC-MS/MS-QTOF to Australian native fruits had allowed us to screen and detect 307 phenolic and non-phenolic compounds with their product ions. No single study had been conducted yet for the characterization of all these Australian native fruits in such a comprehensive way for their phenolic compounds. The screening of these bioactive constituents in these selected fruits can establish a new science to understand their potent health benefits. There is a considerable potential to identify novel unknown bioactive compounds by using this advanced analytical approach. One limitation of using low collision energies is that they could not localize the position of the native phenolic ring in the study of LC-MS/MS that went under modification. To solve

this limitation, Nuclear magnetic resonance (NMR) would be useful because it can identify the responsible compounds for modification.

3.6. Distribution of phenolic metabolites

Venn diagram analysis was conducted to find the diversity and distribution of phenolic metabolites in Kakadu plum (blue), Davidson plum (yellow), quandong peach (red) and muntries (green) (Fig. 3). Australian native commercial fruits contain a diverse range of different phenolic compounds including total number of polyphenols, total phenolic acids, total flavonoids, and total anthocyanins. Furthermore, visual distribution of total number of metabolites based on the presence of individual metabolites identified in Kakadu plum, Davidson plum, quandong peach and muntries (Fig. 3).

Fig. 3A shows that the Kakadu plum has the highest number of unique metabolites (77, 25.2 %) while Davidson plum has the least number of unique metabolites (2, 1.4 %). There were 31 (10.2% individual metabolites that were commonly identified in Kakadu plum, quandong peach and muntries while 14 (4.6 %) individual phenolic metabolites were identified in all four fruits. Fig. 3B depicts that Kakadu plum has the highest number of unique total phenolic acids 21 (39.6 %) while quandong peach has only one individual unique phenolic acid. Moreover, a total of 6 (11.3 %) phenolic acids were overlapped in all four fruits. Fig. 3C shows that Kakadu plum has 43 (30.7 %) individual unique non-anthocyanin flavonoids while Davidson plum has only two (1.4 %) unique non-anthocyanin flavonoids. Whereas, Kakadu plum, muntries and quandong peach shared 22 (15.7 %) unique non-anthocyanin flavonoids. Furthermore, a total of 6 (4.3 %) non-anthocyanin flavonoids were overlapped in all four fruits. Fig. 3D indicates that no anthocyanin metabolite was identified in Kakadu plum while Davidson plum has 13 (21.0%) unique anthocyanins. Interestingly, a total of 6 (9.7%) unique anthocyanins were identified common in Davidson plum, quandong peach and muntries while 10 (16.1 %) anthocyanins were overlapped in muntries and quandong peach.

3.7. Quantification/Semi-Quantification of individual phenolic metabolites

The quantification of individual metabolites from Australian native fruits was achieved through LC-MS/MS-QTOF. LC-MS/MS is a much accurate and more reliable technique for the quantification purposes compared to HPLC. In this study, we semi-quantified a total of 41 phenolic metabolites from Australian native Kakadu plum, Davidson plum, quandong peach and muntries (Table S3).

3.7.1. Anthocyanins

In this study, a total of 9 anthocyanins were semi-quantified. Delphinidin 3-*O*-sambubioside and cyanidin 3-rhamnoside were only quantified in Davidson plum (528.14 ± 34.82 μg/g) and quandong peach (88.56 ± 4.95 μg/g). Cyanidin 3-sambubioside was quantified in Davidson plum (67.60 ± 5.30 μg/g) and muntries (91.28 ± 1.83 μg/g). Cyanidin 3-glucoside was quantified in quandong peach (85.13 ± 1.86 μg/g), muntries (125.01 ± 3.69 μg/g), and Davidson plum (153.73 ± 19.61 μg/g). Previously, Konczak et al. (2010a) were also quantified

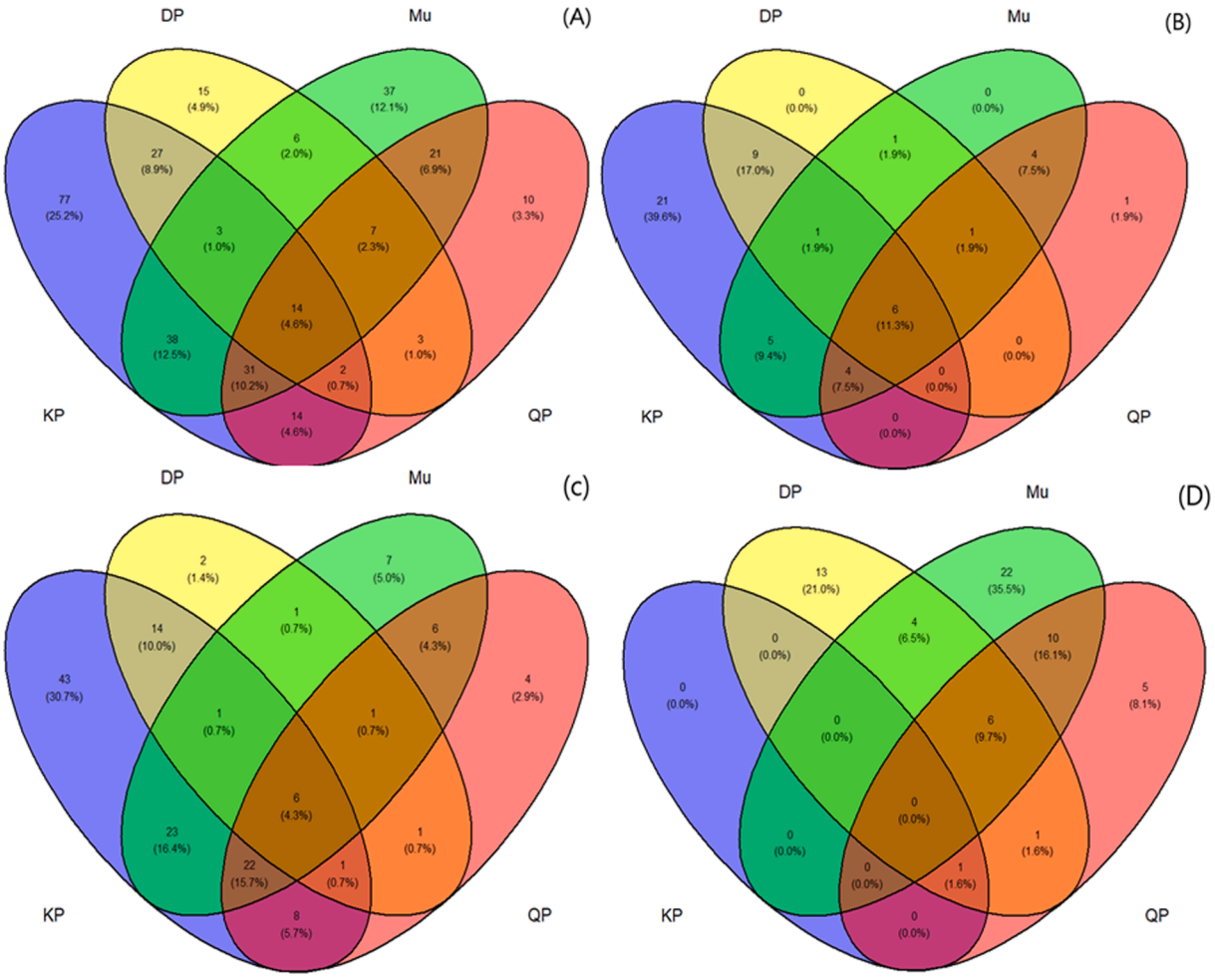

Fig. 3. Venn diagram analysis of phenolic metabolites (A) total number of polyphenols (B) total phenolic acids (C) total non-anthocyanin flavonoids (D) total anthocyanins in Australian native fruits.

delphinidin 3-sambubioside (0.16 ± 0.04 mg/g) and cyanidin 3-sabubioside (0.02 ± 0.001 mg/g) from Davidson plum while cyanidin 3-glucoside (0.13 ± 0.005 mg/g) was quantified in quandong peach. Delphinidin 3-*O*-glucoside was also quantified in muntries (132.28 ± 7.77 μg/g), quandong peach (379.89 ± 26.86 μg/g) and Davidson plum (391.76 ± 15.47 μg/g). Delphinidin was quantified in Davidson plum (90.51 + 3.83 μg/g), quandong peach (175.95 ± 20.01 μg/g) and muntries (321.98 ± 27.02 μg/g). Cyanidin was quantified in Davidson plum (57.23 ± 11.61 μg/g) and quandong peach (82.37 ± 3.83 μg/g). Cyanidin 3-sambubioside was quantified in Davidson plum (67.60 ± 5.30 μg/g) and muntries (91.28 ± 1.83 μg/g). Delphinidin 3-rutinoside (226.81 ± 30.04 μg/g 342.45 ± 11.59 μg/g) and Cyanidin 3-rutinoside (307.76 ± 14.06 μg/g and 81.13 ± 7.45 μg/g) were quantified in quandong peach and muntries, respectively. Previously, delphinidin 3-glucoside (0.3 ± 0.01 μg/g) and cyanidin 3-glucoside (0.8 ± 0.05 μg/g) were quantified in muntries (Tan et al., 2011a, 2011b).

3.7.2. Non-Anthocyanin flavonoids

A total of 16 non-anthocyanin flavonoids were also quantified from selected Australian native fruits. Quercetin-3-glucoside (154.37 ± 8.89 μg/g), kaempferol (193.85 ± 12.33 μg/g), genistein (108.32 ± 5.43 μg/g), chrysin (100.34 ± 10.19 μg/g), phloretin (120.73 ± 7.50 μg/g) and 3′-*O*-methylviolanone (372.43 ± 28.10 μg/g) were only quantified in Kakadu plum while taxifolin (101.80 ± 7.73 μg/g) and procyanidin B2 (94.01 ± 2.24 μg/g) were only quantified in muntries. Moreover, naringin (105.75 ± 7.14 μg/g) was only quantified in Davidson plum. The higher concentration of quercetin (408.14 ± 10.93 μg/g) was measured in Kakadu plum while the least concentration of quercetin (122.55 ± 11.15 μg/g) was detected in quandong peach. Quercetin was also quantified in muntries (236.44 ± 23.46 μg/g) and Davidson plum (126.09 ± 18.08 μg/g). The higher concentration of diosmin (274.78 ± 15.81 μg/g) was quantified in muntries while the least value of diosmin (199.78 ± 10.81 μg/g) was found in Kakadu plum. Luteolin was also quantified in Kakadu plum (250.09 ± 9.70 μg/g), quandong peach (106.44 ± 6.46 μg/g) and muntries (105.64 ± 16.67 μg/g) Previously, kaempferol (0.61 ± 0.01 mg/g) and rutin (0.53 ± 0.01 mg/g) were quantified in quandong peach (Konczak et al., 2010a). Previously, Mani et al. (2021) were also reported luteolin, quercetin, hesperetin and kaempferol glycosides in Kakadu plum.

3.7.3. Phenolic acids and derivatives

A total of 16 phenolic acids were quantified from Kakadu plum (11), quandong peach (10), Davidson plum (5) and muntries (9). The higher concentration of cinnamic acid (460.63 ± 31.94 μg/g) and syringic acid (445.21 ± 32.77 μg/g) was quantified in Kakadu plum. Cinnamic acid (193.32 ± 18.44 μg/g) and syringic acid (61.30 ± 17.31 μg/g) were also quantified in muntries and quandong peach, respectively. Caffeic acid was also quantified in Kakadu plum (102.13 ± 2.97 μg/g) and Davidson plum (78.95 ± 1.98 μg/g) while protocatechuic acid was quantified in Kakadu plum (54.16 ± 3.65 μg/g) and quandong peach (391.61 ± 23.75 μg/g). Moreover, 2,3-dimethylellagic acid and 3-sinapoylquinic acid were only quantified in Kakadu plum (379.75 ± 14.29 μg/g) and muntries (82.24 ± 6.62 μg/g), respectively. Cinnamic acid was quantified in Kakadu plum (748.68 ± 64.07 μg/g) and muntries (801.42 ± 50.44 μg/g). 3-*p*-Coumaroylquinic acid was quantized in muntries (85.70 ± 4.15 μg/g) and quandong peach (86.95 ± 5.40 μg/g).

The highest concentration of ellagic acid (155.86 ± 9.18 μg/g) was quantified in muntries while the least amount of ellagic acid (76.25 ± 3.61 μg/g) was measured in Kakadu plum. Ellagic acid was also quantified in Davidson plum (150.73 ± 5.85 μg/g) and quandong peach (91.61 ± 5.85 μg/g). Previously, Cheesman et al. (2019) were also quantified ellagic acid (1.05%) and gallic acid (4.70%) in methanolic extract of Kakadu plum fruit. Ferulic acid was also quantified in Davidson plum (18.07 ± 3.41 μg/g) and Kakadu plum (12.17 ± 3.11 μg/g). Chlorogenic acid was quantified in quandong peach (80.98 ± 6.91 μg/g) and muntries (142.18 ± 14.01 μg/g). Previously, chlorogenic acid was also identified in quandong peach (Konczak et al., 2010a).

3.7.4. Heatmap clustering of quantified individual phenolic metabolites

Heatmap column and row wise clustering was conducted using MetaboAnalyst (https://www.metaboanalyst.ca) given in Fig. 4. It indicates that delphinidin 3-*O*-sambubioside, delphinidin 3-*O*-glucoside, cyanidin 3-glucoside and ellagic acid have higher concentration in Davidson plum while isorhamnetin, delphinidin 3-*O*-glucoside and cyanidin 3-rutinoside have higher concentration in quandong peach. Davidson plum and quandong peach are correlated to each other. Procatechuic acid, epicatechin, *p*-coumaric acid, delphinidin, delphinidin 3-rutinoside, procyanidin B2, *p*-hydroxybenzoic acid and diosmin were observed with higher concentration in muntries while cinnamic acid, syringic acid, epicatechin, quercetin, *p*-coumaric acid, gallic acid, 2,3-dimethyellagic acid and 3-*O*-methyviolone.

3.8. Molecular docking

Molecular docking has great importance in drug discovery. The calculated binding energies, glide score and glide energy of selected compounds of Australian native fruits in related enzymes (7E3I and 5NN8) are presented in Table S4 while the estimated binding geometry and 3D of delphinidin 3-sambubioside and cyanidin 3-sambubioside in AChE while punicafolin and cyanidin 3-sambubioside in α-glucosidase are given in Fig. 5A and Fig. 5B.

All the compounds were well locked in 7E3I and 5NN8. Delphinidin 3-sambubioside in 7E3I made hydrogen bonds with TRP 86, TYR 341 and SER 203 while π − π stacking was observed with TYR 124 and TYR 341. Cyanidin 3-sambubioside in 7E3I made hydrogen bonds with HID 447, TYR 337, TYR 341, TYR 83, ASN 87, TRP 86, GLY 120 and TYR 133 while π − π interaction with TYR 341. Rutin in 7E3I made hydrogen

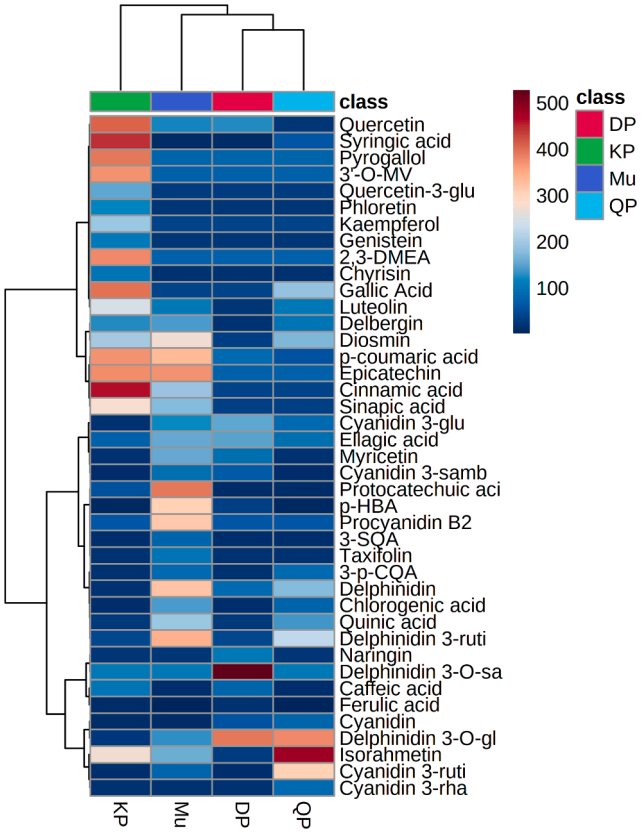

Fig. 4. Heatmap clustering of quantified phenolic metabolites from Australian native Kakadu plum (KP), Davidson plum (DP), quandong peach (QP) and muntries (Mu).

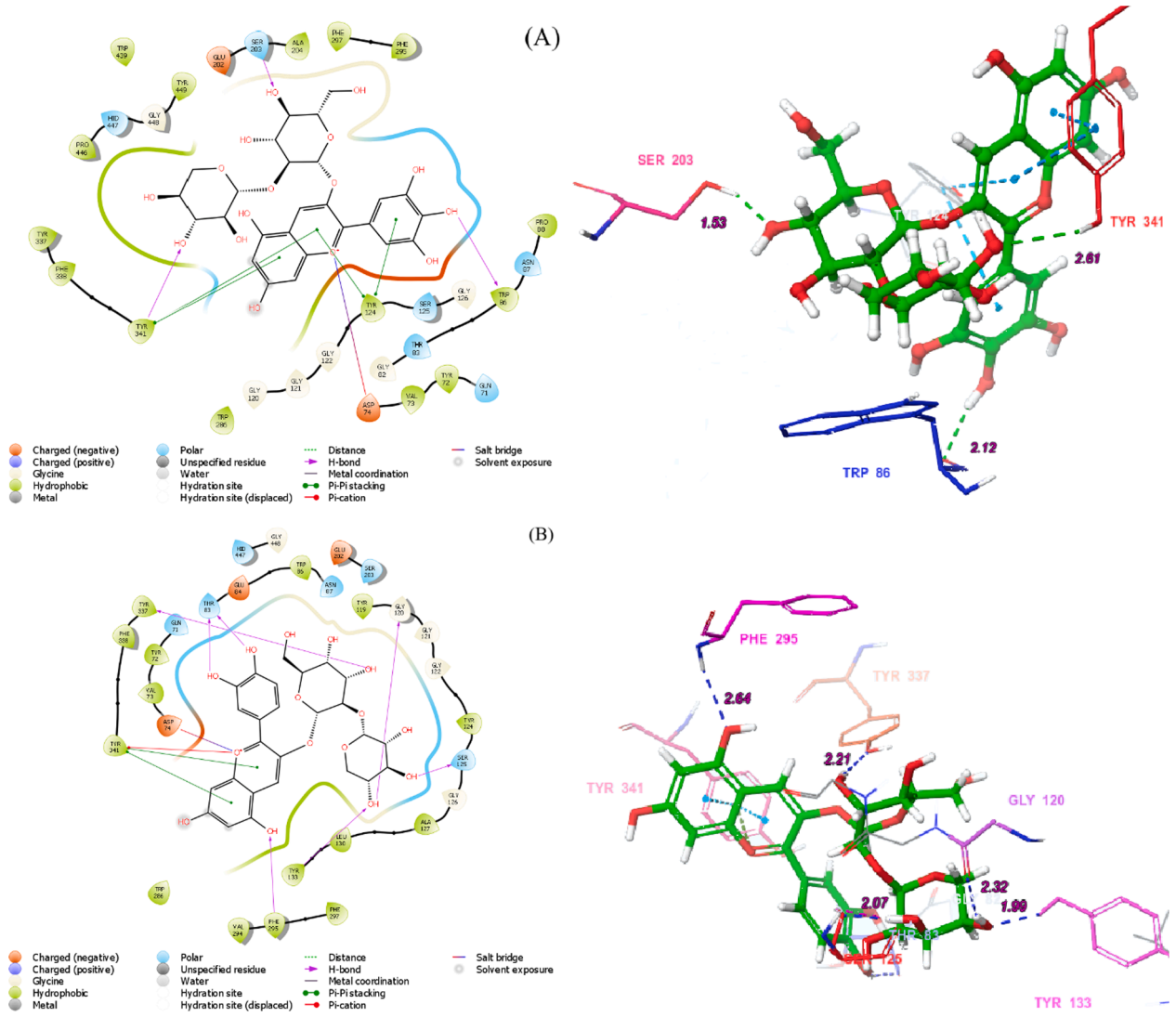

Fig. 5A. The estimated binding geometry and 3D view of delphinidin 3-sambubioside (A) and cyanidin 3-sambubioside (B) in AChE. The active side residues are named with three letters.

bonds with GLU 202, GLY, ASN 87 and TYR 72 while π – π interactions was observed with TYR 337 and TRP 86 (Fig. S4). Docking binding energy of delphinidin 3-sambubioside, cyanidin 3-sambubioside and rutin in 7E3I was – 15.59, −17.77 and – 17.77, respectively. From the given results, it was observed flavonoids especially anthocyanins are potent metabolites to inhibit the AChE. Generally, the docking binding energy of anthocyanins were observed higher than other compounds. Non-anthocyanin flavonoids including procyanidin B2, rutin, vitexin, myricetin, quercetin taxifolin, luteolin, phloretin, naringenin, (-)-epicatechin and kaempferol in AChE (7E3I) have higher binding energies (−15.26 kcal/mol, −13.27 kcal/mol, −12.16 kcal/mol, −10.84 kcal/mol, −9.93 kcal/mol, −9.83 kcal/mol, −9.82 kcal/mol, −9.72 kcal/mol, and – 9.52 kcal/mol, respectively). Interestingly, 3-*O*-sinapoylquinic acid 3-*p*-Coumaroylquinic acid are the phenolic acids which have higher binding energies (−10.20 kcal/mol and – 10.10 kcal/mol) than taxifolin, luteolin, phloretin, naringenin, (-)-epicatechin and kaempferol (Table S4). Punicafolin in 5NN8 made hydrogen bons with ARG 411, SER 676, ASP 518, ASP 616, PHE 525 while π – π stacking was observed TRP 481 and PHE 649. Cyanidin 3-sambubioside made two double hydrogen bonds with negatively charged ASP 282 and one hydrogen bond with ASP 404 while two π – π bonds with PHE 649 and one with TRP 376 (Fig. 5B). Cyanidin 3-glucoside in 5NN8 (Fig. S4) was observed hydrogen bonding with ASP 282, ASP 404, ASP 616, ALA 284, and LEU 677 while π – π interactions was made with PHE 649 and TRP 376. Diosmin in 5NN8 made hydrogen bonds with ASP 404, ASP 518, ASP 616 and EDO 1024 while π – π stacking was observed with TRP 481 and PHE 525 (Fig. S4). Punicafolin, cyanidin 3-sambubioside and cyanidin 3-glucoside in 5NN8 have higher binding energies than acarbose (standard) Table S3. Rutin and diosmin are non-anthocyanin flavonoids that have higher binding energies in 5NN8 compared to all other selected non-anthocyanin flavonoids. Chlorogenic acid and 3-*p*-coumaroylqunic acid are phenolic acids which have higher binding energies in 5NN8 compared to other phenolic acids and flavonoids (Table S4).

It is well established that, molecular docking is a virtual method to predict the possible interactions between potential inhibitors and respective enzymes (AChE and α-glucosidase). Thus, it is necessary to conduct the enzyme inhibitory activities of individual bioactive compounds to establish the actual inhibitory activity of individual compounds (Li et al., 2022).

4. Conclusions

In this study, Australian native fruits (Kakadu plum, Davidson plum, quandong peach and muntries) were comprehensively analysed for phenolic compounds using LC-ESI-QTOF-MS/MS. A total of 307 bioactive metabolites were putatively identified in selected Australian native

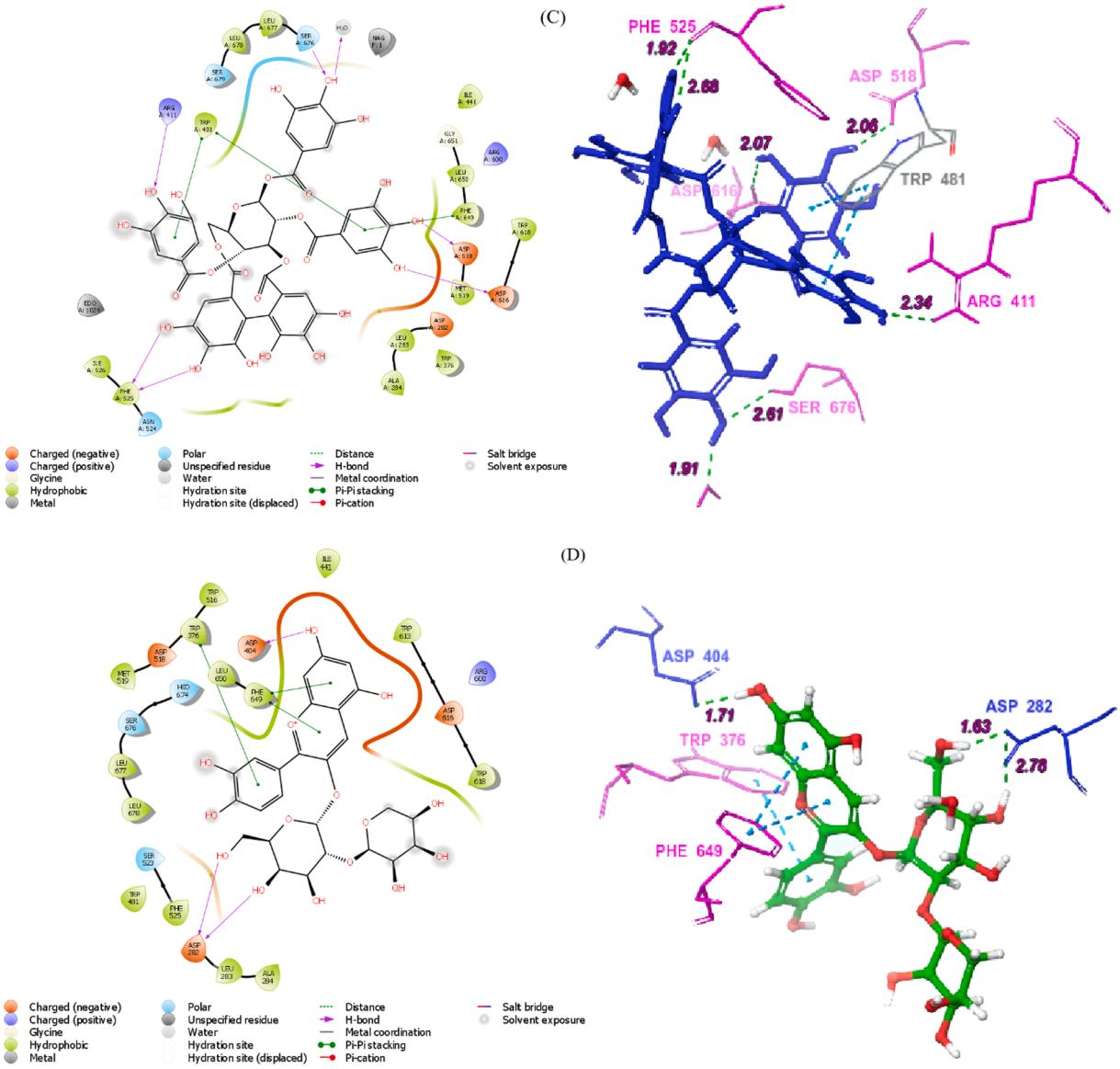

Fig. 5B. The estimated binding geometry and 3D view of punicafolin (C) and cyanidin 3-sambubioside in α-glucosidase. The active side residues are named with three letters.

fruits. Overall, Kakadu plum was observed with higher total phenolic content and biological activities. Davidson plum was observed with higher total monomeric anthocyanin content than berries. Molecular docking reveals that flavonoids especially anthocyanins are potent biological compounds with significant α-glucosidase and acetylcholinesterase inhibitory activities. The higher concentration of anthocyanins and non-anthocyanin flavonoids in these selected Australian native fruits than berries are in consistence with their traditional usage for healing properties. The obtained results demonstrated that these Australian native fruits could be used in food and medicinal products with the further proved clinical data.

Supplementary Materials: The supporting information can be downloaded at: Figure S1: Total Ion Chromatograms (TIC) of Kakadu plum, Davidson plum, quandong peach and muntries in positive (black color) and negative mode (red color); Figure S2: LC-MS/MS identification of delphinidin 3-glucoside (compound 79) in Davidson plum. A chromatogram (A) and a mass spectrum (B) and MS/MS spectra though online library and database (C) of delphinidin 3-glucoside are presented; Figure S3: MS/MS spectra of some selected compounds of Kakadu plum (40 N), quandong peach (42P) and Davidson plum (43P); Figure S4. The estimated binding geometry of rutin (C) in AChE while cyanidin 3-glucoside (A) and diosmin (B) in alpha-glucosidase. The active side residues are named with three letters. Table S1: Biological activities of Australian native fruits; Table S2: LC-ESI-QTOF-MS/MS identification and characterization of phenolic compounds from Australian native fruits; Table S3: Semi-quantification of individual phenolic metabolites in Australian native fruits (μg/g); Table S4: The estimated binding energy (kcal/mol) and glide energy (kcal/mol) of each compound in AChE (7E3I) and α-glucosidase (5NN8).

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Data Availability Statement

The supporting data is provided in the Supplementary Materials.

CRediT authorship contribution statement

Akhtar Ali: Conceptualization, Methodology, Investigation, Formal analysis, Software, Validation, Visualization, Data curation, Writing – original draft. **Jeremy J. Cottrell:** Writing – review & editing, Supervision. **Frank R. Dunshea:** Writing – review & editing, Supervision, Project administration, 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.

Data availability

The supporting data is available in the supplementary file.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111951.

References

Ali, A., Bashmil, Y. M., Cottrell, J. J., Suleria, H. A. R., & Dunshea, F. R. (2021). LC-MS/MS-QTOF screening and identification of phenolic compounds from Australian grown herbs and their antioxidant potential. *Antioxidants (Basel), 10*(11), 1770. https://doi.org/10.3390/antiox10111770

Ali, A., Wu, H., Ponnampalam, E. N., Cottrell, J. J., Dunshea, F. R., & Suleria, H. A. R. (2021). Comprehensive profiling of most widely used spices for their phenolic compounds through LC-ESI-QTOF-MS2 and their antioxidant potential. *Antioxidants (Basel), 10*(5), 721. https://doi.org/10.3390/antiox10050721

Ali, A., Zahid, H. F., Cottrell, J. J., & Dunshea, F. R. (2022). A comparative study for nutritional and phytochemical profiling of coffea arabica (C. arabica) from different origins and their antioxidant potential and molecular docking. *Molecules, 27*(16), 5126. https://doi.org/10.3390/molecules27165126

Anil, D. A., Aydin, B. O., Demir, Y., & Turkmenoglu, B. (2022). Design, synthesis, biological evaluation and molecular docking studies of novel 1H–1,2,3-Triazole derivatives as potent inhibitors of carbonic anhydrase, acetylcholinesterase and aldose reductase. *Journal of Molecular Structure, 1257*, Article 132613. https://doi.org/10.1016/j.molstruc.2022.132613

Ay, M., Charli, A., Jin, H., Anantharam, V., Kanthasamy, A., & Kanthasamy, A. G. (2016). Quercetin. In R. C. Gupta (Ed.), *Nutraceuticals* (pp. 447-452). Academic Press. https://doi.org/10.1016/B978-0-12-802147-7.00032-2.

Bashmil, Y. M., Ali, A., Bk, A., Dunshea, F. R., & Suleria, H. A. R. (2021). Screening and characterization of phenolic compounds from australian grown bananas and their antioxidant capacity. *Antioxidants, 10*(10), 1521.

Boots, A. W., Haenen, G. R. M. M., & Bast, A. (2008). Health effects of quercetin: From antioxidant to nutraceutical. *European Journal of Pharmacology, 585*(2–3), 325–337.

Cáceres-Vélez, P. R., Ali, A., Fournier-Level, A., Dunshea, F. R., & Jusuf, P. R. (2022). Phytochemical and safety evaluations of finger lime, mountain pepper, and tamarind in zebrafish embryos. *Antioxidants (Basel), 11*(7), 1280. https://doi.org/10.3390/antiox11071280

Cheesman, M. J., White, A., Matthews, B., & Cock, I. E. (2019). Terminalia ferdinandiana fruit and leaf extracts inhibit methicillin-resistant Staphylococcus aureus growth. *Planta Medica, 85*(16), 1253–1262.

Chou, O., Ali, A., Subbiah, V., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R. (2021). LC-ESI-QTOF-MS/MS characterisation of phenolics in herbal tea infusion and their antioxidant potential. *Fermentation, 7*(2), 73.

Colović, M. B., Krstić, D. Z., Lazarević-Pašti, T. D., Bondžić, A. M., & Vasić, V. M. (2013). Acetylcholinesterase inhibitors: Pharmacology and toxicology. *Current Neuropharmacology, 11*(3), 315–335. https://doi.org/10.2174/1570159x11311030006

Courtney, R., Sirdaarta, J., Matthews, B., & Cock, I. E. (2015). Tannin components and inhibitory activity of Kakadu plum leaf extracts against microbial triggers of autoimmune inflammatory diseases. *Pharmacognosy Journal, 7*(1), 18–31.

Cuadrado-Silva, C. T., Pozo-Bayón, M., & Osorio, C. (2016). Targeted metabolomic analysis of polyphenols with antioxidant activity in sour guava (Psidium friedrichsthalianum Nied.) fruit. *Molecules, 22*(1), 11. https://doi.org/10.3390/molecules22010011

Dangles, O., & Fenger, J. A. (2018). The chemical reactivity of anthocyanins and its consequences in food science and nutrition. *Molecules, 23*(8), 1970. https://doi.org/10.3390/molecules23081970

Dhakal, S., Kushairi, N., Phan, C. W., Adhikari, B., Sabaratnam, V., & Macreadie, I. (2019). Dietary polyphenols: a multifactorial strategy to target Alzheimer's disease. *International Journal of Molecular Sciences, 20*(20), 5090. https://doi.org/10.3390/ijms20205090

Elwekeel, A., El Amir, D., Mohamed, E. I. A., Amin, E., Hassan, M. H. A., & Zaki, M. A. (2022). Characterization of possible α-Glucosidase inhibitors from trigonella stellata extract using LC-MS and in silico molecular docking. *Plants (Basel), 11*(2), 208. https://doi.org/10.3390/plants11020208

Emir, C., Coban, G., & Emir, A. (2022). Metabolomics profiling, biological activities, and molecular docking studies of elephant garlic (Allium ampeloprasum L.). *Process Biochemistry, 116*, 49–59. https://doi.org/10.1016/j.procbio.2022.03.002

Galiniak, S., Aebisher, D., & Bartusik-Aebisher, D. (2019). Health benefits of resveratrol administration. *Acta Biochimica Polonica, 66*(1), 13–21. https://doi.org/10.18388/abp.2018_2749

Granato, D., Shahidi, F., Wrolstad, R., Kilmartin, P., Melton, L. D., Hidalgo, F. J., Miyashita, K., van Camp, J., Alasalvar, C., & Ismail, A. B. (2018). Antioxidant activity, total phenolics and flavonoids contents: Should we ban in vitro screening methods? *Food Chem., 264*, 471–475. https://doi.org/10.1016/j.foodchem.2018.04.012

Gupta, A., Singh, A. K., Kumar, R., Jamieson, S., Pandey, A. K., & Bishayee, A. (2021). Neuroprotective potential of ellagic acid: A critical review. *Advances in Nutrition, 12*(4), 1211–1238. https://doi.org/10.1093/advances/nmab007

Hou, D. X. (2003). Potential mechanisms of cancer chemoprevention by anthocyanins. *Current Molecular Medicine, 3*(2), 149–159. https://doi.org/10.2174/1566524033361555

Inácio, M. R., de Lima, K. M., Lopes, V. G., Pessoa, J. D., & de Almeida Teixeira, G. H. (2013). Total anthocyanin content determination in intact açaí (Euterpe oleracea Mart.) and palmitero-juçara (Euterpe edulis Mart.) fruit using near infrared spectroscopy (NIR) and multivariate calibration. *Food Chemistry, 136*(3–4), 1160–1164. https://doi.org/10.1016/j.foodchem.2012.09.046

Jankowski, A., Jankowska, B., & Niedworok, J., 2000. The effect of anthocyanin dye from grapes on experimental diabetes. Folia Medica Cracoviensia, *41*(3-4), 5-15. (Wpływ barwnika antocyjaninowego z winogron na doświadczalna cukrzyce.).

Kadam, D., Palamthodi, S., & Lele, S. S. (2018). LC–ESI-Q-TOF–MS/MS profiling and antioxidant activity of phenolics from L. Sativum seedcake. *Journal of food science and technology, 55*(3), 1154–1163.

Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R. (2020). Recent research on the health benefits of blueberries and their anthocyanins. *Advances in Nutrition, 11*(2), 224–236. https://doi.org/10.1093/advances/nmz065

Konczak, I., Zabaras, D., Dunstan, M., & Aguas, P. (2010a). Antioxidant capacity and hydrophilic phytochemicals in commercially grown native Australian fruits. *Food Chemistry, 123*(4), 1048–1054. https://doi.org/10.1016/j.foodchem.2010.05.060

Konczak, I., Zabaras, D., Dunstan, M., & Aguas, P. (2010b). Antioxidant capacity and phenolic compounds in commercially grown native Australian herbs and spices. *Food Chemistry, 122*(1), 260–266.

Leonard, W., Zhang, P., Ying, D., Xiong, Y., & Fang, Z. (2021). Extrusion improves the phenolic profile and biological activities of hempseed (Cannabis sativa L.) hull. *Food Chemistry, 346*, Article 128606. https://doi.org/10.1016/j.foodchem.2020.128606

Li, Y. J., He, F. Q., Zhao, H. H., Li, Y., & Chen, J. (2022). Screening and identification of acetylcholinesterase inhibitors from Terminalia chebula fruits by immobilized enzyme on cellulose filter paper coupled with ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry and molecular docking. *Journal of Chromatography A, 1663*, Article 462784. https://doi.org/10.1016/j.chroma.2021.462784

Lin, C. Z., Zhu, C. C., Hu, M., Wu, A. Z., Bairu, Z. D., & Kangsa, S. Q. (2014). Structure-activity relationships of antioxidant activity in vitro about flavonoids isolated from Pyrethrum tatsienense. *Journal of Intercultural Ethnopharmacology, 3*(3), 123–127. https://doi.org/10.5455/jice.20140619030232

Luo, J., Chen, S., Wang, L., Zhao, X., & Piao, C. (2022). Pharmacological effects of polydatin in the treatment of metabolic diseases: A review. *Phytomedicine, 102*, Article 154161. https://doi.org/10.1016/j.phymed.2022.154161

Maher, P. (2019). The potential of flavonoids for the treatment of neurodegenerative diseases. *International Journal of Molecular Sciences, 20*(12). https://doi.org/10.3390/ijms20123056

Mahmudov, I., Demir, Y., Sert, Y., Abdullayev, Y., Sujayev, A., Alwasel, S. H., & Gulcin, I. (2022). Synthesis and inhibition profiles of N-benzyl- and N-allyl aniline derivatives against carbonic anhydrase and acetylcholinesterase – A molecular docking study. *Arabian Journal of Chemistry, 15*(3), Article 103645. https://doi.org/10.1016/j.arabjc.2021.103645

Makita, C., Chimuka, L., Steenkamp, P., Cukrowska, E., & Madala, E. (2016). Comparative analyses of flavonoid content in Moringa oleifera and Moringa ovalifolia with the aid of UHPLC-qTOF-MS fingerprinting. *South African Journal of Botany, 105*, 116–122. https://doi.org/10.1016/j.sajb.2015.12.007

Mali, A. V., Padhye, S. B., Anant, S., Hegde, M. V., & Kadam, S. S. (2019). Anticancer and antimetastatic potential of enterolactone: Clinical, preclinical and mechanistic perspectives. *European Journal of Pharmacology, 852*, 107–124. https://doi.org/10.1016/j.ejphar.2019.02.022

Mani, J. S., Johnson, J. B., Hosking, H., Ashwath, N., Walsh, K. B., Neilsen, P. M., … Naiker, M. (2021). Antioxidative and therapeutic potential of selected Australian plants: A review. *Journal of Ethnopharmacology, 268*, Article 113580.

Martín, J., Kuskoski, E. M., Navas, M. J., & Asuero, A. G. (2017). Antioxidant capacity of anthocyanin pigments. In Justino, G. C. (Ed.), *Flavonoids-from biosynthesis to human health, Justino* (Vol. 3) (pp. 205-255) London, U.K.: IntechOpen. https://doi.org/210.5772/67718.

Miliauskas, G., Venskutonis, P. R., & van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry, 85*(2), 231–237. https://doi.org/10.1016/j.foodchem.2003.05.007

Oh, Y. S., Lee, J. H., Yoon, S. H., Oh, C. H., Choi, D. S., Choe, E., & Jung, M. Y. (2008). Characterization and quantification of anthocyanins in grape juices obtained from the grapes cultivated in Korea by HPLC/DAD, HPLC/MS, and HPLC/MS/MS. *Journal of Food Science, 73*(5), C378–C389.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science, 5*. https://doi.org/10.1017/jns.2016.41

Peng, D., Zahid, H. F., Ajlouni, S., Dunshea, F. R., & Suleria, H. A. R. (2019). LC-ESI-QTOF/MS profiling of australian mango peel by-product polyphenols and their potential antioxidant activities. *Processes, 7*(10), 764. https://doi.org/10.3390/pr7100764

Polat Kose, L., & Gulcin, İ. (2021). Evaluation of the antioxidant and antiradical properties of some phyto and mammalian lignans. *Molecules, 26*(23), 7099. https://doi.org/10.3390/molecules26237099

Rafi, M., Febriany, S., Wulandari, P., Suparto, I. H., Ridwan, T., Rahayu, S., & Siswoyo, D. M. (2018). Total phenolics, flavonoids, and anthocyanin contents of six Vireya Rhododendron from Indonesia and evaluation of their antioxidant activities. *J. Appl. Pharm. Sci, 8*(9), 49–54.

Reddy, V. P., Aryal, P., Robinson, S., Rafiu, R., Obrenovich, M., & Perry, G. (2020). Polyphenols in Alzheimer's disease and in the gut-brain axis. *Microorganisms, 8*(2), 199. https://doi.org/10.3390/microorganisms8020199

Rossi, A., Serraino, I., Dugo, P., Di Paola, R., Mondello, L., Genovese, T., Morabito, D., Dugo, G., Sautebin, L., Caputi, A. P., & Cuzzocrea, S. (2003). Protective effects of anthocyanins from blackberry in a rat model of acute lung inflammation. *Free Radical Research, 37*(8), 891–900. https://doi.org/10.1080/1071576031000112690

Saeedi-Boroujeni, A., & Mahmoudian-Sani, M.-R. (2021). Anti-inflammatory potential of Quercetin in COVID-19 treatment. *Journal of Inflammation, 18*(1), 1–9.

Sakulnarmrat, K., & Konczak, I. (2012). Composition of native Australian herbs polyphenolic-rich fractions and in vitro inhibitory activities against key enzymes relevant to metabolic syndrome. *Food Chemistry, 134*(2), 1011–1019. https://doi.org/10.1016/j.foodchem.2012.02.217

Sakulnarmrat, K., Srzednicki, G., & Konczak, I. (2014). Composition and inhibitory activities towards digestive enzymes of polyphenolic-rich fractions of Davidson's plum and quandong. *LWT - Food Science and Technology, 57*(1), 366–375. https://doi.org/10.1016/j.lwt.2014.01.002

Sakulnarmrat, K., Srzednicki, G., & Konczak, I. (2015). Bioprospecting Davidson's plum and quandong: Cytoprotective and proapoptotic activities. *LWT - Food Science and Technology, 61*(2), 622–629. https://doi.org/10.1016/j.lwt.2014.12.023

Sharifi-Rad, J., Quispe, C., Castillo, C. M. S., Caroca, R., Lazo-Vélez, M. A., Antonyak, H., Polishchuk, A., Lysiuk, R., Oliinyk, P., De Masi, L., Bontempo, P., Martorell, M., Daştan, S. D., Rigano, D., Wink, M., & Cho, W. C. (2022). Ellagic acid: A review on its natural sources, chemical stability, and therapeutic potential. *Oxidative Medicine and Cellular Longevity, 2022*, 3848084. https://doi.org/10.1155/2022/3848084

Sharifi-Rad, J., Song, S., Ali, A., Subbiah, V., Taheri, Y., & Suleria, H. A. R. (2021). LC-ESI-QTOF-MS/MS characterization of phenolic compounds from Pyracantha coccinea M. Roem. and their antioxidant capacity. *Cellular and Molecular Biology, 67*(1), 201–211. https://doi.org/10.14715/cmb/12021.14767.14711.14729

Simunkova, M., Alwasel, S. H., Alhazza, I. M., Jomova, K., Kollar, V., Rusko, M., & Valko, M. (2019). Management of oxidative stress and other pathologies in Alzheimer's disease. *Archives of Toxicology, 93*(9), 2491–2513. https://doi.org/10.1007/s00204-019-02538-y

Singh, A., Bajpai, V., Kumar, S., Sharma, K. R., & Kumara, B. (2016). Profiling of gallic and ellagic acid derivatives in different plant parts of Terminalia arjuna by HPLC-ESI-QTOF-MS/MS. *Natural Product Communications, 11*(2), 239–244.

Solomakou, N., Loukri, A., Tsafrakidou, P., Michaelidou, A.-M., Mourtzinos, I., & Goula, A. M. (2022). Recovery of phenolic compounds from spent coffee grounds through optimized extraction processes. *Sustainable Chemistry and Pharmacy, 25*, Article 100592.

Sommano, S., Caffin, N., & Kerven, G. (2013). Screening for antioxidant activity, phenolic content, and flavonoids from Australian native food plants. *International Journal of Food Properties, 16*(6), 1394–1406.

Subbiah, V., Zhong, B., Nawaz, M. A., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R. (2020). Screening of phenolic compounds in Australian grown berries by LC-ESI-QTOF-MS/MS and determination of their antioxidant potential. *Antioxidants (Basel), 10*(1), 26. https://doi.org/10.3390/antiox10010026

Suleria, H. A. R., Barrow, C. J., & Dunshea, F. R. (2020). Screening and characterization of phenolic compounds and their antioxidant capacity in different fruit peels. *Foods, 9*(9), 1206. https://doi.org/10.3390/foods9091206

Takanche, J. S., Lee, Y. H., Kim, J. S., Kim, J. E., Han, S. H., Lee, S. W., & Yi, H. K. (2018). Anti-inflammatory and antioxidant properties of Schisandrin C promote mitochondrial biogenesis in human dental pulp cells. *International Endodontic Journal, 51*(4), 438–447. https://doi.org/10.1111/iej.12861

Tan, A. C., Konczak, I., Ramzan, I., & Sze, D. M. Y. (2011a). Antioxidant and cytoprotective activities of native Australian fruit polyphenols. *Food Research International, 44*(7), 2034–2040. https://doi.org/10.1016/j.foodres.2010.10.023

Tan, A. C., Konczak, I., Ramzan, I., & Sze, D. M. Y. (2011b). Native Australian fruit polyphenols inhibit cell viability and induce apoptosis in human cancer cell lines. *Nutrition and cancer, 63*(3), 444–455.

Taslimi, P., Köksal, E., Gören, A. C., Bursal, E., Aras, A., Kılıç, Ö., … Gülçin, İ. (2020). Anti-Alzheimer, antidiabetic and antioxidant potential of Satureja cuneifolia and analysis of its phenolic contents by LC-MS/MS. *Arabian Journal of Chemistry, 13*(3), 4528–4537. https://doi.org/10.1016/j.arabjc.2019.10.002

Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients, 2*(12), 1231–1246. https://doi.org/10.3390/nu2121231

Wang, H., Ye, Y. H., Wang, H. H., Liu, J., Liu, Y. J., & Jiang, B. W. (2019). HPLC-QTOF-MS/MS profiling, antioxidant, and α-glucosidase inhibitory activities of Pyracantha fortuneana fruit extracts. *Journal of food Biochemistry, 43*(5), e12821.

Wang, Z., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R. (2021). A Comparative investigation on phenolic composition, characterization and antioxidant potentials of five different Australian grown pear varieties. *Antioxidants (Basel), 10*(2), 151. https://doi.org/10.3390/antiox10020151

Xiong, Y., Ng, K., Zhang, P., Warner, R. D., Shen, S., Tang, H. Y., … Fang, Z. (2020). In Vitro α-Glucosidase and α-Amylase inhibitory activities of free and bound phenolic extracts from the bran and kernel fractions of five sorghum grain genotypes. *Foods, 9*(9), 1301. https://doi.org/10.3390/foods9091301

Yang, S., & Yuan, C. (2021). Schisandra chinensis: A comprehensive review on its phytochemicals and biological activities. *Arabian Journal of Chemistry, 14*(9), Article 103310. https://doi.org/10.1016/j.arabjc.2021.103310

Youdim, K. A., Martin, A., & Joseph, J. A. (2000). Incorporation of the elderberry anthocyanins by endothelial cells increases protection against oxidative stress. *Free Radic Biol Med, 29*(1), 51–60. https://doi.org/10.1016/s0891-5849(00)00329-4

Yun, E. S., Park, S. K., Kim, B. S., Chae, Y. Z., Cho, S. M., Yi, H., … Shin, H. C. (2012). Determination of the esculetin contents of medicinal plants by liquid chromatography–tandem mass spectrometry. *Biomedical Chromatography, 26*(10), 1247–1251.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*(11), 5165–5170. https://doi.org/10.1021/jf010697n