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Exploring efficient extraction methods: Bioactive compounds and antioxidant properties from New Zealand damson plums

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ABSTRACT

The study determined the best extraction method for phenolic compounds (rutin, catechin, epicatechin, naringenin, neochlorogenic acid, and rosmarinic acid) in the New Zealand damson plums. Accelerated solvent extraction (ASE) using ethanol and water was used to evaluate the solvent efficacy. Further comparisons were made among ASE, ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), and a combined method (E + UAE) using water as the solvent. The findings showed that ASE for 40 min was the most effective method for extracting phenolic compounds (1.76 mg gallic acid equivalent/g) compared to other methods (UAE = 1.17, EAE = 1.3, and E + UAE = 1.45 mg/g). The ASE method also resulted in an extract with a higher antioxidant activity than other methods. The extraction time over 40 min decreased the yield regardless of the solvent used. Therefore, the ASE extraction method for 40 min is recommended as the best method for extraction of phenolic compounds from the New Zealand damson plums.

1. Introduction

Plum fruits are reported to contain high amounts of phenolic compounds, particularly flavonoids and the anthocyanin subclass (Walle et al., 2003), which are linked to some health-promoting properties for bone health (Wallace, 2017), memory function (Shahidi et al., 2013) and cognition improvement (Igwe & Charlton, 2016; Shahidi et al., 2013), antioxidant and anti-inflammatory properties (Popov et al., 2014), and constipation relief (Mahboubi, 2021).

Damson plums, a type of plum that was discovered approximately 2000 years ago (Igwe & Charlton, 2016) and has different varieties such as Reine, Claude and Stanley, are known to possess higher antioxidant capacity compared to the other plum cultivars (Kim et al., 2003). Damson plums contain a higher total phenolic content (TPC) of about 375 mg gallic acid equivalent (GAE) per 100 g of fresh fruit, compared to Stanley (174 mg GAE), Beltsville (332 mg GAE), Cacak Bes (319 mg GAE), and Yugoslavian Elite (217 mg GAE) (Kim et al., 2003). However, to the best of our knowledge, such properties of damson plums are yet to be systematically studied as there is no comprehensive report on the extraction, identification, isolations, or use of phenolic compounds from these plums. In particular, there is a variety of damson plums grown in New Zealand, the phenolic/antioxidant properties of which have not been investigated yet. Although there is limited research specifically focusing on damson plums, there is none available on those grown in New Zealand. Hence, the primary objective of this study was to address the existing knowledge gap by conducting a comprehensive investigation into the efficiency of extraction methods for bioactive compounds and antioxidant properties from the New Zealand damson plums. By exploring the specific phenolic and antioxidant properties of this plum variety, our research provides valuable insights into the potential health benefits it may offer.

The extraction method plays a crucial role in determining the phenolic and bioactive profile of damson plums. Various extraction methods including ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), a combination of both UAE and EAE methods (E + UAE), and accelerated solvent extraction (ASE) have been reported for the extraction of phenolic compounds from other natural sources (González et al., 2022; Jawad et al., 2022; Yun-Chang et al., 2010). A recent study used the E + UAE approach to extract polyphenols from *Prunus domestica* juice (Olawuyi et al., 2021). The study optimised the extraction time while keeping the enzyme concentration and ultrasound frequency constant. Both UAE and EAE methods were considered environmentally friendly as they used water as the solvent and avoided high pressure or temperature that could harm the phenolic compounds.

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In another study (Yang et al., 2017), ASE was found to exhibit better extraction efficiency than UAE for the extraction of phenolic compounds from mulberries. Accordingly, we hypothesised that water-assisted extraction could achieve a high extraction efficacy for phenolic compounds in the New Zealand damson plums. Additionally, a combination of ultrasound and enzyme-assisted extractions could perform better than either of those two extraction methods alone.

Furthermore, what sets our study further apart is the systematic evaluation of various extraction techniques, including accelerated solvent extraction (ASE), ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), and a combined method (E + UAE) using water as the solvent. To the best of our knowledge, no studies have extensively compared these extraction methods for plum samples, let alone damson plums. By doing so, this study contributes to the understanding of the best approach for extracting bioactive compounds from this particular plum variety. This systematic investigation adds a significant layer of novelty to our research and can have implications for other studies in the field.

In the initial phase of this study, our objective was to assess the efficacy of extraction methods utilising water and ethanol as solvents. The focus was to identify the optimal solvent for extracting essential bioactive compounds, such as phenolic acids, flavonoids, and anthocyanins. The second phase aimed to use the best solvent from Phase 1 and compare the extraction efficiency of different methods including EAE, UAE, E + UAE, and ASE. The impact of extraction time on extraction yield was also analysed to identify the optimal conditions for maximum economic benefit for each method. Accordingly, the present study not only explores extraction methods but also investigates the influence of extraction time on bioactive compound yield, offering practical insights for optimizing extraction processes with economic benefits, enhancing the relevance and potential impact of our research in the food and nutraceutical industries, and suggesting damson plums as a valuable source of bioactive compounds for health applications.

2. Methods and materials

2.1. Samples and chemicals

The fresh New Zealand damson plums were generously supplied by FootSteps Ltd. located in Karamu, Hastings, New Zealand, and were stored at −20 °C upon arrival. The fruit samples were later pitted, freeze-dried, and ground to produce the dry sample, which is referred to as the freeze-dried damson plum powder (FDDPP) henceforth. The standards of catechin, epicatechin, naringenin, gallic acid monohydrate, rutin hydrate, quercetin, neochlorogenic acid, rosmarinic acid, and cyanidin 3-glucoside chloride (all HPLC grade) were acquired from Sigma-Aldrich Co., Inc. in Darmstadt, Hesse, Germany. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Folin–Ciocâlteu's reagents were also purchased from Sigma-Aldrich Co., Inc. (Darmstadt, Hesse, Germany) and Merck Co., Inc. (New Jersey, USA) respectively. The Pectinex Ultra SPL from Aspergillus aculeatus (3800 units/mL) was obtained from Sigma-Aldrich Co., Inc. (Darmstadt, Hesse, Germany). All the chemicals and reagents used in the study were of analytical-grade quality.

2.2. Extraction methods

The phenolic compounds of FDDPP samples were analysed using an accelerated solvent extractor (350 Dionex, Thermo Fisher Scientific, Massachusetts, USA) with water and ethanol as the extraction solvents. The extraction was performed for 20, 40 and 60 min at 45 °C. One cycle extraction was performed with 90% manual rinse volume and 5 mL of automatic rinse volume. The extraction cell was equipped with a cellulose filter pad (ASE Extraction Filters, Thermo Scientific, Australia) at the bottom, a layer of 1 cm diatomaceous earth/MAP mixture under the filter pad, a middle layer of 1 cm diatomaceous earth/MAP mixture mixed with 1 g of FDDPP sample, and a top layer filled with white sand (ECP Ltd.). After extraction, the samples were centrifuged (1370×*g*) for 10 min at 22 °C using a Heraeus Multifuge X3R apparatus (Thermo Fisher Scientific, Waltham, MA, USA). The supernatants were then collected and stored in the dark at −20 °C for further analysis.

To investigate the impact of different extraction methods, four techniques were employed: enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE), the combination of enzyme and ultrasound-assisted extraction (E + UAE), and accelerated solvent extraction (ASE). Milli-Q® water was used for EAE, UAE, and E + UAE, while a mixture of ethanol and Milli-Q water was used for ASE, maintaining a constant volume of 20 mL and a temperature of approximately 45 °C for extraction durations of 20, 40 and 60 min.

Following the extraction, the samples underwent mixing with a high shear mixer (3 min, 19000 rpm), followed by centrifugation (1370×*g*, 10 min, and 22 °C). Afterward, the samples were filtered and stored in the dark at −20 °C for further processing and analysis.

2.2.1. Enzyme-assisted water extraction (EAE)

An enzyme extraction was performed using 40 μL of Pectinex Ultra SPL (3800 units/mL) added to 20 mL of sample solution (1 g of FDDPP in 20 mL Milli-Q water). The enzyme concentration was 0.2% v/v, as per previous research (Olawuyi et al., 2021). The extraction was conducted at 45 °C for 20, 40 and 60 min in an incubator (Infors HT Incubator, Bottmingen, Arlesheim Switzerland).

2.2.2. Ultrasound-assisted water extraction (UAE)

The temperature of both the ultrasound bath and water bath was set at 45 °C during the entire extraction time. The samples were subjected to ultrasound treatment (38 kHz) for 20, 40 and 60 min using a Sonorex Digitec DT 255 H apparatus (Bandelin Instruments, Berlin, Berlin, Germany), and then kept in a water bath (45 °C) for an additional 20, 40 and 60 min.

2.2.3. Enzyme and ultrasound-assisted water extraction (E + UAE)

Samples were prepared in the same manner as the enzyme-assisted extraction (EAE). The extraction was carried out in an ultrasound bath at 45 °C, and the sample solutions were transferred to a 90 °C water bath for 2 min after the ultrasound extraction at 38 kHz for 20, 40 and 60 min. Afterward, the samples were kept in a 45 °C water bath for 20, 40 and 60 min. The samples were then centrifuged at 1370×*g* for 10 min (22 °C). The resulting supernatants were collected and stored in the dark at −20 °C for further analysis.

2.3. Bioactive analysis

2.3.1. Total phenolic content (TPC)

TPC was determined using the Folin–Ciocâlteu method reported previously (Singleton et al., 1999, pp. 152–178) with slight modifications. A 96-well plate (Thermo Scientific, Oxoid, USA) was used for reading the absorbance at 725 nm. A 50 μL aliquot of diluted sample solutions or gallic acid standard solutions was mixed with 25 μL of Folin–Ciocâlteu reagent, and 150 μL 2% (w/v) Na_2CO_3 and kept in the dark for 15 min (Olawuyi et al., 2021). The sample solution was diluted with 50% of ethanol and the absorbance was measured using the microplate reader of a UV spectrophotometer (GENESYS 10 Series, UV–vis, USA) at 725 nm. The results were expressed as mg gallic acid equivalent (mg GAE)/g of FDDPP, according to the absorbance of the gallic acid standard curve.

2.3.2. Total flavonoid content (TFC)

An aluminium colorimetric assay was used to analyse TFC (Teng et al., 2009). Sample solutions and rutin standard solutions were loaded onto a 96-well plate. To each well, 19 μL of the solution was mixed with 116 μL of 50% ethanol, 14 μL of NaNO2, and 14 μL of 10% Al(NO3)·9H2O. After the plate was kept in the dark at 22 °C (25 °C) for 6 min (Olawuyi et al., 2021), 135 μL of NaOH was added. The absorbance was

measured at 510 nm using the microplate reader of the spectrophotometer (GENESYS 10 Series, UV–vis, USA). The results were expressed as mg rutin equivalent (mg RE)/g of FDDPP, based on the absorbance of the rutin standard curve.

2.3.3. Total anthocyanins content (TAC)

The TAC was determined using the AOAC pH-differential method (Lee et al., 2005). Two 96-well plates (A & B) were used. Plate A was filled with a 20 μL diluted sample or cyanidin-3-glucoside standard solution and 180 μL potassium chloride buffer (pH 1.0, 0.025 M) for 15 min. Plate B was filled with a 20 μL diluted sample and 180 μL sodium acetate buffer (pH 4.0, 0.4 M) and incubated for 15 min. Absorbances at 520 and 700 nm were read by a microplate reader for both plates. TAC values were calculated using the following equation:

$$Cyanidin-3-glucoside\ equivalent\ (mg) = \frac{Ab \times MW \times DF \times 1000}{\varepsilon} \quad (1)$$

$$Ab = (Ab_{520nm} - Ab_{700nm})(pH\ 1.0) - (Ab_{520nm} - Ab_{700nm})(pH\ 4.5) \quad (2)$$

where, MW refers to the molecular weight of cyanidin-3-glucoside is 449.2 g/mol, and the molar extinction coefficient is 26900/L/M/cm. ε refers to the molar absorptivity of the reference anthocyanin. Results are reported as mg cyanidin-3-glucoside per g of FDDPP, using the dilution factor (DF) based on the original extraction ratio (mL/mg) (Lee et al., 2005).

2.3.4. ABTS radical-scavenging activity

The ABTS radical-scavenging activity was determined using a modified method reported earlier (Arnao et al., 2001; Olawuyi et al., 2020). The ABTS cation was generated by mixing 2.45 mM potassium persulfate and 7 mM ABTS solution in a 1:1 ratio and incubating the mixture in the dark at 22 °C for over 16 h. The mixture was then diluted 50-fold with 50% ethanol to reach an absorbance of 0.7 ± 0.02 at 734 nm. The assay was performed in a 96-well plate, with 20 μL of 50% ethanol (blank), sample solutions, or ascorbic acid standards mixed with 280 μL ABTS cation solution. The mixture was incubated in the dark at 22 °C for 6 min and absorbance was read at 734 nm. The results were expressed as mg ascorbic acid equivalent (AAE)/g FDDPP.

2.3.5. DPPH radical-scavenging activity

The DPPH radical scavenging activity was measured using a modified version of the Blois (1958) method. A 0.1 mM DPPH solution was made in ethanol and allowed to react for 2 h before use. A 20 μL of ascorbic acid standard, 50% ethanol (blank), and sample solutions were mixed with 280 μL of the DPPH solution in a 96-well plate and left to react in the dark (22 °C) for 30 min. The absorbance was recorded at 517 nm using a GENESYS 10 Series UV–Vis spectrophotometer (Thermo Scientific, MA, USA). The results were expressed as mg ascorbic acid equivalent per g of FDDPP.

2.3.6. High-performance liquid chromatography (HPLC) analysis

The HPLC method (Podsędek et al., 2006) was used to determine the concentration of phenolic compounds (neochlorogenic acid, gallic acid, catechin, epicatechin, rutin, rosmarinic acid, naringenin, and quercetin) in the FDDPP sample extract. For this purpose, an Agilent 1200 HPLC machine with Kinetex® 2.6 μm XB-C18 column (100 × 4.6 mm) was used. Sample solutions were diluted, ultra-centrifuged, and filtered, before being injected into the HPLC system. The mobile phase consisted of 6% acetic acid in 2 mmol/L sodium acetate (A) and 100% acetonitrile (B). A 20% methanol mix was used as the blank. The injection volume was 5 μL, the flow rate was 1 mL/min, and the total run time was 70 min. A gradient programme was applied (100%–85% A 0–45 min, 85%–70% A 45–60 min, 70%–50% A 60–65 min, and 50%–0% A 65–70 min) and the column temperature was kept at 25 °C. The UV detector was set at 280, 320, 360, and 520 nm to determine the phenolic compounds using their retention times and spectra compared to standards. The calibration curves of corresponding standards (0–100 μg/mL) were used for the calculations.

3. Results and discussion

The current research investigated the impact of using ethanol and water as extraction solvents. Based on the results, the most suitable solvent was chosen to further examine the effect of various extraction techniques, including enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE), and a combination of both EAE and UAE (E + UAE). Thus, in the first part of the results, we discuss the effect of the extraction solvent before discussing the effect of extraction methods in the second part.

3.1. Effect of extraction solvent

Fig. 1 presents the impact of ethanol and water (as the solvents) on TPC, TFC, TAC, antioxidant activity (DPPH and ABTS), and rutin content. The results indicated a significant increase in TPC when water was used as the solvent, compared to ethanol extraction (Fig. 1). The values for TPC, TFC, TAC, antioxidant activity, and rutin content also varied based on the extraction time. When water was used, a minimum of 40 min was needed to achieve the highest extractable yield. A lower yield was observed with longer extraction times over 60 min (Fig. 1), indicating that longer extraction times might lead to the degradation of phenolic compounds.

The results of this study corroborate previous research highlighting the significance of selecting an appropriate solvent and extraction duration for extracting phenolic compounds from natural sources. For instance, a study conducted on plums found that the addition of water along with an organic solvent, such as methanol or ethanol, resulted in a higher yield of extracted phenolic content compared to using the organic solvent alone. These findings underscore the importance of optimizing extraction conditions to achieve maximum yields of bioactive compounds from natural sources (Seke et al., 2021). Another study found that increasing the extraction time beyond 60 min did not significantly increase the yield of phenolic compounds from whole grain and bran of soft wheat (DiNardo et al., 2019). Maximum yield of TPC content *sparganii rhizoma* was obtained when the samples were extracted for 40 min, which is consistent with the findings of the current study (Wang et al., 2013).

The observed rise in total phenolic content (TPC) upon using water as a solvent can be ascribed to the higher solubility of polar compounds in water as compared to ethanol. This finding aligns with earlier studies emphasizing the influence of solvent polarity on the extraction of phenolic compounds. Specifically, the use of a solvent with higher polarity, such as water, can facilitate the extraction of more polar phenolic compounds, thereby increasing the overall yield of TPC. These results underscore the significance of selecting an appropriate solvent based on the chemical properties of the target compounds to achieve optimal extraction efficiency (Brahmi et al., 2012). Moreover, the observation that longer extraction times resulted in a lower yield of phenolic compounds is consistent with previous research that has suggested that prolonged extraction times can lead to the degradation of phenolic compounds due to oxidation or other chemical reactions (DiNardo et al., 2019; Niazmand et al., 2021). These findings collectively indicate that the selection of solvent and extraction duration plays a crucial role in determining the quantity and quality of extracted compounds. Specifically, our study identified water as an optimal solvent, along with a minimum extraction time of 40 min, for extracting bioactive compounds from natural sources, specifically phenolic compounds from plum samples. These results highlight the importance of optimizing extraction conditions to obtain maximum yields of desired compounds from natural sources.

Plant-derived phenolic compounds with redox properties have a

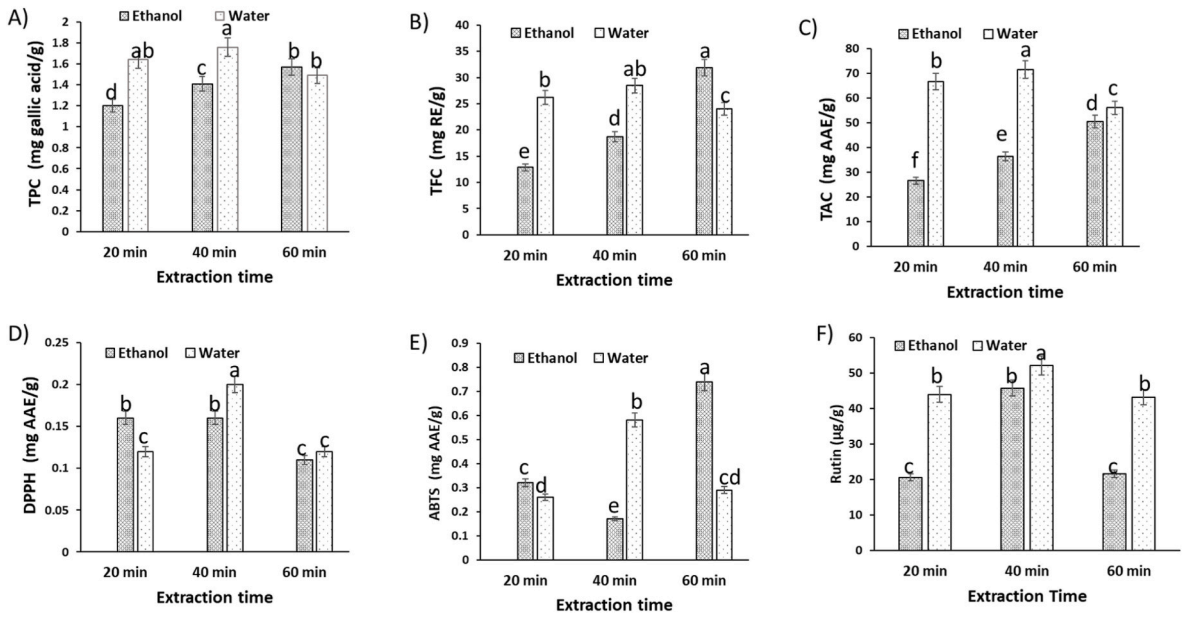

Fig. 1. Effect of extraction solvents (ethanol and water) and time (20, 40 and 60 min) on total phenolic content (A), total flavonoid content (B), total anthocyanin content (C), DPPH antioxidant activity (D), ABTS antioxidant activity (E), and rutin concentration (F) of the New Zealand damson plums. Values with different superscripts (a–j) within the same column are significantly different ($p < 0.05$, Tukey's test). Freeze-dried damson plum powder was incubated at 45 °C either with water or ethanol.

crucial role in reducing oxidative stress, making them potential candidates as antioxidant therapeutic agents (Soobrattee et al., 2005). In the current study, the TPC of the New Zealand FDDPP varied based on different extraction methods, with the TPC of the water-extracted sample (1.63 ± 0.01 mg/g of FDDPP) being significantly higher than that of the ethanol-extracted sample (1.39 ± 0.03 mg GAE/g). This aligns with the findings of the previous research by Yang et al. (2017), who discovered that the extraction efficiency of phenolic compounds using the ASE method was lower with 50% organic solvent compared to water. However, a mixture of water and methanol as the solvent (16.8%) is reported to show higher extraction efficiency for phenolic compounds from algae compared to water (16.6%), methanol (13.3%), or ethanol (2.36%) alone (López et al., 2011).

Total flavonoid content (TFC) is an important parameter used to evaluate the presence and quantity of flavonoids in a sample. Flavonoids are a group of bioactive compounds found in a variety of plant-based foods and beverages, including fruits, vegetables, tea, wine, and beer. They have been associated with a range of health benefits, including antioxidant, anti-inflammatory, and anti-cancer effects. The TFC content in the New Zealand FDDPP (fruit-derived dietary polyphenols) varied significantly with extraction solvent and time, as shown in Fig. 1B. Water extraction was more effective in producing TFC from FDDPP at 40 min, but ethanol was better at 60 min. The increased TFC content in ethanol-extracted samples may be due to ethanol's greater protection against oxidation (Pires & Brányik, 2015). Ethanol aids in solubilising and stabilising flavonoid compounds throughout the extraction process, safeguarding them from degradation and conserving their antioxidant properties and other bioactive compounds during both extraction and processing. This proves especially vital for samples that are susceptible to oxidation, like those containing catechins or other flavanols.

Most flavonoids are polar compounds, meaning that they have a positive and negative end and can form hydrogen bonds with water molecules. This makes them more soluble in water than in nonpolar solvents such as ethanol or chloroform. Water is readily available and non-toxic, making it a more desirable solvent for food and beverage processing. Furthermore, water extraction is a simple and cost-effective method that can be easily scaled up for commercial production. It is also a non-destructive method that preserves the quality of the sample and can be used to extract a wide range of flavonoids from different plant sources. However, it is important to note that the effectiveness of water extraction may vary depending on the type of flavonoid being extracted and the material being used. For example, some flavonoids, such as quercetin, may be more readily extracted using organic solvents like ethanol or methanol (Meng et al., 2018), while others, such as anthocyanins, may require acidic solvents for optimal extraction (Metivier et al., 1980). It is therefore important to carefully select the extraction solvent and method based on the specific flavonoids of interest and the properties of the source material.

The TAC was determined using the pH differential method, which changes the chemical structure of anthocyanins. These compounds are responsible for the characteristic blue-black or purple colour, and it is known that a diet rich in anthocyanins can help prevent inflammation, cancer, cardiovascular disease, and type 2 diabetes (Lin et al., 2016). In the case of the current study, as shown in Fig. 1C, water was a more effective solvent than ethanol. Muangrat et al. (2018) found that a mixed solvent of 50% ethanol and 50% water was more efficient for extracting anthocyanins from purple waxy corn than water or ethanol alone. This is because anthocyanins contain hydroxyl groups in their chemical structure and are highly soluble in water (Zhang et al., 2014), explaining the greater extraction efficiency of water compared to ethanol. Overall, in the current experiment, the FDDPP sample extracted for 40 min showed a significantly ($p < 0.05$) higher TAC value than that extracted using the same method but for 20 or 60 min (Fig. 1B). Similar to other bioactive compounds, increasing the extraction time up to 40 min could be beneficial for TAC extraction.

Antioxidants play a crucial role in maintaining health by preventing the formation of reactive oxygen species and protecting cells against oxidative stress. Therefore, the effect of extraction solvent and time on the total antioxidant activity (TAA) of plums was examined using ABTS and DPPH methods. There was a continuous increase when the extraction time was prolonged from 20 to 40 min. However, when the extraction time was extended from 40 to 60 min, a significant reduction was observed (Fig. 1D). Regardless of the extraction solvent (water or ethanol), the antioxidant activity obtained from the ABTS assay followed a similar trend to the results from the DPPH assay (Fig. 1D and E). Nonetheless, this similarity was not maintained when comparing the

increase in time from 40 to 60 min. In the case of ABTS values, a significant decrease occurred from 40 min (3.13 ± 0.11 mg AAE/g) to 60 min (2.46 ± 0.09 mg AAE/g), while the effect was reversed for the DPPH values (Fig. 1D ad 1 E). The results of this study suggest that plums, which are rich in phenolic antioxidants, have the potential to effectively neutralise harmful free radicals and reduce oxidative damage. The impact of extraction time on the TAA measured through the ABTS assay was inconsistent, as a continuously rising trend was observed when the extraction time was prolonged from 20 to 40 min. However, when the extraction time was extended from 40 to 60 min, a significant reduction in TAA was observed. Regardless of the extraction solvent (i.e., water or ethanol), the antioxidant activity obtained from the ABTS assay followed a similar trend to the results from the DPPH assay. However, the similarity was not maintained when comparing the increase in time from 40 to 60 min. In the case of ABTS values, a significant decrease occurred from 40 to 60 min, while the effect was reversed for the DPPH values. This indicates that the two methods may measure different aspects of antioxidant activity and should be used in combination to provide a more comprehensive assessment of TAA. Overall, the results of this study suggest that plums are a good source of antioxidants and can have beneficial effects on the immune system, aging, and skin health. However, the optimal extraction conditions for maximum TAA may vary depending on the specific antioxidant being targeted, and a combination of different methods may be necessary to obtain a more accurate assessment of TAA. Further research is needed to fully understand the mechanisms underlying the antioxidant activity of plums and to develop optimal extraction methods for maximum health benefits.

Rutin is a flavonoid that is widely distributed in fruits and vegetables. This flavonoid has been shown to have several potential health benefits, which make it an important compound to study and incorporate into natural products for potential health benefits. The results of this study suggest that the choice of extraction solvent and time can significantly affect the efficiency of rutin extraction (Fig. 1E). Regardless of the extraction time, water was more effective at extracting rutin (20 min: 43.97 μg/g; 40 min: 52.08 μg/g; 60 min: 43.15 μg/g) compared to ethanol (20 min: 20.59 μg/g; 40 min: 45.73 μg/g; 60 min: 21.65 μg/g). Furthermore, the study found that the optimal extraction time for rutin was 40 min (Fig. 1E). Similar to the other bioactive compound, we observed that extending the extraction time beyond 40 min did not increase the rutin content, but instead decreased it, regardless the solvent (Fig. 1E).

The observed decline in rutin content with longer extraction times, regardless of the solvent used, suggests that this phenomenon is not solely attributed to a specific chemical interaction between the solvent and the plant material. Instead, it could be linked to physical or biological factors, such as the saturation of the plant material with rutin or the compound's degradation over time. These findings have significant implications for the development of extraction protocols for rutin, especially in the context of industrial-scale production. For instance, our results imply that utilising shorter extraction times with larger volumes of plant material may be more efficient than attempting to extract the maximum amount of rutin from a smaller sample using longer extraction times. Considering these factors could lead to improvements in the efficiency of industrial extraction processes for rutin and other bioactive compounds.

The study also found that catechin and epicatechin were extracted more efficiently using water compared to ethanol, while naringenin, neochlorogenic acid, and rosmarinic acid were extracted better using ethanol (Table 1). This suggests that the extraction solvent plays a critical role in determining the yield of target compounds. Moreover, we found that a longer extraction time of more than 40 min had a negative impact on the yield of phenolic compounds, regardless of the solvent used. This could be due to the degradation or transformation of these compounds at prolonged extraction times, resulting in reduced yield. Thus, it is suggested that there is no single solvent that can provide maximum efficiency for all phenolic and flavonoid compounds. This implies that the choice of solvent should be tailored to the specific target compounds to obtain the maximum yield (DiNardo et al., 2019; Niazmand et al., 2021).

Furthermore, the study findings suggest that a combination of ethanol and water could potentially improve extraction efficiency of different phenolic compounds. Ethanol is known to be more effective in extracting hydrophobic compounds, while water is more effective in extracting hydrophilic compounds. Therefore, a combination of these solvents may provide a more comprehensive extraction of phenolic compounds, leading to a higher yield.

3.2. Effect of extraction methods

The previous experiment aided in identifying the ideal extraction solvent, and water was selected as the solvent due to its superior extraction results compared to ethanol. The most effective extraction time was determined to be 40 min (Table 1). Despite the negative effect seen in most cases with longer extraction times, this study was still carried out to consider all possibilities. The effect of different extraction methods including ASE, UAE, EAE, and U + E on TPC, TFC, TAC, and ABTS is shown in Fig. 2, and each is discussed in the following sections.

3.2.1. Total phenolic content (TPC)

The findings of the current study showed that the TPC values of the New Zealand damson plum samples varied depending on the extraction method used. Samples extracted using ASE with water had significantly higher TPC (1.76 mg gallic acid equivalent/g) compared to those extracted using EAE (1.3 mg/g), UAE (1.17 mg/g), and the combination of EAE and UAE methods (1.45 mg/g) (Fig. 2A). TPC values were lower for samples extracted for 20 min than for those extracted for 40 or 60 min (Fig. 2A). There was no significant difference ($p > 0.05$) in TPC values between samples extracted for 40 and 60 min across all extraction methods, implying that the efficiency of extraction methods may reach its highest yield at 40 min and longer extraction is not substantially effective. In some cases, longer extraction than 40 min may decrease TPC values; e.g., TPC decreased for samples extracted for 60 min using both EAE and ASE in water compared to 40 min (Fig. 2). This decrease could be due to instability in the lateral glycosidic chains of polyphenolic compounds and enzymatic hydrolysis after extended extraction (Saldarriaga-Hernández et al., 2020). The TPC content in samples extracted with the E + UAE method for 60 min was higher than that for those extracted for 40 min, due to the effect of ultrasonication; i.e.,

Table 1
Effect of extraction solvents (water and ethanol) and time (20, 40 and 60 min) on different phenolic acids (gallic acid, neochlorogenic acid and rosmarinic acid) and flavonoids (catechin, epicatechin and naringenin) in the New Zealand damson plum extract.

Extraction solvent and time	Catechin (μg/g)	Epicatechin (μg/g)	Naringenin (μg/g)	Gallic acid (μg/g)	Neochlorogenic acid (μg/g)	Rosmarinic acid (μg/g)
Water-20	20.64 ± 0.34^{e}	37.55 ± 1.48 ^{c}	44.51 ± 1.22 ^{c}	48.58 ± 0.09^{c}	1008.1 ± 44.7^{b}	8.44 ± 0.88^{c}
Ethanol-20	45.25 ± 1.37^{b}	24.48 ± 0.40^{f}	**52.91 ± 1.42** ^{a}	36.29 ± 0.18^{e}	1295.9 ± 25.9^{ab}	10.32 ± 0.59^{b}
Water-40	**66.51 ± 2.42**^{a}	**56.51 ± 2.35**^{a}	46.67 ± 2.53 ^{c}	52.39 ± 0.93^{b}	1099.4 ± 47.0^{b}	9.91 ± 0.22^{b}
Ethanol-40	42.00 ± 0.60^{b}	28.13 ± 1.11^{d}	**51.23 ± 1.39**^{a}	37.62 ± 0.19^{e}	**1393.2 ± 29.7**^{a}	**13.01 ± 0.44**^{a}
Water-60	29.44 ± 1.61^{d}	50.24 ± 1.16^{b}	42.72 ± 1.53 ^{d}	**58.14 ± 1.76**^{a}	1105.0 ± 84.5^{b}	**12.85 ± 0.75**^{a}
Ethanol-60	36.69 ± 1.53^{c}	19.52 ± 0.55^{g}	47.52 ± 0.32^{b}	39.67 ± 0.16^{d}	1158.3 ± 40.2^{b}	8.91 ± 0.60^{c}

Values with different superscripts (a-g) are significantly different ($p < 0.05$, Tukey's test).

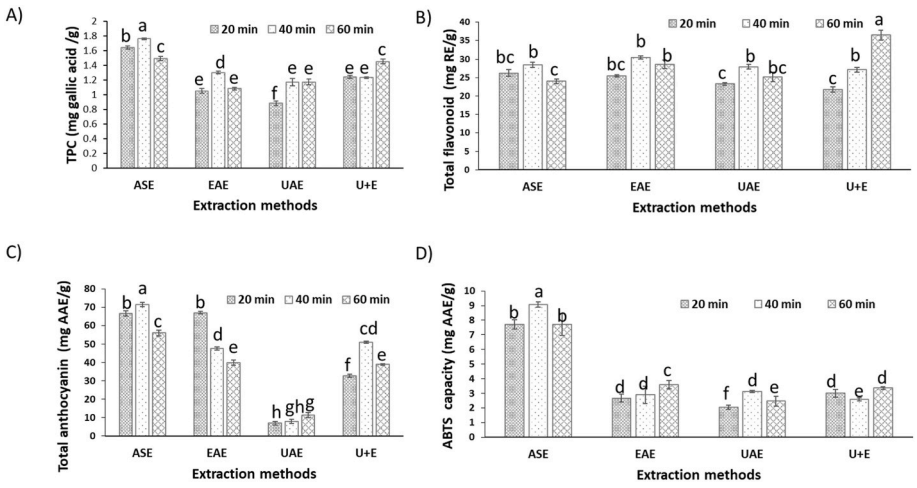

Fig. 2. Effect of different extraction methods (ASE, UAE, EAE, U + E) and time (20, 40 and 60 min) on total phenolic content (A), total flavonoid content (B), total anthocyanin content (C), and ABTS antioxidant activity (D) of the New Zealand damson plums. Values with different superscripts (a–i) are significantly different ($p < 0.05$, Tukey's test). Abbreviations: ASE = accelerated solvent extraction, UAE = ultrasound-assisted extraction, EAE = enzyme-assisted extraction, and E + UAE = a combined extraction method consisted of both enzyme and ultrasonication.

breaking down cell walls and allowing better access for the extraction solvent (Butnariu et al., 2013; Machado et al., 2019; Nguyen & Nguyen, 2018b).

3.2.2. Total flavonoid content (TFC)

In the present study, there were no significant ($p < 0.05$) differences among the four extraction methods using water as the solvent in the TFC content of the damson plum extract at 40 min (Fig. 2B). The ASE method showed slightly higher TFC than UAE at 20 min but did not perform better results than EAE or the combined E + UAE method. After 60 min of extraction, the E + UAE method showed the highest TFC value (36.47 mg RE/g), significantly ($p < 0.05$) higher than the UAE method alone (25.13 mg RE/g) (Fig. 2B). Ultrasonication has been shown to increase extraction yield by promoting the propagation of ultrasound waves and cavitation, causing the plant cell wall to break down and allowing for improved solvent diffusion (Vilkhu et al., 2008). However, previous research (Dent et al., 2015) found that a high frequency of UAE could decrease TFC extraction efficiency from sage when the frequency was changed from 24 kHz to 30 kHz, resulting in a reduction of TFC content in 10 and 11 min of extraction. A similar trend was observed in the present study, with a significant decrease ($p < 0.05$) in TFC quantified by ASE method, when the extraction time was extended from 40 min (0.71 mg RE/g) to 60 min (0.61 mg RE/g) (Fig. 2B).

The EAE method has also been shown to achieve a higher extraction yield from plant by-products, as the indigenous enzyme breaks down the plant cell wall and structural polysaccharides under moderate conditions, leading to an increase in the effectiveness of secondary plant metabolites with antioxidant characteristics, including flavonoids (Selvamuthukumaran & Shi, 2017). However, it was noted that a prolonged extraction time of 60 min could lead to a decrease in TFC content, potentially due to the activation of enzymes that hydrolyse the TFC.

3.2.3. Total anthocyanins (TAC)

The TAC of the sample extracted with the UAE method (8.83 ± 0.18 mg cyn-3-glu/g) in this investigation was lower than those extracted with EAE (51.49 ± 1.21 mg cyn-3-glu/g) and their combined (E + UAE) method (40.88 ± 0.83 mg cyn-3-glu/g). The TAC of the E + UAE method was also lower than EAE alone. This is consistent with the results of a previous research (Olawuyi et al., 2021), in which it was found that the TAC of plum juice extracted with UAE was significantly ($p < 0.05$) lower than that extracted with EAE or their combined method. Such low TAC value is likely due to the degradation of anthocyanin glucosides caused by polyphenol oxidase (PPO) activity. Enzymatic treatment may increase TAC by inhibiting PPO. We also found that the New Zealand FDDPP sample extracted using the ASE method for 40 min (71.5 mg AAE/g) had higher TAC than for 20 min (66.68 mg AAE/g) or 60 min (56.04 mg AAE/g). The E + UAE method showed an upward trend in TAC from 20 to 40 min, but a significant ($p < 0.05$) drop was seen when it was extended to 60 min. This drop may be explained by decreased TAC as when the ultrasound amplitude level increased from 50% to 100%, there was an initial increase in TAC from 20 to 40 min. Such a decrease in TAC may be due to the increase in ultrasound amplitude level; i.e., the ultrasound waves used to facilitate the extraction may have been too strong, which led to a decrease in the concentration of antioxidants extracted from the substance.

3.2.4. Total antioxidant activity (TAA)

The TAA of the damson plum samples extracted using ASE, EAE, UAE, and U + E methods is shown in Fig. 2D. The results show that both extraction method and time had a significant impact on the antioxidant capacity of the plum samples. Samples extracted using the ASE method showed higher antioxidant activity compared to those extracted using the EAE, UAE, and U + E methods. A longer extraction time of more than 40 min with water and UAE had a negative impact on the antioxidant activity (Fig. 2D). However, the presence of the enzyme increased the antioxidant capacity, which could be due to the generation of bioactive peptides during proteolytic hydrolysis. The extraction method and time had a significant effect ($p < 0.05$) on total antioxidants determined by the ABTS assay, with slightly different results than the DPPH assay. The E + UAE method had the best results compared to single extraction of UAE or EAE, which was also found in a previous study (Olawuyi et al., 2021). There was a strong correlation ($r^2 > 0.95$) between antioxidant capacity assessed by ABTS and TFC, but not with ASE methods. The antioxidant capacity of FDDPP extract is related to the number of phenolic compounds and anthocyanins, which are important antioxidants in various plum cultivars (the New Zealand damson plums being no exception).

3.2.5. Effect of extraction methods on the concentration of phenolic compounds

The findings of the present research confirmed that the FDDPP from New Zealand contains various other phenolic compounds and flavonoids (Table 2). Phenolic compounds are relevant to the appearance (anthocyanin and pigment accumulation), taste (astringency), and antioxidant capacity of original damson plums (Tomás-Barberán et al., 2001). In this research, the highest concentration of phenolic compounds in FDDPP samples was related to neochlorogenic acid, which was found at the concentration of 1158.3–1393.2 μg/g, compared to other phenolic acids and flavonoids (Table 2). Overall, in the current experiment, after neochlorogenic acid, as the principal phenolic acid and antioxidant in the New Zealand damson plums, the maximum and minimum extracted phenolic compounds were gallic acid (40.66–67.34 μg/g) and

Table 2
Effect of extraction methods (ASE, UAE, EAE, U + E) and time (20, 40 and 60 min) on different bioactive compounds in the New Zealand damson plum extract.

Extraction methods	Neochlorogenic acid (μg/g)	Gallic acid (μg/g)	Rosmarinic acid (μg/g)	Rutin (μg/g)	Catechin (μg/g)	Epicatechin (μg/g)	Naringenin (μg/g)
ASE-20	1008.1 ± 44.7^{a}	48.58 ± 0.09^{g}	8.44 ± 0.88^{c}	11.17 ± 0.03^{h}	20.64 ± 0.34^{c}	37.55 ± 1.48^{a}	44.51 ± 1.22^{a}
UAE-20	185.35 ± 11.78^{e}	60.26 ± 0.59^{d}	8.45 ± 0.80^{c}	35.17 ± 1.45^{f}	46.51 ± 1.85^{a}	25.52 ± 1.16^{b}	46.43 ± 0.51^{a}
EAE-20	896.0 ± 55.8^{b}	63.75 ± 1.59^{c}	11.33 ± 0.22^{a}	37.49 ± 2.99^{f}	46.99 ± 1.27^{a}	37.85 ± 2.91^{a}	44.52 ± 1.32^{a}
U + E-20	752.5 ± 48.7^{c}	40.66 ± 1.33^{h}	9.52 ± 0.43^{b}	44.81 ± 2.38^{de}	43.65 ± 2.30^{b}	36.64 ± 1.99^{a}	30.37 ± 1.19^{b}
ASE-40	**1099.4 ± 47.0^{a}**	52.39 ± 0.93^{f}	**9.91 ± 0.22^{a}**	56.88 ± 2.93^{bc}	**66.51 ± 2.42^{a}**	**56.51 ± 2.35^{a}**	46.67 ± 2.53^{b}
UAE-40	242.13 ± 20.63^{d}	61.75 ± 0.30^{d}	10.93 ± 0.75^{a}	**67.51 ± 1.52^{a}**	43.49 ± 1.56^{c}	23.84 ± 0.71^{c}	**49.13 ± 0.74^{a}**
EAE-40	1038.2 ± 58.7^{a}	**67.34 ± 0.89^{a}**	6.83 ± 0.27^{b}	20.08 ± 1.34^{g}	56.33 ± 2.36^{b}	43.88 ± 2.27^{b}	45.11 ± 1.39^{b}
U + E-40	861.5 ± 39.2^{b}	58.33 ± 0.43^{e}	9.88 ± 0.42^{a}	51.35 ± 2.27^{cd}	43.76 ± 2.59^{c}	44.56 ± 0.66^{b}	32.29 ± 2.04^{c}
ASE-60	1105.0 ± 84.5^{a}	58.14 ± 1.76^{e}	12.85 ± 0.75^{a}	55.57 ± 2.77^{bc}	29.44 ± 1.6^{d}	50.24 ± 1.16 ^{a}	42.72 ± 1.53^{b}
UAE-60	204.35 ± 10.2^{d}	64.46 ± 1.32^{b}	8.67 ± 0.60^{b}	61.43 ± 2.88^{b}	40.96 ± 0.90^{c}	23.17 ± 1.36^{c}	49.12 ± 1.95^{a}
EAE-60	769.7 ± 42.7^{c}	52.08 ± 2.38^{e}	11.57 ± 0.71^{a}	43.97 ± 3.41^{ef}	44.65 ± 1.66^{b}	33.36 ± 2.97^{b}	41.20 ± 1.86^{b}
U + E-60	967.5 ± 39.2^{b}	61.63 ± 0.28^{d}	9.71 ± 0.66^{b}	52.08 ± 3.10^{cd}	49.44 ± 2.56^{a}	52.33 ± 1.23 ^{a}	30.33 ± 1.18^{c}

Values with different superscripts (a-g) are significantly different ($p < 0.05$, Tukey's test). Abbreviations: ASE = accelerated solvent extraction, UAE = ultrasound-assisted extraction, EAE = enzyme-assisted extraction, and E + UAE = a combined extraction method of enzyme and ultrasonication method.

rosmarinic acid (6.83–12.85 μg/g), respectively (Table 2).

The extraction method was found to significantly ($p < 0.05$) affect the yield of neochlorogenic acid (Table 2). ASE extraction was found to be the most effective method for extracting neochlorogenic acid compared to other methods used in this study (Table 2). Neochlorogenic acid has been previously identified as a major phenolic compound in damson plums (Chun et al., 2003), so the current investigation on the New Zealand damson plums confirms these previous findings. The UAE method was found to be less effective in extracting neochlorogenic acid and its extraction was significantly impacted by the prolonged ultrasonication (Table 2). These findings align with those reported in a previous study by Muangrat et al. (2018), who found that phenolic content extracted from dried cob of purple waxy corn decreased as the amplitude level of ultrasound increased up to 46.21%. In addition, rutin, catechin, epicatechin, and naringenin were predominantly found as flavonoids in damson plums (Table 2). These results agree with those found in a previous investigation (Will & Dietrich, 2006), where rutin and catechin were found as the major flavonoids in *Prunus domestica* samples.

Phenolic acids including gallic acid, neochlorogenic acid, and rosmarinic acid were significantly ($p < 0.05$) affected by solvents and extraction methods (Tables 1 and 2). Enzyme-assisted extraction for 40 min showed a significantly ($p > 0.05$) higher extraction efficiency than the other treatments for gallic acid (Table 2). Apart from the ASE treatment, the EAE also performed as the best extraction method in the case of neochlorogenic acid (1038.2 ± 58.7 μg/g) and rosmarinic acid (11.57 ± 0.71 μg/g). However, in the trials conducted previously (Olawuyi et al., 2021), the combination of EAE and UAE methods showed a relatively higher extraction efficiency than other methods for the content of phenolic acids including chlorogenic acid, caffeic acid, and ferulic acids from *Prunus salicina*. Different plum cultivars could be a reason for this difference compared to the findings of our research, because some other researchers (Azlim Almey et al., 2010; Johnson et al., 2020) showed that different extraction methods showed various extraction effectiveness on distinct plant materials and damson plum cultivars, respectively.

Table 2 presents different phenolic acids (gallic acid, neochlorogenic acid, and rosmarinic acid) and flavonoids (catechin, epicatechin, and naringenin) extracted under different extraction methods and times. ASE method with water in 40 min extraction time showed the highest extraction efficiency for catechin (66.51 ± 2.42 μg/g) and epicatechin (56.51 ± 2.35 μg/g). The ASE method showed the highest content of extracted naringenin (52.91 ± 1.92 μg/g). Rutin was relatively higher in the case of EAE treatment (67.51 ± 1.52 μg/g) (especially for 40 min) than in other samples (Table 2).

Apart from the ASE method, extraction conducted with the EAE method for 40 min achieved the highest catechin (44.65 μg/g) and rutin (20.08 μg/g). However, the E + UAE method for 15 min showed the highest extraction efficiency for these phenolics in the study reported previously (Olawuyi et al., 2021). These discrepancies could be due to the various frequencies of ultrasound during the extraction. There are limited studies that compared the effectiveness of different frequencies of UAE on the extraction of phenolic compounds from damson plums or even other fruits. A previous study compared the effectiveness of extraction with the UAE method on phenolic compounds from sage (*Salvia officinalis* L.) with two different frequencies (24 and 30 kHz) (Dent et al., 2015). The mass fraction of rosmarinic acid extracted with 30 kHz was significantly higher than that extracted with 24 kHz frequency ($p < 0.05$), as the extraction time was extended to 11 and 12 min. However, the total phenols extracted under 24 kHz were significantly higher than that extracted under 30 kHz for the whole range of extraction time (8, 10, 11, and 12 min). Prolonged extraction time resulted in higher extraction capacity of phenolic compounds, while excessively prolonged extraction time in their study could also significantly reduce the content of extracted phenolic compounds including romantic acid, hydroxy-cinnamic acid, hydroxy-benzoic acid, and flavonoids. For example, the content of romantic acid dropped from 3991.59 ± 12.18 to 3549.36 ± 13.75 mg/100 g of dry sage when the extraction time was extended from 11 to 12 min at 24 kHz (Dent et al., 2015). Additionally, the higher ultrasound frequency also improved the extraction capacity of phenolic compounds. For example, the concentration of extracted hydroxycinnamic acids and benzoic acids was capped at 45.14 ± 1.57 and 43.80 ± 5.35 mg/100 g with the frequency of ultrasound of 24 kHz, and they were enhanced to 117.30 ± 8.47 and 120.22 ± 4.59 mg/100 g dry sage, respectively, with the frequency of 30 kHz. The timing of the extraction in the previous studies (Dent et al., 2015; Nguyen & Nguyen, 2018a) was grouped in a shorter time interval, so the phenolic compounds could be suppressed or degraded by the longer time of extraction from 15 to 20 min.

In the current study, the E + UAE method showed the highest efficiency among the UAE, EAE, and E + UAE methods for extracting epicatechin from the New Zealand damson plum samples. It showed a stable increase trend for catechin (43.65–49.44 μg/g), epicatechin (36.64–52.33 μg/g), and rutin (51.35–61.43 μg/g) when the extraction time was extended from 20 to 60 min. However, the other methods mostly presented an increase when the extraction time was extended from 20 to 40 min, but a decreasing trend as the extraction time was further extended to 60 min (Table 2).

4. Conclusions

Taken together, this study demonstrated that the New Zealand damson plums are a valuable source of bioactive compounds with potent antioxidant properties. The study also evaluated the impact of different solvents and extraction methods on the phenolic compounds present in these plums. The results showed that the most effective method for the extraction of phenolic compounds from damson plums was ASE with water as the solvent for 40 min, which resulted in the highest yields of polyphenolic compounds. Furthermore, the combination of ASE and UEA for 60 min was found to be the best method for extracting total flavonoid content. These findings are important for food manufacturers and pharmaceutical companies seeking to use phenolic compounds from damson plums in functional foods and nutraceuticals. Moreover, the study highlights the significance of the extraction method when assessing the antioxidant properties of phenolic compounds and provides valuable information for future research in this field. Future research could focus on investigating the impact of other factors, such as temperature and pressure, on the extraction efficiency of phenolic compounds. This may lead to the development of more efficient and sustainable methods for extracting bioactive compounds from natural sources, such as damson plums, and their application in various industries, including functional foods, nutraceuticals, and pharmaceuticals. Overall, this study significantly contributes to a deeper understanding of the potential applications of the New Zealand damson plums as a natural source of bioactive compounds with potent antioxidant properties.

Author statement

P.X.: Methodology; Investigation; Data Analysis; Project administration; Writing—Original Draft; Writing—Review and Editing, M.K.A.: Methodology; Data Analysis; Data Visualization Writing—Original Draft; Review and Editing, A.R.: Conceptualization; Writing— Review and Editing; Resources; Project administration; Supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The Authors declare no competing interest.

Data availability

Data will be made available on request.

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