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# Characteristics, Bioactives and Antioxidant Activity of Illawarra Plum (*Podocarpus elatus*) Fruit

Rebecca McCullum[1] | Md Saifullah[2] | Michael Bowyer[1] | Quan V. Vuong[1]

[1]College of Engineering, Science and the Environment, University of Newcastle, Ourimbah, Australia | [2]Centre for Food Innovation, Tasmanian Institute of Agriculture, University of Tasmania, Newnham, Australia

**Correspondence:** Rebecca McCullum (Rebecca.richmond@uon.edu.au) | Quan V. Vuong (vanquan.vuong@newcastle.edu.au)

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## ABSTRACT

Illawarra plums (IP) are native to Australia, having been used as bush food for centuries. This study characterized the physicochemical and antioxidant properties of mature IP and explored the efficacy of aqueous ethanol for extracting bioactives. The height, width, diameter, and weight of the fruit are 14.78 ± 3.1 mm, 17.45 ± 2.7 mm, 14.69 ± 3.0 mm, and 2.59 ± 1.2 g, respectively. The fruits were categorized into three ripening stages: unripe(green), almost-ripe(blushing), and ripe(red). The ripe fruit had a pH of 4.37 ± 0.03, total soluble solids of 9.3 ± 0.5°Brix, and a titratable acidity of 0.25 ± 0.01% w/v. The extraction solvents significantly influenced the yield of bioactives and antioxidant activity. The most effective solvent was 50% ethanol, which had total phenolics 123.93 ± 10.81 mg, flavonoids 130.58 ± 23.33 mg, proanthocyanins, and anthocyanins 16.12 ± 0.69 mg per gram of dried fruit. The extract exhibited potent DPPH radical scavenging properties (153.22 ± 39.67 mg TE/g). IP had five times more phenolics than African and American plums. Fourteen peaks were isolated by HPLC-PDA, with three tentatively identified as chlorogenic acid, epicatechin, and p-coumaric acid. IP shows great potential for the development of natural functional ingredients. Future research could explore individual phenolics and investigate the potential applications of IP in the food industry.

## 1 | Introduction

Illawarra Plum (IP), *Podocarpus elatus* R. Br. Ex Endl, is a native edible bush fruit belonging to the family Podocarpaceae from the plant group of gymnosperms (Jiménez-Aspee et al. 2019; Richmond et al. 2019). The botanical name *Podocarpus* has etymological roots in Greek from "podos" meaning "foot" and "karpos" meaning fruit. This is because the edible portion or 'fruit' is the fleshy stalk of the seed. The term "elatus" is Latin for "tall." The evergreen diecious subtropical tree typically grows 32–40 m high, though the trees are reported to be shorter in cultivation (Hegarty et al. 2001; Mpala et al. 2024; Witjuti Grub Bushfood Nursery 2015). The IP is known colloquially by other names, including the Daalgall, Goongum, Gidneywallum, Plum Pine, and Brown Pine, previously also known as *Nageia elata* (Endl.) F. Muell. It fruits between March to July in the Southern hemisphere (Ahmed and Johnson 2000; Mpala et al. 2024). The fruit has a natural distribution through the Northern Territory of Australia, and from Cape York Peninsula along the East Coast towards the Southern end of New South Wales (Enright and Jaffré 2011; Hegarty et al. 2001). It can now also be found in Papua New Guinea, the closest Northern neighbor of Australia. The ripe fruit was an esteemed part of the diet of the Aboriginal people local to New South Wales and Queensland (Hegarty et al. 2001).

The IP has been labeled as an 'emerging' commercial variety of native fruit (O'Brien 2008) and has great potential for further development in food or nutraceutical products. Most of the work on the IP was initiated in the late 1980s and consists of

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studies identifying basic nutritional composition and comparison between other varieties of edible native foods (Brand-Miller and Holt 1998; Brand-Miller et al. 1993; Cribb and Cribb 1987). Preliminary screening studies of the IP alongside a number of other plants for antimicrobial, anticancer, or anti-inflammatory activity have occurred, indicating the Plum is rich in anthocyanins, has high antioxidant activity and demonstrates limited antimicrobial activity (Tan et al. Konczak, Ramzan, & Sze 2011a, 2011b; Tan et al. 2011; Wood and Cock 2022; Wright et al. 2016). Due to the nature of the screening work, little focus has been given to the IP and there is only brief information available on its visual appearance. No work to undertake basic characterization, morphology, or chemical properties has occurred through its stages of maturity.

Preliminary studies show that the IP is a rich source of phytochemicals including phenolics, antioxidants and anthocyanins. Solvent selection is a key factor influencing the extraction efficiency of its phytochemicals. From the preliminary studies, there is not one common solvent that has been evaluated across the range of studies. One study identified that an extract prepared from IP using dichloromethane has a higher level of antioxidant activity as compared to an extract prepared using methanol, acetone, or water (Cayot et al. 2016; Forbes-Smith and Paton 2002). Dichloromethane is classified as "Class 2," meaning its use should be limited due to its inherent toxicity. Safer solvents such as water, ethanol, or acetone should be substituted, where possible (U.S. Department of Health and Human Services 2018). To our knowledge, the comparison of water, ethanol, or their combination as an extraction solvent for IP has not been evaluated. In terms of cost-effectiveness and benefit to commercial production, water, ethanol, or aqueous ethanol formulations should be comparatively assessed as solvents for the extraction of phenolics and antioxidants from IP. Ethanol and water are preferred solvents for end processing in food, as they are accessible, safe, have low cost for upscale, are odorless and have no maximum residue limit in food (preparation of flavorings, extraction solvents) (Plaskova and Mlcek 2023; U.S. Department of Health and Human Services 2018).

This study aimed to (i) characterize the physicochemical properties including color, size, weight, moisture, soluble solids, acidity, and pH of the fruits at three stages of maturity, and (ii) determine the efficacy of aqueous ethanol or water on the extraction of total phenolics, flavonoids, proanthocyanidins, anthocyanins and antioxidant properties from the ripe fruit to provide information for further studies on processing and applications of this fruit.

## 2 | Materials and Methods

### 2.1 | Materials

#### 2.1.1 | IP Fruit

Mature fruits and immature IP were harvested from Newcastle, NSW, Australia (Latitude: S32°53'40.67" Longitude: E151°43'17.87"). The plums were authenticated by a botanist at University of Newcastle, and a sample deposited in the University of Newcastle herbarium (voucher number 10763 and 10764). After collection, the fruits and seeds were immediately transferred to the laboratory where they were classified as mature, or immature based on a visual assessment of the skin color compared to the seed. Immature fruit skin had red or green appearance, and immature seeds were green. The seeds were removed easily from the plum by twisting, and the whole fruits and seeds were stored separately at −18°C.

#### 2.1.2 | Chemicals

High-performance liquid chromatography (HPLC) grade solvents (e.g., acetonitrile) were used. Solvents were de-gassed using an Ultrasonic water bath for a minimum of 20 min at room temperature (Soniclean, 220 V, 50 Hz, 250 W, Thebarton, Australia). All other chemicals used in this study were analytical grade. Methanol, ethanol, vanillin, NaOH were purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu's reagent, $Na_2CO_3$, $NaNO_2$, $C_2H_3Na$, $AlCl_3$, DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, and catechin were sourced from Sigma-Aldrich Pty Ltd. (Castle Hill, Sydney, Australia). HCl and KCl were purchased from ChemSupply (Port Adelaide, South Australia, Australia). Colorless, flat-bottom microplates were used in the spectrophotometric assays. All dilutions were made using deionised water unless otherwise specified.

### 2.2 | Measurement of Morphological Properties

The weight of fruits and their external seeds were measured using an analytical balance. Their dimensions were measured using Vernier callipers. Measurements were taken from the widest part of the fruit's vertical diameter and labeled "height", and two from the equatorial diameter with approximately 90° rotation difference, labeled "width" and "depth". The fruits were bisected using a scalpel to allow classification of the ripening stages, which were named ripe, almost ripe, and unripe. Measurement of the height and width of the fruit's endocarp (internal seed) were taken from the cut section. In some cases, the fruit contained two internal seeds that were partially fused. Measurements of both height and width were taken, along with an additional measurement of the widest part of the fused seed's width.

The external seed was similarly measured and weighed. The seed measurements are similarly described as the height, width, and depth, with additional measurements of the exocarp thickness, pericarp diameter, and endocarp height and width taken.

The surface color was measured using a colourimeter (model CR-20, Konica Minolta, Japan) expressed using the CIELAB scale ($L^*$, $a^*$, $b^*$ along with hue angle (h°) and chroma (C). Six measurements (including the distal area and equatorial zone) in each of three fruits.

The moisture content of fruit and bisected seed (5 g units of each) was independently determined using pre-dried moisture dishes. Drying was conducted for 24 h in a laboratory oven (LABEC Laboratory Equipment Pty Ltd., Marrickville, NSW, Australia) set at 105°C. The difference in weight was determined to be the moisture content and expressed as g moisture/100 g.

The thawed fruit was juiced in an electronic juicer (Sunbeam JuiceDrop Model JE4800, China), filtered through four layers of cheesecloth, and analyzed for total soluble solids content (TSS), pH and titratable acidity (TA) in three replicates. TSS was determined at 22°C using a hand-held temperature-compensated refractometer (Atago Co., Japan), and expressed as °Brix. The pH of the juice was recorded at 22°C with a pH-meter (model Aqua-pH, TPS, Queensland, Australia). Titratable acidity was determined by titration of an aliquot of 10 mL of fruit juice with 0.1 M NaOH using phenolthalein indicator (colorimetric end-point pink to purple). Results were expressed as meq. of citric acid per g of fresh weight (FW) (Valdenegro et al. 2012).

## 2.3 | Determination of Bioactives and Antioxidant Properties

### 2.3.1 | Preparation of Extracts for Further Analysis

Frozen fruits were freeze-dried in a single layer on pre-dried trays at −45°C, 135 millitorr (Benchtop Pro Freeze Drier, Scitek, Lane Cove, NSW, Australia). After drying, the samples were ground using a spice grinder (Breville BCG200BSS, NSW, Australia). Samples were sieved using a 500 μm mesh and kept at −18°C for further extraction.

Ethanol 95%, ethanol 50% and water were compared to determine the solvent of choice for the preparation of the extracts for further analysis. Extracts were prepared with a ratio of 1:100 g/mL using Ultrasonic Assisted Extraction with the setting of 60 min, 40°C, and power of 150 W (Soniclean, 220 V, 50 Hz, 250 W, Thebarton, Australia). Samples were agitated every 5 min using a vortex mixer (McCullum et al. 2024b; Nguyen et al. 2015; Vuong et al. 2018). After extraction, the samples were placed in an ice bath to cool and centrifuged at 3100*g* for 5 min. The extracts were then filtered, and the solvent was removed using a rotary evaporator with a 40°C water bath, while the pressure was gradually reduced to 60 milliBar. The concentrates were then freeze dried at −45.2°C, 236 milliTorr to yield a powdered extract, which was stored at −18°C until required.

### 2.3.2 | Determination of Bioactive Compounds

Total phenolic content was determined using the Folin-Ciocalteu (FC) method adapted for microplate analysis (Horszwald and Andlauer 2011; McCullum et al. 2024a; McCullum et al. 2024b). Briefly, 20 μL of diluted sample was mixed with 200 μL 10% FC reagent, 20 μL 20% (w/v) $Na_2CO_3$ on a plate shaker and the absorbance read at 765 nm in a microplate reader after 20 min rest. Gallic acid was used as the standard. Results are expressed as mg of gallic acid equivalents per gram of dried sample (mg GAE/g DW).

Total flavonoid content was determined according to the spectrophotometric method described by (McCullum et al. 2024a) adapted for the microplate analysis. First, 20 μL of diluted sample was added to microplate wells containing 88 μL of DI water. Then 6 μL of 5% (w/v) $NaNO_2$ was added, followed by 6 μL 10% (w/v) $AlCl_3$, and then 80 μL of 4% (w/v) NaOH. The plate was mixed on a plate shaker for 30 s between solvent additions, and the absorbance was measured at 515 nm in a microplate reader. Catechin was used as the standard. The results are expressed as mg of catechin equivalents per gram of dried sample (mg CE/g DW).

Proanthocyanidin (tannin) content was determined as described by (McCullum et al. 2024a) with a minor modification. For this method, dilutions of all solutions and the sample were made with methanol. Briefly, the sample was diluted and 20 μL mixed with 120 μL 4% vanillin (w/v) and 60 μL 36% hydrochloric acid on a 96-well microplate. The absorbance was measured at 500 nm. Catechin was used as the standard and results are expressed as mg of catechin equivalents per gram of dried sample (mg CE/g DW).

Total monomeric anthocyanin content of the fruit was measured using the pH differential method as described by (McCullum et al. 2024a; Sadowska et al. 2017) with a minor modification. 10 μL of sample was mixed with 190 μL of buffer at pH 1.0 (25% 0.2 N KCl and 75% 0.2 N HCl) and a duplicate sample was mixed with a buffer at pH 4.5 (0.14% 1 M sodium acetate, 0.34% 0.2 N HCl and water). Absorbance was measured at 515 nm, a turbidity reading at 700 nm was subtracted from the reading. To calculate anthocyanin content, the cyanidin-3-glucoside extinction coefficient $\varepsilon$ of 26,900 L/cm-mol, path length of 1 cm and molecular weight of 449.2 g/mol was used (McCullum et al. 2024b). Results are expressed as mg cyanidin-3-glucoside equivalents per gram of dried sample (mg C3G/g DW).

### 2.3.3 | Determination of Antioxidant Properties

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay to determine antioxidant activity was performed according to the method described by (McCullum et al. 2024a; Papoutsis et al. 2016). Briefly, 30 μL of diluted sample was mixed with 220 μL DPPH working solution in a 96-well microplate, rested for 10 min and the absorbance was measured at 515 nm. Trolox was the standard and results are expressed as mg Trolox equivalents per gram of dried sample (mg TE/g DW).

### 2.3.4 | Determination of Major Bioactive Compounds

HPLC-PDA was performed on the 50% ethanol IP extracts for putative identification of its constituents. The HPLC system (Xcalibur) used an injector volume of 20 μL, column oven temperature of 30°C, and tray temperature of 15°C. A Phenomenex Luna C18 RP column was used. Solvent A (0.2% formic acid) and B (100% acetonitrile) were run in a gradient in terms of solvent A. The gradient started at 100% from 0 to 5 min, 5–15 min decreasing to 85%, from 15 to 20 min decreasing to 50%, from 20 to 25 min maintaining 50%, from 25 to 30 min decreasing to 20%, then maintaining 20% until 34 min, then from 34 to 34.5 min increase to 100% and was maintained for a total run time of 40 min. The PDA detector was set for 280 nm. Software used was Xcalibur v2.0, Inst hardware version 4.1. Reference standards of gallic acid (standard curve), coumaric acid, caffeic acid, quercetin, coumarin, kaempferol, rutin, tannic acid syringic acid, escin, chlorogenic acid, epicatechin,

naringenin, ECGC, and β-sitosterol were run for putative identification based on peak retention time.

### 2.4 | Statistical Analysis

Data was analyzed using JMP software (JMP Pro v17.2. 2022, JMP Statistical Discovery LLC, USA). Data is presented as the mean ± standard deviation. Differences between fruit ripening stages, solvents, or plant parts were analyzed using one-way analysis of variance, comparing all group means using the Tukey's HSD post hoc test. Statistical differences were considered at the significance level of $p < 0.05$. Pearson's correlation was performed to determine if there is a relationship between the groups of phytochemical and antioxidant capacity. All chemical data is presented on a fresh weight (FW) basis, except moisture, and bioactive content which is presented on a dry weight (DW) basis.

## 3 | Results and Discussion

### 3.1 | Morphological Characterization of IP Fruit and Ripening Stages

The tree *P. elatus* is a conifer within the gymnosperm plant group. The female cone consists of a fleshy appendage at the base of the ovule/seed and is what is referred to as the IP fruit (Figure 1A). Each tree yields approximately 6 kg of fruit or 1.65 ton per hectare (Graham and Hart 1997) The fruit can be described as softer than its hard-coated seed which is attached at the outer end of the fruit. The fruit and seed both consist of a skin (exocarp), and a pericarp which is the combination of the flesh (mesocarp) and the internal pip (here referred to as pip for the fruit, or endocarp for the seed) (see Figure 1B). The fruit is asymmetrical, and the pip starts from the base of the fruit and is on average approximately 50% of the total height and 12%–15% of the width of the cross-section (Tables 1 and 2). The seed displays symmetry, and the endocarp appears more centrally located both vertically and horizontally within the pericarp of the seed. Some fruits have multiple seeds attached. These contain two pips both starting from the base of the stalk where they appear fused and separate towards the outer end forming a V shape (Figure 1C).

In the early developmental stage of the fruit, the fruit has green skin and is similar in color to the outer seed. The immature fruit also has green colored flesh and is typically smaller than the external seed. The color of the fruit's skin starts to blush and turn red, then further changes to dark blue as the fruit develops and starts to swell. Postharvest, fruit may have a stalk at one end of the fruit from where it was connected to the branch, and an oblate end with one (or more) cavity at the top where the seed(s) attached. At all stages of maturity, there is a pine aroma to the fruit and seed. The mature fruit is equal to or greater in width than the external seed(s) (Table 2).

The ripening stages from immature to mature are visually presented in Figure 2 and mature fruit can be categorized in at least three distinct ripening stages based on the internal flesh color: unripe, almost ripe (turning/blushing) and ripe. Unripe fruit flesh is firm, has greenness and no visual pinkness, and contrasts with the pip and skin which are both similarly colored and are dark pink/red (Table 1). The almost ripe fruit has blushing/pinkness developing around the pip and ripe fruit has an evenly distributed deep red flesh color. The change in texture from firm to soft during ripening may be attributed to changes in the degree of pectin esterification and hydrolysis of pectin (Li et al. 2024). This process occurs during their storage after harvest and is typical of plums. More work is required to conduct additional analysis on the plums to characterize the starches, sugars, and fibers.

While the fruits swell as they mature, the size of mature fruit is highly variable and there is similarity in size between the ripening stages ($p > 0.05$); that is, bigger fruit doesn't always correlate to increased ripeness. The ripe fruit measures $14.78 \pm 3.1$ (h) $\times$ $17.45 \pm 2.7$ (w) $\times$ $14.69 \pm 3.0$ mm (d), and weighs $2.5958 \pm 1.2$ g (Table 2). There is no statistical difference ($p > 0.05$) between the weight, height, or depth of the fruit between the ripening stages Table 2. This is also apparent in the image of the ripe fruit given in Figures 1B and 2. The unripe fruit is statistically smaller ($p < 0.05$) in width than the other two stages, indicating the fruit swelling during ripening favors

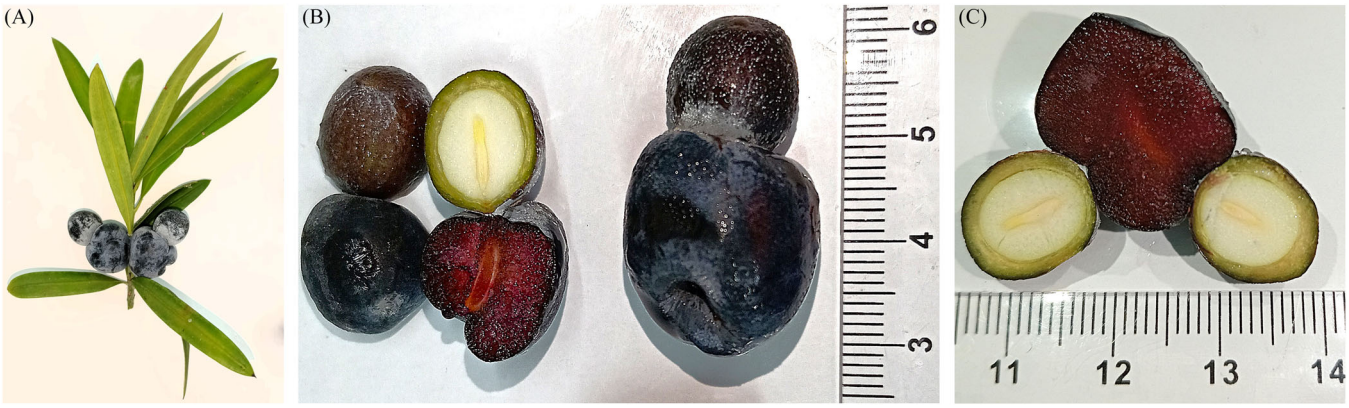



**FIGURE 1** | (A) The cone of a female *Podocarpus elatus* tree, consisting of a hard-coated seed and a fleshy appendage referred to as the Illawarra plum. (B) The cross section outlines the structure of the two sections, a fleshy appendage and a hard-coated seed, which both consist of the exocarp, mesocarp and endocarp (pip). The mesocarp of the fleshy appendage is the edible "fruit". (C) Cross section of a fruit consisting of two seeds connected to the same stalk.

**TABLE 1** | Visual description of the shape, color and texture of Illawarra plum fruit and seed at defined ripening stages.

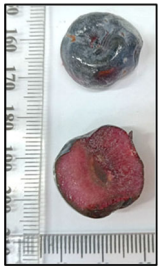
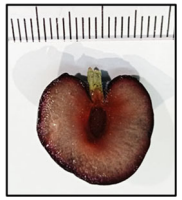
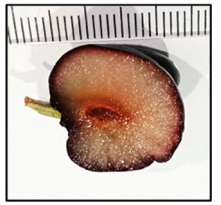
| | Fruit ($n = 10$) | | | Seed ($n = 5$) | |
|---|---|---|---|---|---|
| | **Ripe** | **Almost Ripe** | **Unripe** | **Mature** | **Immature** |
| **Observations** |  |  |  |  |  |
| Shape | Oblate, round or heart shaped | Oblate, round or heart shaped | Oblate, round or heart shaped | Round | Oval, tapered |
| Exocarp | Edible; thin, strong, blue/black, smooth, waxy external surface, internal edge is dark red | Thin, strong, blue/black, smooth, waxy | Thin, strong, blue/black, smooth, waxy | Not eaten; Brown, waxy, aromatic, thick, hard, inside is green to dark yellow | Green, waxy, aromatic, inside is green to dark yellow |
| Mesocarp | Edible; dark pink/red swollen, juicy, some sliminess, some browning evident after cutting. Soft flesh | Blushing yellow to pink, swollen, juicy, some sliminess. Some browning evident after cutting. Some softness to the flesh | Pale green to yellow swollen, juicy, some sliminess, some browning after cutting. Firm flesh | Not eaten; Pale creamy white | Pale creamy white |
| Endocarp | Inedible; dark red to brown | Concentrated dark pink/red color | Concentrated dark pink/red color | Not eaten; Yellow | Yellow |

**TABLE 2** | Characterization of weight (g), size (cm), and moisture content (%) of Illawarra plum fruit and seed at defined ripening stages.

| Morphological properties | | Fruit ($n = 10$) | | | | | | | | | Seed ($n = 5$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ripe | | | Almost ripe | | | Unripe | | | Mature | Immature |
| Moisture content (g/100g) $n = 3$ | x̄ | $85.80_a$ | | | $85.67_a$ | | | $86.15_a$ | | | $57.23_a$ | $58.93_a$ |
| | σ | 2.9 | | | 0.2 | | | 4.7 | | | 1.1 | 1.5 |
| Weight (g) | x̄ | $2.5958_a$ | | | $2.3883_a$ | | | $1.8069_a$ | | | $1.7440_a$ | $1.1028_b$ |
| | σ | 1.23 | | | 0.72 | | | 0.65 | | | 0.36 | 0.14 |
| Height (mm) | x̄ | $14.78_a$ | | | $14.44_a$ | | | $13.65_a$ | | | $15.55_a$ | $13.53_b$ |
| | σ | 3.1 | | | 1.4 | | | 1.5 | | | 0.7 | 0.8 |
| Width (mm) | x̄ | $17.45_a$ | | | $16.72_a$ | | | $14.25_b$ | | | $13.47_a$ | $11.53_b$ |
| | σ | 2.7 | | | 1.4 | | | 2.3 | | | 0.9 | 0.5 |
| Depth (mm) | x̄ | $14.69_a$ | | | $13.98_a$ | | | $12.79_a$ | | | $13.38_a$ | $11.69_b$ |
| | σ | 3.0 | | | 1.8 | | | 2.1 | | | 1.0 | 0.6 |
| Pip(s)/endocarp height (mm) | | *(1)* | | *(2)* | *(1)* | | *(2)* | *(1)* | | *(2)* | | |
| | x̄ | $7.10_a$ | | $6.57_a$ | $6.955_a$ | | $7.135_a$ | $7.077_a$ | | $6.350_a$ | $6.898_a$ | $5.772_a$ |
| | σ | 1.5 | | 1.5 | 1.0 | | 1.1 | 1.4 | | — | 1.3 | 0.9 |
| Pip(s) /endocarp width (mm) | | *(1)* | *(2)* | *fused* | *(1)* | *(2)* | *fused* | *(1)* | *(2)* | *fused* | | |
| | x̄ | $2.48_a$ | $2.07_a$ | $4.39_b$ | $2.123_a$ | $1.73_a$ | $3.93_b$ | $2.403_a$ | $1.73_a$ | $4.81_b$ | $1.448_a$ | $1.402_a$ |
| | σ | 0.7 | 0.3 | 0.9 | 0.5 | 0.2 | 0.8 | 0.5 | — | — | 0.2 | 0.2 |
| Seed exocarp thickness (mm) | x̄ | | | | | | | | | | $2.094_a$ | $1.402_a$ |
| | σ | | | | | | | | | | 0.8 | 0.3 |
| Seed pericarp width (mm) | x̄ | | | | | | | | | | 9.308 | 8.11 |
| | σ | | | | | | | | | | 0.6 | 0.8 |

*Note:* Different subscript letters within a row and plant part indicate significant difference at ($p < 0.05$).




**FIGURE 2** | Visual presentation of whole and bisected Illawarra plum mature fruit at three stages of ripening, and immature fruit. We have categorized the mature fruit as ripe, almost ripe and unripe.

one direction. The immature fruit swells in size around the pip to become a round or heart-shaped mature fruit. The skin of the mature fruit appears blue/back, is thin, strong and has a waxy outer appearance. The flesh of mature fruit is like a plum or grape in texture, described as mucilaginous, starchy, and giving a slimy mouthfeel. The physical description of the fruit is given in Table 1. The IP is approximately the diameter and weight of a large blueberry, and four to five times smaller in diameter than an apple. A seedless blueberry (Northern Highbush variety), by comparison, is between 1.3 and 1.8 cm and 1.1–2.6 g and a "red

delicious" cv apple is between 6.66 and 6.74 cm diameter and 164–166 g (Ganai et al. 2018; Jorquera-Fontena et al. 2017).

The ripe fruit is deep purple, has strong to subtle pine and juniper flavors, and is sweet, resinous, astringent and bitter. It has soft flesh, and the mouthfeel offers slight sliminess (sometimes referred to as gluey). The flesh has variability in the intensity of flavors and characteristics and gives a lingering pine/resin aftertaste (Hegarty et al. 2001; Li et al. 2024; Richmond et al. 2019). From our collection, the taste and flavor of the almost ripe and unripe fruit was considerably unpleasant, strongly astringent, resinous, bitter, and had little to no sweetness. For the ripe fruit, there was sweetness, astringency, resin flavor and chewing around the fruit pip gave more flavor, and more bitterness and astringency. Chewing around the pip is a less pleasant experience than eating the rest of the flesh. A previous report has mentioned the "particularly resinous core that is first removed with an olive corer" (Hegarty et al. 2001). The pip is not eaten and is discarded.

Manually picked fruit may present a postharvest challenge to visually distinguish between fully ripe and unripe whole fruit. Once the fruit is cut, this is easier. The IP used in this study was wild harvested and had no method for sorting or grading applied at harvest. From our collection, it is apparent that fully ripe fruit will fall to the ground, allowing catch nets as a harvest method to be used. Ripe fruit can be easily plucked from the tree, however, the unripe and almost ripe fruit does not take much effort to remove from the tree. Further studies on postharvest ripening of the fruit would be beneficial to fully characterize cultivation factors affecting harvest, for example, understanding if the fruit is climacteric like a stone fruit or non-climacteric like a grape or strawberry. Previous measurements of the IP have suggested the fruit is 2–3 cm in diameter (Ahmed and Johnson 2000). The fruits used in this study are smaller than previously reported can be due to several variables, including geographical location, age of the tree, pruning differences, fertilization or soil quality, and weather.

The pip in each ripening stage appears to have the same deep red color and there is no difference in the dimensions of (h) and (w) of the pip between the three ripening phases (Table 2). After cutting, some browning develops around the seed which is likely due to oxidation and enzymatic activity. Visually, the fruit appears yellow/green with a brown/red pip. The presence of a pip(s) is a challenge for commercialization of processed fruit products due to requirements for seed removal steps. Solid–liquid extraction can be completed with minimal prior processing such as slight maceration to allow solvent penetration.

The color of the fruits flesh and skin (plant parts) at each of the three ripening stages were measured using a colorimeter Table 4. The lightness $L^*$ was highest ($38.57 \pm 1.26$) for the unripe fruit flesh. Lightness for the fruit was significantly different between the three ripening stages and decreased with increasing ripening ($p < 0.05$). There were no significant differences in $a^*$ (yellow/blueness), browning index, or $a^*/b^*$ between the three ripening stages. The values for color of almost ripe and unripe fruit were significantly different between the flesh and the skin for all parameters (Table 4). The ripe fruit flesh was not significantly different to the skin for hue angle. Chroma and hue angle is significantly higher in the ripe fruit skin than other the skin of the fruit in the other two ripening stages.

Between the other two ripening stages, the unripe fruit flesh was significantly different for hue angle, chroma, $b^*$ (yellow blueness) and $L^*$ (lightness) ($p < 0.05$). Hue angle, $b^*$ and chroma of the almost ripe flesh was significantly different to the unripe flesh but not to ripe flesh. The fruit flesh was significantly different ($p < 0.05$) in color to the fruit skin for all ripening stages and parameters except for the hue angle of the ripe skin which was not different to the ripe flesh ($p > 0.05$). Freezing can sometimes have detrimental effects to Western fruit quality, particularly color, sometimes causing bruising or browning in the case of bananas. For IP, the frozen fruit flesh has no observed ill effects of bruising or browning that can be attributed to freezing. In some cases freezing caused in fully ripe fruit to swell and split. Split fruits were not used for measurements.

The ripe fruit has an average moisture of $85.80 \pm 2.9$ g/100 g. There were no significant differences ($p < 0.05$) in moisture content across the three ripening stages (Table 2). The IP has been analyzed for moisture in 1998 and found similar values (Fresh frozen, cored fruit) 87.6 g/100 g water and (wild fresh frozen whole fruit) 85.6 g/100 g (Brand-Miller and Holt 1998).

Chemical properties of pH, titratable acidity, total soluble solids and TSS:TA ratio were evaluated for the fruits in the three ripening stages (Table 3). The pH of the ripe fruit was $4.37 \pm 0.03$ and TSS $9.3 \pm 0.5$ °Brix and were not significantly different between the ripening stages. The titratable acidity of the ripe fruit was $0.25 \pm 0.01$% w/v and was lower than the titratable acidity of the almost ripe fruit $0.34 \pm 0.02$% w/v.

The seeds were classified as mature or immature (Figure 3). There is a significant difference in green color between mature and immature seeds. In the color values, this is particularly reflected in the value for $a^*$ and $b^*$ which was higher in the immature seed ($p < 0.05$) than the other two ripening stages see Table 4. The mature seed is significantly ($p < 0.05$) bigger in

**TABLE 3** | Chemical properties of moisture, pH, total soluble solids (TSS), titratable acidity (TA) and TSS:TA of ripe, almost ripe and unripe mature IP fruit.

| Chemical properties ($n = 3$) | | Fruit | | |
|---|---|---|---|---|
| | | Ripe | Almost ripe | Unripe |
| TSS (°Brix) | $\bar{x}$ | $9.3_1$ | $9.3_1$ | $9.10_1$ |
| | $\sigma$ | 0.5 | 0.2 | 0.000 |
| pH | $\bar{x}$ | $4.37_1$ | $4.39_1$ | $4.39_1$ |
| | $\sigma$ | 0.03 | 0.03 | 0.03 |
| TA (% w/v) | $\bar{x}$ | $0.25_2$ | $0.34_1$ | $0.21_2$ |
| | $\sigma$ | 0.01 | 0.02 | 0.02 |
| TSS:TA | $\bar{x}$ | $37.44_2$ | $27.97_3$ | $43.70_1$ |
| | $\sigma$ | 0.91 | 2.31 | 3.43 |

*Note:* Different numerical subscript indicates a significant difference between a ripening stage $p < 0.05$.
Abbreviations: TA, titratable acidity; TSS, total soluble solids.




**FIGURE 3** | Latitudinal and longitudinal cross section of the hard-coated seed of *Podocarpus elatus* at mature and immature stages of development.

height, width and depth and pericarp width compared to the immature seed, however there is no difference in the dimensions (h) and (w) of the endocarp (Table 2). The exocarp is also significantly thicker in the mature seed at $2.094 \pm 0.8$ mm compared to $1.402 \pm 0.3$ mm in the immature seed ($p < 0.05$). The mature seed is heavier at $1.7440 \pm 0.6$ g compared to the immature seed which is $1.1028 \pm 0.14$ g. The moisture content of the mature seed was $57.23 \pm 1.1$ g/100 g and the immature seed $58.93 \pm 1.5$ g/100 g (not different $p > 0.05$).

### 3.2 | Bioactives and Antioxidant Activity of IP

Plums were extracted with 50% ethanol, 95% ethanol and water to understand the potential for ethanol, water, or their combination to be used as a greener solvent alternative.

Ethanol and water are predominant solvents used for extracting phenolic compounds (Routray and Orsat 2022). Ethanol, water, and their mixture significantly affected extraction yields of bioactive compounds from the ripe IP. Ripe plums extracted with 50% ethanol (v/v) gave significantly higher levels of total phenolics ($123.93 \pm 10.81$ mg GAE/g), proanthocyanins ($130.58 \pm 23.33$ mg CE/g), monomeric anthocyanins ($16.12 \pm 0.69$ mg C3G/g) and antioxidant scavenging activity ($153.22 \pm 39.67$ mg TE/g) ($p < 0.05$) than the IP extracted with 95% ethanol or water (Figure 4). The levels of total phenolics recovered by 50% ethanol is higher than similar fruits, such as other types of plums or grapes. A native West African plum had TPC levels of 2.03–4.63 mg GAE/g DW (Traore et al. 2020). In six plum cultivars harvested in New York, the phenolic content ranged from 1.74 to 3.75 mg GAE/g FW (Kim et al. 2003). In five varieties of Australian grown green grapes, the TPC level was 1.73–4.31 mg GAE/g FW (Vo et al. 2022). Note that some of these values are reported on a FW basis. Assuming 85% moisture in both these plums and grapes this would equal a phenolic content of roughly 11–25 mg GAE/g DW. Thus, the IP has roughly 25, 5 and 5 times more TPC than the West African plum, six American plum varieties, and five Australian green grape varieties, respectively. It is possible that the difference in extraction technique and solvent contributes to these differences. Both studies on plums used the ultrasonic-assisted extraction technique. However, they used 80% aqueous methanol as the extraction solvent. The study on the grapes used a different extraction technique; and solvent (70% aqueous ethanol). However, there are many other factors that influence the yield of phenolics, alongside extraction technique and solvent choice, cultivar, climate, stressors such as pests, growing seasons, agricultural practices and fertilization may all contribute as reasons for the large differences observed between the plants. It is proposed that Australian native plants are naturally high in phenolic compounds. Other Australian native plums such as the Davidson's Plum and Kakadu Plum also have high levels of total phenolics with 48.6–949.0 and 158.57 mg GAE/g DW for each, respectively (Mani et al. 2020). The levels of phenolics from this study are thus consistent with levels of phenolics in other native Australian plums. Polyphenols are important phytochemicals that contribute to bitterness, color, flavor, odor and oxidative stability of fruits (Vo et al. 2022). Considering IP is high in phenolics, it is suggested that IP phenolics could be applied to foods to improve color, flavor, or oxidative stability. The functional potential of IP phenolics is an avenue for further research.

It was observed that total flavonoids extracted from IP was significantly higher when 95% ethanol was the solvent ($163.99 \pm 19.71$ mg CE/g DW) compared to 50% ethanol ($54.42 \pm 4.75$ mg CE/g DW) ($p < 0.05$) (Figure 4). This was not reflected in the total phenolic recovery, which did not see the same increase with increasing ethanol concentration. This has been observed in other studies, where increasing concentrations of ethanol have given increasing yields of total flavonoids without the same increase in total phenolics (Arya et al. 2024; Dirar et al. 2019).

Flavonoids are a class of phenolic compounds, and includes many sub-groups such as flavonols, flavones, flavanones, isoflavones, proanthocyanins and anthocyanins (Seleem et al. 2017). Compared to the extraction efficiency of phenolics, 95% ethanol can extract higher levels of flavonoids than 50% ethanol. This can be explained by their polarity and electric constant of the solvents (Vuong et al. 2013). Despite extracting more total flavonoids with 95% ethanol, this solvent was less effective than 50% ethanol in extracting anthocyanins or proanthocyanins ($p < 0.05$). This difference is due to varying solubility between the different phenolic groups. For instance, flavonoids such as luteolin, naringenin, quercetin, and hesperidin have low water solubility (Dong et al. 2023), whereas anthocyanins and proanthocyanins are more water-soluble. Alcohol solvents such as ethanol and methanol are

**TABLE 4** | Color CIELAB properties of Illawarra plum fruit and seed at defined ripening stages.

| Color $n = 3$ | | Fruit | | | | | | Seed | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ripening stage | | Ripe | | Almost ripe | | Unripe | | Mature | | Immature | |
| Plant part | | Flesh | Skin | Flesh | Skin | Flesh | Skin | Pericarp | Exocarp | Pericarp | Exocarp |
| $L^*$ Lightness | x̄ | $20.14_{b3}$ | $24.17_{a2}$ | $29.05_{a2}$ | $26.88_{b1}$ | $38.57_{a1}$ | $24.43_{b2}$ | $76.73_{a1}$ | $36.47_{b1}$ | $72.47_{a1}$ | $37.00_{b1}$ |
| | σ | 2.70 | 0.72 | 0.54 | 0.57 | 1.26 | 1.95 | 7.04 | 2.22 | 4.76 | 2.67 |
| $a^*$ Yellow (+)/blue (−) | x̄ | $10.93_{a1}$ | $-0.32_{b3}$ | $8.87_{a1}$ | $1.92_{b1}$ | $11.62_{a1}$ | $0.30_{b2}$ | $0.27_{b2}$ | $0.93_{a1}$ | $1.48_{a1}$ | $-0.13_{b2}$ |
| | σ | 3.93 | 0.08 | 0.51 | 0.64 | 1.45 | 0.11 | 0.42 | 0.34 | 0.20 | 0.32 |
| $b^*$ Red (+)/green (−) | x̄ | $0.72_{a2}$ | $-3.20_{b2}$ | $1.32_{a2}$ | $-1.93_{b1}$ | $9.01_{a1}$ | $-2.45_{b1,2}$ | $12.18_{a1}$ | $-0.73_{b2}$ | $12.35_{a1}$ | $2.37_{b1}$ |
| | σ | 1.53 | 0.42 | 0.44 | 0.67 | 2.22 | 0.50 | 1.94 | 0.31 | 0.90 | 0.45 |
| h° | x̄ | $0.02_{b2}$ | $1.47_{a1}$ | $0.15_{a2}$ | $-0.78_{b2}$ | $0.65_{a1}$ | $-1.44_{b3}$ | $1.53_{a1}$ | $-0.66_{b1}$ | $1.45_{a2}$ | $-0.27_{b1}$ |
| | σ | 0.13 | 0.03 | 0.04 | 0.33 | 0.14 | 0.07 | 0.06 | 0.26 | 0.01 | 1.58 |
| Chroma | x̄ | $11.03_{a2}$ | $3.22_{b1}$ | $8.97_{a2}$ | $2.85_{b1,2}$ | $14.83_{a1}$ | $2.47_{b2}$ | $12.20_{a1}$ | $1.22_{b2}$ | $12.44_{a1}$ | $2.39_{b1}$ |
| | σ | 4.01 | 0.42 | 0.57 | 0.17 | 1.71 | 0.49 | 1.91 | 0.37 | 0.92 | 0.45 |
| Browning index | x̄ | $587.8_{b1}$ | $588.4_{a1}$ | $588.0_{b1}$ | $588.3_{a3}$ | $587.8_{b1}$ | $588.3_{a2}$ | $588.1_{b1}$ | $588.2_{a1}$ | $588.0_{b2}$ | $588.2_{a2}$ |
| | σ | 0.27 | 0.02 | 0.02 | 0.04 | 0.09 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 |

*Note:* x̄- mean, σ- standard deviation, h°- hue angle. Different subscript letters indicate a significant difference between the part of the plant within a ripening stage, different numerical superscript indicate significant difference between ripening stage within a plant part $p < 0.05$.

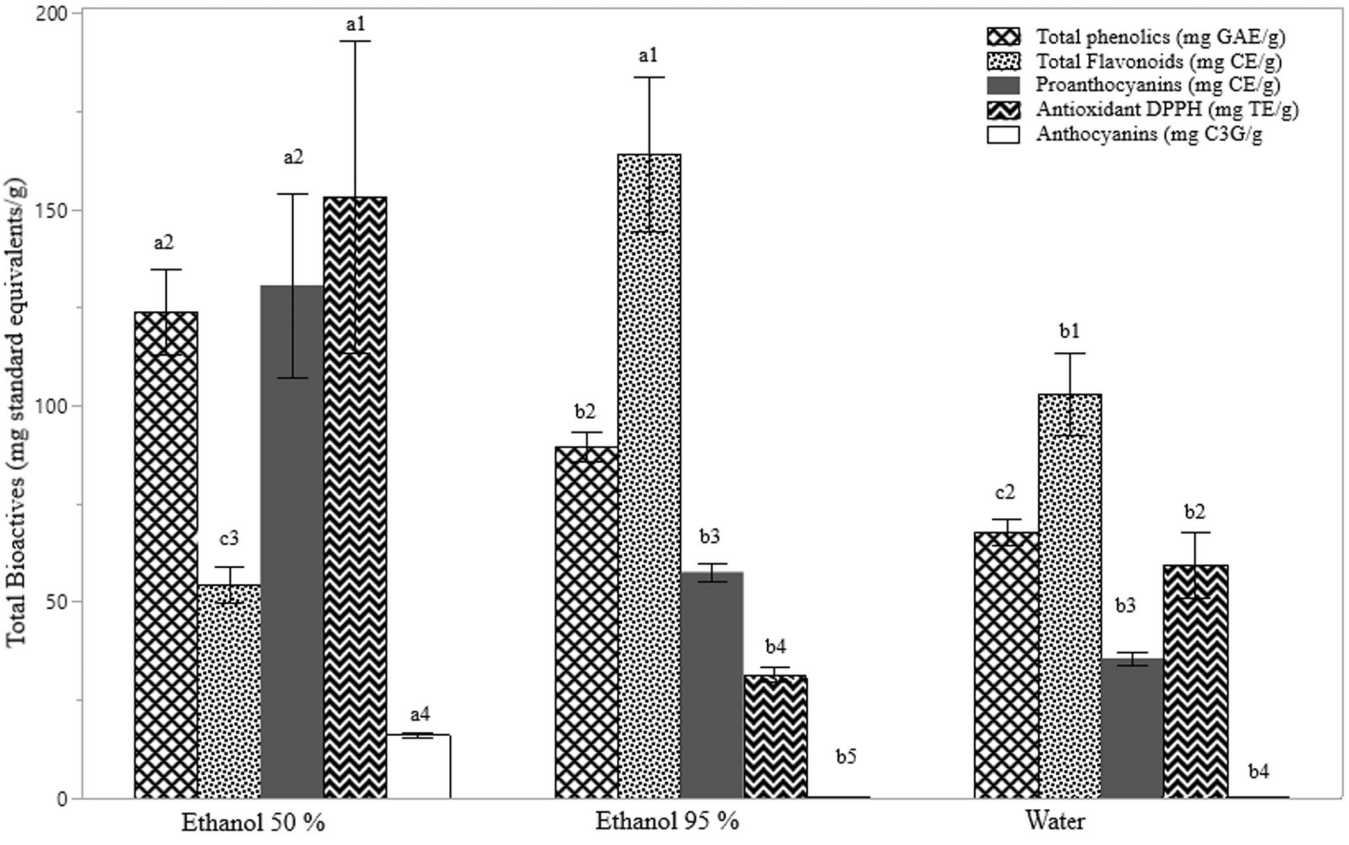



**FIGURE 4** | Total phenolics, total flavonoids, proanthocyanins, antioxidant scavenging activity as measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and monomeric anthocyanins in Illawarra plum fruit extracted using water, or 50% and 95% v/v ethanol concentrations. Columns not sharing a letter within an assay type are significantly different at $p < 0.05$. Columns not sharing a number within a solvent type are significantly different at $p < 0.05$.

polar-protic and hydrogen bond donors, which enhance the extraction of certain flavonoids when combined with water. Binary solvents, such as mixtures of ethanol or acetone with water (e.g., 30%) are often more efficient than single solvent systems for extracting antioxidants (Efenberger-Szmechtyk et al. 2021; Silva et al. 2017). Catena et al. (2020) also reported that 50-60% ethanol resulted in better extraction of anthocyanins compared to pure water or 90% ethanol. This study indicates that 50% ethanol is

more suitable than 95% ethanol or water and is recommended as a green extraction solvent to achieve high extraction yields of total phenolics, including antioxidants, anthocyanins and proanthocyanins from IP.

### 3.3 | Screening for Key Bioactive Compounds in IP

HPLC-PDA evaluation of IP extracted with 50% ethanol had 14 identified key peaks (Figure 5). These were compared with a range of phenolic standards and three are suggested as chlorogenic acid, epicatechin, and p-coumaric acid based on peak retention time. An earlier study of IP has previously reviewed the composition of the plum by HPLC-DAD and LC-PDA-MS to understand if the plum holds significant cyto-protective activity against oxidative stress. In that study, the authors identified 10 major peaks, identifying five of them as: delphinidin 3-glucoside, cyanidin 3-glucoside, pelargonidin 3-glucoside, luteolin/kaempferol glucoside and quercetin glucoside (Tan et al. 2011a). It should be noted that three identified compounds, including chlorogenic acid, epicatechin, and p-coumaric acid, along with 11 unidentified compounds, have not been quantified from the fruits. Further work is required to fully identify and quantify the phytochemical and bioactive composition of IP, taking care to consider the impact of extraction solvent, particularly to flavonoids.

### 3.4 | Correlation Between Bioactive Compounds and Antioxidant Properties

Nonlinear regression with Pearson's pairwise correlation was used to analyse the influence of phenolic groups on antioxidant activity in IP (Table 5). Total phenolics exhibited a positive correlation with DPPH scavenging activity (0.6447, $p < 0.05$), consistent with previous studies (Li et al. 2023; Netzel et al. 2006). Flavonoids also had a positive correlation with antioxidant capacity. These findings are similar to those reported by (Zhao et al. 2024). Anthocyanins also revealed a strong positive correlation with antioxidant activity (0.8518, $p < 0.05$). It is noted that while phenolic content contributes to antioxidant activity, it is not the only factor. Non-phenolic antioxidants like ascorbic acid, tocopherols and carotenoids also play a role (Khan et al. 2010).

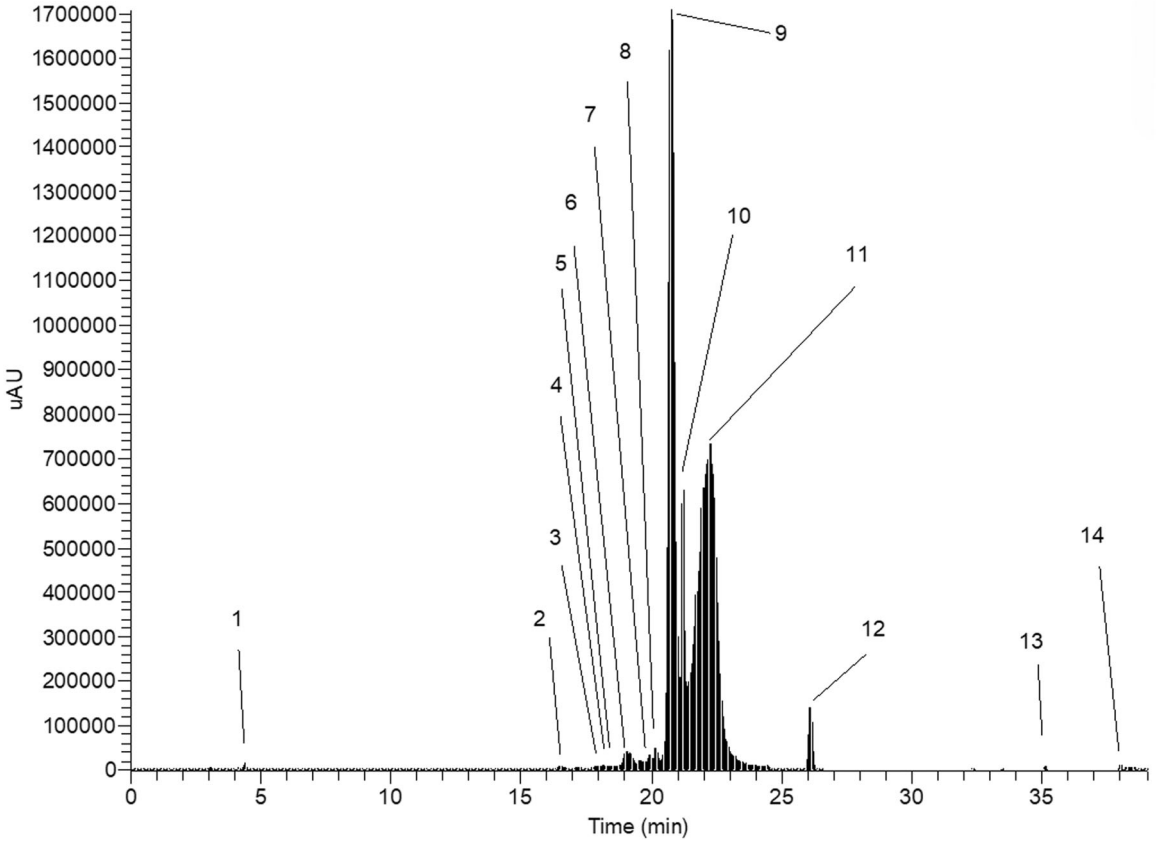



**FIGURE 5** | Major peaks identified in high performance liquid chromotagraphy (HPLC) chromatogram at 280 nm of Illawarra Plum extracted using 50% ethanol v/v. Three peaks were putatively identified as chlorogenic acid (Peak 9), epicatechin (Peak 10), and p-coumaric acid (Peak 11).

**TABLE 5** | Correlation of DPPH scavenging activity to phytochemical groups in Illawarra plum (all solvents).

| Fruit | Correlation | Total phenolics | Total flavonoids | Proanthocyanins | Anthocyanins |
|---|---|---|---|---|---|
| IP | Coefficient | 0.6447 | −0.8501 | 0.5852 | 0.8518 |
| | Probability | 0.0002 | < 0.0001 | 0.0009 | < 0.0001 |

This study is the first to report a strong positive correlation between anthocyanins and antioxidants in IP, highlighting the significant functional potential of its anthocyanins.

Phenolics are a large group of secondary metabolites of plants and are attributed with bioactivity including antioxidant function, antimicrobial activity, and health benefits. There are many studies assessing the impact of solvents on extraction of this group of phytochemicals, and the observations in this study can be easily explained from previous studies (Arya et al. 2024; Catena et al. 2020; Dirar et al. 2019; Mokrani and Madani 2016; Silva et al. 2017). Extracts from IP have functional potential. In one study, anti-inflammatory action in mice was observed from an extract and its isolated fraction (Tan et al. 2011). Antimicrobial activity against *Bacillus anthracis* was achieved causing bacterial growth inhibition, while a separate study found no effect on *Shewanella putrefaciens*, bacteria that commonly cause fish spoilage (Wright et al. 2016; Wright et al. 2015).

It is important to iterate that antioxidant content, in vitro antioxidant activity, antioxidants as food additives and antioxidants that offer cellular protective effects or health benefits are all separate properties. Additional research is required to investigate the functional potential, limitations, and underlying mechanisms of the IP's bioactivity. It is crucial to carefully consider the choice of solvent for extraction to ensure it is suitable for food applications and achieves appropriate recovery yields of bioactive compounds.

## 4 | Conclusions

The morphological properties of IP were characterized in this study for the first time. The color, size, weight, moisture, soluble solids, acidity, and pH of IP fruit at three distinct ripening stages based on the internal flesh color: unripe (green flesh), almost ripe (turning/blushing) and ripe (deep red) were characteristics evaluated in this study. The ripe fruit measures $14.78 \pm 3.1$ (h) $\times$ $17.45 \pm 2.7$ (w) $\times$ $14.69 \pm 3.0$ mm (d) and weighs $2.5958 \pm 1.2$ g and its size in each ripening stage is not significantly different by height, width, or weight. The ripe fruit pH was $4.37 \pm 0.03$, TSS was $9.3 \pm 0.5$ °Brix, and TA $0.25 \pm 0.01$% w/v. Extraction solvent and concentration significantly affected the total phenolic content, flavonoids, proanthocyanins, scavenging antioxidant activity and anthocyanins in the ripe fruit. Ethanol 50% v/v is recommended as the extraction solvent, as it is more efficient than 95% ethanol or water. IP is rich in phenolics with 14 major peaks isolated by HPLC-PDA. Three peaks were putatively identified as chlorogenic acid, epicatechin and p-coumaric acid. Correlation confirmed the anthocyanin content as the main group of phenolics in IP contributing to the antioxidant scavenging activity. Additional research is required to investigate the functional potential, limitations, and underlying mechanisms of the IP's bioactivity.

### Author Contributions

**Rebecca McCullum:** conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, writing – original draft, writing – review and editing. **Md Saifullah:** writing – review and editing. **Michael Bowyer:** validation, writing – review and editing. **Quan V. Vuong:** conceptualization, methodology, writing – review and editing.


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### Ethics Statement

The authors have nothing to report.

### Consent

All images are used are original images from the author.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

Data will be made available upon request.

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