INTRODUCTION

Kiwifruit, belongs to the family Actinidiaceae, is one of the most popular fruits today. It is the common name of the edible berry of cultivars of the woody vine of several Actinidia species. Even though kiwifruit is native to China, they are now well distributed throughout the world, especially in eastern Asia, Europe, United States, and New Zealand[1,2]. The most commonly consumed kiwifruits in the world are green-fleshed cultivar (Actinidia delicosa) (A. Chev.) C.F.Laing et A.R. Ferguson ‘Hayward’) and gold-fleshed cultivar (A. chinensis Planch. ‘Hort16A’)[3]. A few other Actinidia species exist, and they are currently being assessed for potential commercialization or are used as genetic resources for new cultivar development through interspecific hybridization[4]. A. chinensis cv Sungold (Gold 3) which belongs to the genus Actinidia is a new kiwifruit variety commercialized in 2010 by ZESPRI (New Zealand) after 10 years of development and is marketed internationally under the name of Sungold. This fruit had shown a greater level of tolerance to Pseudomonas syringae pvactinidiae than the original ZESPRI gold kiwifruit variety. The second green variety which forms part of the ZESPRI suite of products is the sweet green (sp. A. delicosa) kiwifruit, which has a similar shape and visual appeal but offers a smoother, sweeter taste[5]. A. macroperma is a non-commercial type kiwifruit with orange-colored flesh, small size fruit with large seeds, and relatively thick, hairless skin. This is a well known medicinal plant which has been extensively employed in Chinese traditional medicine[6-9]. In terms of nutritional value, kiwifruit has a reputation as being particularly nutritious and excellent sources of vitamin C, carotenoids, polyphenols, flavonoids, folate, potassium, and dietary fiber.

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ABSTRACT

The aim of this study was to assess phenolic profiles, in vitro radical scavenging activity and antioxidant activity of five different kiwifruit cultivars namely, A. delicosa cultivar Hayward, A.deliciosa cv Sweetgreen, A.chinensis cv Hort16A (Gold), A.chinensis cv Sungold (Gold 3) and A. macrosparma grown in New Zealand. Phenolic compounds were extracted using 70% acetone by steeping technique, and the content of total phenol, flavonoid, flavanol were determined spectrometrically. Antioxidant capacity and radical scavenging activity were determined by ferric reducing antioxidant power (FRAP) assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, respectively. Among the five different kiwifruit cultivars tested, A. macrosparma resulted in the significantly higher values of total phenolic (TP) content (823±14.4 mg gallic acid equivalent/100 g dry weight [DW]), total flavonoid (TFO) content (171±1.9 mg catechin equivalent/100 g DW), total flavanol (TFA) content (82.6±0.6 mg CE/100 g DW), radical scavenging activity (5.1±0.1 mmol Trolox equivalent/100 g DW) and the antioxidant capacity (8.3±0.1 mmol Fe (II) equivalent/100 g DW). High correlation (R²= 0.942) observed between FRAP and DPPH antioxidant capacities implied that antioxidants in these kiwifruit extracts were capable of scavenging free radicals (DPPH) and reducing oxidants. The presented result along with the qualitative analysis on high-performance liquid chromatography (HPLC) coupled with diode array detector (DAD) and quantitative analysis on Liquid chromatography electrospray ionization coupled with mass spectrometry (UPLC-ESI-MS/MS) revealed that A. macrosparma is an excellent source of potential phenolic antioxidants compared to commercially available kiwifruits cultivars.

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although the levels vary among the different genotypes of Actinidia fruit. Actinidia species have been widely used in traditional medicine and abound spectrum of pharmacological and biological properties such as antioxidants, antimicrobials, antinecancer, anti-inflammatory and anti-hypertensive have been reported. They also possess rich phytochemical profiles.

Many different plant sources have recently become of great interest to scientific research as a result of their naturally occurring phenolic compounds. It is reported that phenolics among different substances contained in the kiwifruits are the major compounds that are responsible for most of the antioxidant capacity in kiwifruit. Even though intensive studies on the polyphenolic antioxidant constituents in numerous plant sources have been conducted, the composition data for new and wild kiwifruit is limited.

Therefore, the objective of this study was to compare the total phenolic, flavonoid, flavonol contents, phenolic profiles, radical scavenging activity and antioxidant capacity of A. Macroasperma fruit with four different commercial kiwi fruit varieties namely, A. delicosa cultivar Hayward, A.deliciosa cv Sweetgreen, A.chinesis cv Hort16A (Gold), A.chinesis cv and Sungold (Gold 3) grown in New Zealand. Findings from this study will help to evaluate the best among the five different varieties in terms of antioxidant capacity and phenolic composition and also helps horticulturists to develop new kiwifruit cultivars with specific traits to be marketed as commercial cultivars.

MATERIALS AND METHODS

PLANT MATERIALS

The fruits of A. delicosa cv Hayward (Green kiwifruit), A.deliciosa cv Sweet green, A. chinesis cv Hort 16A (Gold kiwifruit) and A. chinesis cv Sungold were donated by ZESPRI-New Zealand. The fruits of A. macroasperma reaching physiological maturity were collected at the Plant and Food research orchard in Te Puke Bay, New Zealand. The fruits with defects were discarded, and the remaining fruits were cut into small parts, freeze-dried and stored at -80 °C. The samples were prepared by grinding the lyophilized fruit samples in the mortar using a pestle prior to the extraction.

Chemicals

Folin-Ciocalteu phenol reagent, iron(III) chloride 6-hydrate, hydrochloric acid, ferulic acid, caffeic acid, chlorogenic acid, p-coumaric acid, syringic acid, catechin, epicatechin, rutin, quercetin-3-O-glucoside, quercetin, luteolin, 2,2-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), Trolox and p-dimethyaminocinna maldehyde (DMACA) were purchased from Sigma, St Louis, USA. Sodium carbonate, sodium hydroxide, sodium nitrite, formic acid, and aluminum hexahydrate, were from Scharlau, Spain. gallic acid (ACROS, USA), iron(II) sulfate 7-hydrate (BDH Chemicals, England), HPLC grade methanol, HPLC grade acetonitrile, ethanol, methanol, hexane, and all other chemicals were purchased from ECP Ltd, Auckland, New Zealand.

Preparation of extracts from different kiwifruit cultivars

Extraction was carried out according to the method described by steeping each lyophilized ground kiwifruit cultivar sample (5 g) in 70% aqueous acetone (100 mL) for 6 hours in the dark with nitrogen gas purging at room temperature (23±2 °C). Total phenolic content (TP), total flavonoid content (TFO), total flavanol content (TFA), radical scavenging activity and total antioxidant capacity of each extract were determined.

Determination of total phenolic content

The Folin-Ciocalteu assay was performed to estimate the total phenol content (TP) of defatted mixtures as described, and the results were expressed as mg gallic acid equivalents (GAE)/100 g DW of the fruit.

Determination of total flavonoid content

The aluminum chloride colorimetric method was performed, and the results were expressed as mg catechin equivalents (CAE)/100 g DW of the fruit.

Determination of total flavanol content

The p-dimethyaminocinna maldehyde (DMACA) method was performed, and the results were expressed as mg catechin equivalents (CAE)/100 g DW of the fruit.

Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was used to determine the electron-donating potential of the fruit extracts based on the assay described and the results were expressed in mmolFe (II) equivalents/100 g DW of the fruit.

Antioxidant capacity by DPPH assay

The antioxidant capacity of all extracts was determined using DPPH assay according to the method described, and the results were expressed in mmol Trolox equivalents/100 g DW of the fruit. The DPPH radical-scavenging activity (%) of each kiwifruit extract having serial concentrations (0, 1, 2, 3, 4, and 5 mg/mL) was assayed according to the method described. The radical-scavenging activity (%) was calculated by the following equation:

Scavenging activity (%) = \( \frac{1-(Ab \ sample-Ab \ control)}{Ab \ sample} \times 100\% \)

Ab sample is the absorbance of the solution with different concentrations of extract, and Ab control is the absorbance of the DPPH solution without sample extract. The scavenging activity was expressed as 50% effective concentration (EC50), which represented the concentration of the sample having 50% of DPPH radical scavenging effect.

High performance liquid chromatography coupled to diode array detection (HPLC-DAD) fingerprint analysis

The phenolic profiles in each defatted crude extract were determined according to the procedure described. Since there are a large number of different types of antioxidants and phenolic compounds that might contribute to the total antioxidant capacity, all kiwifruit extracts were analyzed on HPLC-DAD, and the chromatograms of all defatted extracts (50 mg/mL) (DW/volume) were recorded at four different wavelengths.
RESULTS AND DISCUSSION

The total phenolic contents of different kiwifruit cultivars were investigated. The results were expressed as mean±SD. The effect of the kiwifruit varieties tested on the TP, TFO, TFA, and AA values were analyzed by analysis of variance (ANOVA) using Originpro8 software. Pairwise multiple comparisons were evaluated by Tukey’s’s significance difference test used in Originpro8 software. Differences at $p=0.05$ were considered significant.

### Statistical analysis

All measurements were conducted in triplicates, and the results were expressed as mean±SD. The effect of the kiwifruit varieties tested on the TP, TFO, TFA, and AA values were analyzed by analysis of variance (ANOVA) using Originpro8 software. Pairwise multiple comparisons were evaluated by Tukey’s’s significance difference test used in Originpro8 software. Differences at $p=0.05$ were considered significant.

### RESULTS AND DISCUSSION

**Extract yield and total phenolic content**

The yields of the extracts obtained from different kiwifruit varieties varied from 16.0±1.1 to 58.4±1.4g/100g DW of the fruit. Sweet green kiwifruit had the highest extraction percentage yield followed by sungold, A.macrosperma, gold and green varieties. The yields from sweet green and sungold were not significantly different at $p=0.05$ level. Variation in the yields of the extracts obtained in this study could be resulted due to the polarity of various phytochemicals present in different kiwifruit cultivars tested. Similar observations are reported in the literature.[21-24]

This study showed that total phenolic contents were dependent on the variety of kiwifruit and ranged from 111±0.5 to 823±14.4 mg GAE/100 g DW of the fruit with a descending order of A.macrosperma>sungold>sweetgreen> gold > green (Figure 1). Interestingly, it is noticed that A.macrosperma kiwifruit extract had the highest amount of total phenols, which is significantly different at $p=0.05$ level. The TP of the extracts obtained from A. delicosa cv Hayward (Green kiwifruit) and A. chinensis cv Hort 16A (Gold kiwifruit) were significantly lower than the new commercial kiwifruits namely, sweet green and sungold. However, the total phenolic contents of different kiwifruit cultivars previously reported using Folin-Ciocalteu method was cultivar dependent.[25] Their results showed that TP of A. delicosa cv Hayward and A. macrosperma fruits were 129 mg GAE/100 g FW and 165 mg GAE/100 g FW of the fruit, respectively.[26] Research studies carried out by Du et al. in 2009 on the determination of total phenols extracted into ethanol: acetone (70:30) of different kiwifruits, showed that the TP values varied from 41.7 to 710 mg/100g fresh weight[17]. Bursal and Gulcin (2011) reported that the total phenolic content determined in water extract from A. delicosa (Green kiwifruit) in 1 mg of lyophilized kiwifruit extract is 16.7±2.83 µg GAE.[26] The total phenolic content determined for A. chinensis kiwifruit under slightly different conditions of Folin-Ciocalteu method showed 9.3±0.4 µg GAE/mL.[26] The observed differences in TP could be related to the result of different methods of extraction, solvents, and analysis used.

**Liquid chromatography coupled to mass spectrometry (UPLC-ESI-MS/MS) analysis**

Analyses of major individual phenolic compounds present in all extracts obtained were performed as described[29].

### Total flavonoid and total flavanol content

The total flavonoid contents of the extracts tested were significantly different at $p=0.05$ level and varied from 12.9±0.74 to 171± 1.9 mg CAE/100 g of DW of the fruit (Figure 2). Similar to the total phenolic content measured by the Folin-Ciocalteu method, A.macrosperma kiwifruit extract had the highest total flavonoid content, which was significantly different at $p=0.05$ level from the TFO values obtained from all commercial cultivars tested. Extracts obtained from green and gold kiwifruit exhibited the lowest. By comparing with the literature, the flavonoid content of A. delicosa cv Hayward (Green kiwifruit) was reported as 6.69±0.08 mg rutin/100 g fresh weight of the fruit among different kiwifruit cultivars tested using the same method.[17]. Bursal and Gulcin (2011) reported that the total flavonoid content detected in water extract from A. delicosa (Green kiwifruit) in 1 mg of lyophilized kiwifruit extract is 13.0±0.52 µg quercetin equivalent (QE) /100 g FW[26]. Research studies carried out by Bekhradnia et al., in 2011 reported that the flavonoid content of A. chinensis kiwifruit was 7.9±0.63 µg QE/ mL[21].

**Figure 1 Total phenolic content of different kiwifruit cultivars**

**Figure 2 Total flavonoid and total flavanol contents of different kiwifruit cultivars**

Total flavanol (TFA) content of the extracts tested was determined using the p-dimethylaminocinnamaldehyde (DMACA) method, which is based on the reaction between the reagent and flavanol compounds to form a blue complex that

wavelengths: 280 nm (flavan-3-ols, proanthocyanidins and benzoic acids), 320 nm (hydroxycinnamic acids), 360 nm (flavonols) and 520 nm (anthocyanidins). The UV spectrum was recorded between 200-550 nm. In addition, a mixture of the most abundant standard phenolic compounds (0.1 mg/mL each) namely gallic acid, ferulic acid, caffeic acid, chlorogenic acid, p-coumaric acid, syringic acid, catechin, epicatechin, rutin, quercetin-3-O-glucoside, quercetin, and luteolin which are common to many fruits was also separated under the same conditions.

**Liquid chromatography coupled to mass spectrometry (UPLC-ESI-MS/MS) analysis**

Analyses of major individual phenolic compounds present in all extracts obtained were performed as described[29].

### Statistical analysis

All measurements were conducted in triplicates, and the results were expressed as mean±SD. The effect of the kiwifruit varieties tested on the TP, TFO, TFA, and AA values were analyzed by analysis of variance (ANOVA) using Originpro8 software. Pairwise multiple comparisons were evaluated by Tukey’s’s significance difference test used in Originpro8 software. Differences at $p=0.05$ were considered significant.

**RESULTS AND DISCUSSION**

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This study showed that total phenolic contents were dependent on the variety of kiwifruit and ranged from 111±0.5 to 823±14.4 mg GAE/100 g DW of the fruit with a descending order of A.macrosperma>sungold>sweetgreen> gold > green (Figure 1). Interestingly, it is noticed that A.macrosperma kiwifruit extract had the highest amount of total phenols, which is significantly different at $p=0.05$ level. The TP of the extracts obtained from A. delicosa cv Hayward (Green kiwifruit) and A. chinensis cv Hort 16A (Gold kiwifruit) were significantly lower than the new commercial kiwifruits namely, sweet green and sungold. However, the total phenolic contents of different kiwifruit cultivars previously reported using Folin-Ciocalteu method was cultivar dependent[25]. Their results showed that TP of A. delicosa cv Hayward and A. macrosperma fruits were 129 mg GAE/100 g FW and 165 mg GAE/100 g FW of the fruit, respectively.[26] Research studies carried out by Du et al. in 2009 on the determination of total phenols extracted into ethanol: acetone (70:30) of different kiwifruits, showed that the TP values varied from 41.7 to 710 mg/100g fresh weight[17]. Bursal and Gulcin (2011) reported that the total phenolic content determined in water extract from A. delicosa (Green kiwifruit) in 1 mg of lyophilized kiwifruit extract is 16.7±2.83 µg GAE.[26] The total phenolic content determined for A. chinensis kiwifruit under slightly different conditions of Folin-Ciocalteu method showed 9.3±0.4 µg GAE/mL.[26] The observed differences in TP could be related to the result of different methods of extraction, solvents, and analysis used.
can be measured at 640 nm. The total flavanol contents of the extracts tested varied from 1.2±0.1 to 82.6±0.6 mg CAE/100 g DW of the fruit (Figure 2) with the ranking order of *A. macroasperma>*sungold>*sweet green>*gold>*green. The new commercial kiwifruits namely, sungold and sweet green were second and third to *A. macroasperma* while green and gold kiwifruit exhibited the lowest. Only a few studies on the total flavanol content of kiwifruits were reported in the literature. TFA value reported by Du et al. (2009) for *A. delicosa cv* Hayward (Green kiwifruit) originated in China was 4.34±0.25 mg CAE/100 g FW of the fruit[17].

Antioxidant capacity

Radical scavenging activity and antioxidant capacities of all *Actinidia* extracts selected for this study were evaluated with *in vitro* assays namely DPPH and FRAP assays respectively. The Antioxidant capacity values by FRAP assay, varied from 1.3±0.1 to 8.3±0.1 mmol Fe (II) equivalent/100 g DW of the fruit (Figure 3) which were significantly different at p=0.05 level with the order of *A. macroasperma>*sungold>*sweet green>*gold>*green. Radical scavenging activity measured by DPPH values varied from 0.6±0.1 to 5.1±0.1 mmol Trolox equivalent/100 g DW of the fruit with the order of *A. macroasperma>*sweet green>*sungold>*gold>*green (Figure 3).

In the DPPH and FRAP assays, the antioxidant capacity of *A. delicosa cv* Sweet green, and *A. chinensis cv* Sungold was stronger than those of other commercial varieties namely, *A. delicosa cv* Green and *A. chinensis cv* Hort 16 A. It is interesting to observe that *A. macroasperma* had the highest FRAP and DPPH values compared to commercial varieties tested.

![Figure 3](image-url) Antioxidant capacities assayed by FRAP and DPPH of different kiwifruit cultivars

The DPPH radical scavenging capacity of each defatted kiwifruit extract measured was dose-dependent in the concentration range used in this study (1-5 mgmL⁻¹) as presented in the Figure 4. The EC₅₀ values from DPPH assay for five different kiwifruit cultivars tested namely, *A. macroasperma, A. chinensis cv* sungold, *A. delicosa cv* sweetgreen, *A. chinensis cv* Hort 16 A and *A. delicosa cv* Hayward were 13.0, 14.8, 14.8, 17.5 and 41.0 mg/mL, respectively.

![Figure 4](image-url) Antiradical-scavenging activity (%) of 70% aqueous acetone extracts obtained from five different kiwifruit cultivars

There are few previous studies reported on the antioxidant capacity of *Actinidia* fruits in the literature. However, the antioxidant activity of different kiwifruit cultivars previously reported in the literature using DPPH assay was cultivar dependent [25]. Their results showed that DPPH values of *A. delicosa cv* Hayward and *A. macroasperma* fruits were 117 mg ascorbic acid equivalent (AAE)/100 g FW and 27.1 mg AAE/100 g FW of the fruit, respectively [25]. The results for the antioxidant activity of ethanol: acetone (70:30) extracts from different kiwifruit cultivars reported by Du and colleagues was cultivar and assay dependent [27]. Bursal and Gulcin (2011) showed that the antioxidant activity detected in water extract from *A. delicosa* (Green kiwifruit) was dependent on laboratory assays [26]. The reported antioxidant capacities of ‘Hayward’ and ‘Hort 16A’ kiwifruit consistently ranged from 6.0 to 9.2 and 12.1 µmol Trolox equivalent (TE)/g FW, when assayed by the ORAC method [14]. In a study of six *Actinidia* species, the higher levels of vitamin C, phenolic compounds and antioxidant activity measured by the DPPH assay, were found in *A. kolomikta cv* ‘Dr Szymanowski’ [25]. The observed differences in antioxidant activity could be related to the result of different methods of extraction, solvents, analysis and the origin of the kiwifruit used for the analysis.

Correlation between antioxidant capacity and phenolic composition

To explore the influence of the phytochemical constituents on antioxidant capacity in different kiwifruit extracts tested, the correlations were analyzed in the present study. The results obtained imply that the total phenol, flavonoid, flavanol, contents, and antioxidant capacity obtained from different kiwifruit extracts tested were strongly correlated with each other. Therefore, our research study reveals that high phenolic, flavonoid, and flavanol contents are important factors in determining the antioxidant activities of kiwifruits studied. This observation was well supported by the research study carried out by Park et al. (2011) on a comparison of bioactive compounds and antioxidants in different kiwifruit cultivars grown in South Korea[27]. A strong relationships between phenolic composition and antioxidant activity of different kiwifruit cultivars were identified by Du et al. in 2009[17]. Our data are in agreement with other former reports that showed the
antioxidant activity of fruits and vegetables significantly increases with the presence of a high concentration of total phenol content [28,29]. The antioxidant activities obtained from FRAP and DPPH assay were well correlated (R² = 0.942), which implied that antioxidants in these fruit extracts were capable of scavenging free radicals (DPPH) and reducing oxidants (ferric ions). Furthermore, it is reported in the literature that phenolic acids and flavonoid compounds are the main phytochemicals responsible for the antioxidant capacity of fruits and radical scavenging capacity and might be mostly related to their concentration of phenolic hydroxyl group, the molecular structure, the availability of phenolic hydrogens and the possibility for stabilization of the resulting phenoxyl radicals formed by hydrogen donation[30].

**High-performance liquid chromatography coupled to diode array spectrometry (HPLC-DAD) fingerprint analysis**

By comparing the retention times of the standard compounds and the absorption spectra of the peaks with that of each component in the defatted extracts of kiwifruits, it could be preliminarily concluded that there was correspondence to some of standard compounds in the extracts analyzed. Gallic acid, caffeic acid, chlorogenic acid, p-coumaric acid, and ferulic acid were detected in the defatted extract of Actinidia macrosperma kiwifruit. HPLC-DAD profile of Actinidia macrosperma cultivar showed unidentified peaks (from the retention time 15-34 minutes on the chromatogram) representing high peak areas that might contribute to the antioxidant activity. Gallic acid and catechin were identified in sweet green cultivar while catechin, caffeic acid, and chlorogenic acid were identified in sungold cultivar. Several peaks detected at 360 nm of the defatted extracts from Actinidia chinensis cv Sungold and Actinidia deliciosa cv Sweet green kiwifruits were unidentified flavonoids aswll. There was no correspondence to any of the standard phenolics detected in Actinidia chinensis cv Hort 16A (Gold) while a very weak correspondence to the catechin in Actinidia deliciosa cv Hayward (Green) kiwifruits at 50 mg/mL (DW/volume) concentration. This observation further supported and confirmed the presence of poor antioxidant capacity, and the phenolic profiles found in gold and green kiwifruits in this study could be due to the lack of phenolics in them. The crude extracts analyzed on analytical HPLC-DAD without further purification allowed us to identify only a few phenolic compounds with the aid of the spectrum at 280, 320, and 360 nm due to their co-eluted and superimposed peaks of the chromatogram obtained from Actinidia macrosperma kiwifruit. This observation was well supported by the recent studies reported by Latocha et al. (2010) showed that the fruit of Actinidia macrosperma contains phenolic acids such as tannic acid, gentisic acid, hydroxy benzoic acid, chlorogenic acid, p-coumaric acid, and caffeic acid[25]. However, only a few flavonoids, catechin, epicatechin, and quercetin were identified in Actinidia macrosperma fruit due to the complications raised[25]. The HPLC-DAD analysis further revealed that the extract obtained from Actinidia macrosperma kiwifruit is rich in mainly flavonoids other than kiwifruit varieties tested.

**Liquid chromatography coupled to mass spectrometry (LC-ESI-MS/MS) analysis**

The sub-class, flavonol was identified as the most abundant group of flavonoids detected by liquid chromatography coupled to mass spectrometry (UPLC-ESI-MS/MS) analysis in tested kiwifruit cultivars. Quercetin, quercetin-3-O-galactoside, quercetin-3-O-rhamnoside, quercetin-3-O-rutinoside, quercetin-arabinogalactoside, catechin, epigallocatechin gallate, epigallocatechin, chlorogenic, ferulic, isoeufural and caffeic acids were found in Actinidia macrosperma fruit.

**CONCLUSION**

In conclusion, the investigation of five different types of kiwifruits demonstrated that the antioxidant activities and the phenolic composition showed great variation among Actinidia varieties and high correlation among them. To the best of our knowledge, this is the first study that investigated the antioxidant capacity and the phenolic profiles of Actinidia deliciosa cv Sweet green, and Actinidia chinensis cv Sungold kiwifruits. This study shows that Actinidia macrosperma is an excellent source of potential phenolic antioxidants compared to commercially available kiwifruits cultivars. Thus, it will be of interest to isolate and characterize these potent antioxidants in future studies.

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**References**


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