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In vitro antioxidant activities and phenolic profiles of different solvent extracts of a wild kiwifruit (*Actinidia macrosperma)*

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Abstract
The aim of this study was to study the effect of different solvents on extraction of polyphenols and characterization of antioxidant capacity *in vitro* of a wild kiwi (*Actinidia macrosperma*) fruit. The total phenolic, flavonoid, flavonol contents were determined by Folin-Ciocalteu, aluminiumchloride colorimetric, and *ρ*-dimethylaminocinnamaldehyde (DMACA) methods, respectively. The radical scavenging activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and antioxidant activity was determined by ferric reducing power activity (FRAP) assay. Polyphenols of the extracts were quantified using high performance liquid chromatography coupled to diode array detector (HPLC-DAD) and identification of the compounds was validated by HPLC-electrospray ionization coupled with mass spectrometry (HPLC-ESI-MS/MS). Among the extraction solvents tested, 70% aqueous acetone showed the highest values of total phenolic (823±14 mg GAE/100 g DW), total flavonoid (171±2 mg CAE/100 g DW), total flavanol (83±0.6 mg CAE/100 g DW) contents and the radical scavenging activity (5.1±0.1 mmolTrolox equivalent/100 g DW) and antioxidant capacity (8.3±0.1 mmol Fe (II) equivalent/100 g DW) by DPPH and FRAP assays, respectively. Analysis of each extract on LC-DAD and LC-ESI-MS/MS revealed the presence of many flavonoids such as quercetin-3-*O*-galactoside (most abundant), quercetin-3-*O*-glucoside, and quercetin-3-*O*-arabinoglucoside in 70% aqueous acetone extract obtained. Therefore, 70% aqueous acetone can be suggested as the best extraction solvent to extract polyphenols of *A. macrosperma* fruit.

Keywords: antioxidants, extracts, flavonoids, kiwifruit, polyphenols

1. Introduction
Antioxidants play a vital role in foods as well as in the human body to reduce oxidative processes and harmful effects of reactive oxygen species (ROS) [1, 2]. In biological systems during respiration, a significant fraction of oxygen is incompletely reduced, leading to ROS, which includes free radicals (superoxide anion, alkoyl, hydroxyl, and peroxyl) and non-radical derivatives of oxygen (singlet oxygen and hydrogen peroxide). ROS can cause functional damage to biological systems due to the unbalance between ROS and antioxidants, triggering a number of degenerative processes such as carcinogenesis, mutagenesis, and aging [2, 3].

Polyphenol-based natural antioxidants have recently attracted considerable to reduce the risk of oxidative stress-mediated diseases such as certain cancers [2, 4]. Many different plant sources have recently become of great interest to scientific research as a result of their naturally occurring antioxidants with lesser side effects. As safe sources of phenolic antioxidants, edible fruits have been investigated for their antioxidant properties, for example, pomelo (*Citrus grandis* (L) beck) [5], sour cherries (*Prunus cerasus* L.) [6], berries [7], mango (*Mangifera indica*, L.) [8], bitter gourd (*Memordica charantia* L.) [9], snake fruit [10], and kiwifruits [11, 12, 13, 14, 15].

Actinidia macrosperma L. belongs to the family *Actinidiaceae* is a non-commercial fruit with orange-colored flesh, small size fruit with large seeds, and relatively thick, hairless skin. This plant is popularly called 'cat ginseng' due to its attractant effect on cats by giving off a specific odor, and then cats preferred to eat fresh leaves of the plant or twigs to excite themselves and cure wounds [13]. This plant has a reputation for treating various diseases in Chinese traditional medicine [13, 16, 17]. Recent studies have shown that the different parts of the *A. macrosperma* plant exhibit various biological activities including angiotensin converting enzyme (ACE) inhibitory activity [2], immunomodulatory [16], antioxidant, antibacterial and antifungal activities [13, 16, 18]. There is a great interest in the evaluation of the chemical composition and biological activities of *A. macrosperma* plant attributed by bioactive compounds and their high potential as a source for nutraceuticals and functional foods [2, 19]. A number of bioactive constituents extracted from stem and leaves of *A. macrosperma* have been reported, including polysaccharides, alkaloids, saponinns, and organic acids [13]. Recent studies have reported that the fruit of *A. macrosperma* includes compounds such as lutein, β-carotenes, zeaxanthin, violaxanthin, chlorophyll a and b, catechin, epicatechin, quercetin, tannic acid, gentisic acid, hydroxybenzoic acid, chlorogenic acid, *p*-coumaric acid, and caffeic acid [20]. However, phenolic compounds, mainly flavonoid glycosides present in this fruit have not been fully characterized. Therefore, the extraction of antioxidants from *A. macrosperma* can be considered to contribute to the value of these fruits.

Extraction is one of the most important steps in the isolation, identification, and quantification of phenolic compounds from plant materials. The efficacy of the extraction is affected by several factors such as the extraction method employed, type of solvent, the chemical nature of the compounds (simple and complex phenolics),

the storage or extraction time and the conditions (pH, temperature) [21]. Among the above-mentioned factors, the polarity of the solvent plays an important role in the selective extraction of different flavonoids [22]. Therefore, the objective of this research work was to study the effect of extraction solvents on biological active compounds of *A. macrosperma* fruit by evaluating their polyphenol profiles by HPLC-UV-DAD and HPLC-ESI-MS/MS as well as determining their antioxidant capacity.

2. Materials and methods

2.1. Plant materials

The fruits of *A. macrosperma* reaching physiological maturity were collected at the Plant and Food Research Institution's orchard in Te Puke Bay, New Zealand. Defective fruits 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 using a pestle and mortar prior to the extraction.

2.2. 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, myricetin, luteolin, 2,2-diphenyl-2-picylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), Trolox and *p*-dimethylaminocinnamaldehyde (DMACA) were purchased from Sigma, St Louis, USA. Sodium carbonate, sodium hydroxide, sodium nitrite, formic acid, and aluminium 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. HPLC grade methanol used for LC-ESI-MS/MS analysis was purchased from Sigma Aldrich, Oakville, ON, Canada.

2.3. Preparation of extracts from *A. macrosperma* fruit

Five different solvent systems, namely 70% acetone, 80% methanol, 80% ethanol, 100% methanol, and 100% water were selected based on their wide use in the extraction of phenolics from plant material [21]. Extraction was carried out by steeping each lyophilized ground kiwifruit sample (3 g) in each solvent (50 mL) in a Scott Duran bottle for 6 h in the dark with nitrogen gas purging at room temperature (23±2 °C) to prevent phenolic oxidation during extraction. The extracts were filtered through a sintered glass filter and collected into a conical flask in an ice bath. The residue was subjected to re-extraction, and the filtrates were combined and concentrated on a rotary evaporator (Buchi, Switzerland) below 35 °C under vacuum.

Defatted crude extracts were prepared by partitioning the crude extracts with hexane in a separating funnel and subjected to freeze-drying and stored at -80 °C. Total phenolic (TP), total flavonoid (TFO), total flavanol (TFA) contents and antioxidant activity (AA) of each extract were determined to select the extraction solvent.

2.4. Determination of total phenolic, flavonoid and flavanol contents

The Folin-Ciocalteu assay, aluminum chloride colorimetric method, and *p*-dimethylaminocinnamaldehyde (DMACA) method were performed to estimate the total phenol (TP), total flavonoid (TF), and total flavonol (TFO) contents of defatted crude mixtures respectively as described [2].

2.5. Determination of ferric-reducing antioxidant power activity (FRAP assay)

The FRAP assay was used to determine the electron donating potential of the fruit extracts based on the assay described [2] and the results were expressed in mmol Fe(II)equivalents/100 g DW of the fruit.

2.6. Determination of antioxidant capacity (DPPH assay)

The antioxidant capacity of all extracts was determined using the DPPH assay according to the method described [2] and the results were expressed in mmolTrolox equivalents/100 g DW of the fruit.

2.7. High performance liquid chromatography coupled to diode array detection (HPLC-DAD)

The phenolic profiles in each defatted crude extract were determined according to the procedure described [2].

2.8. Liquid chromatography coupled to mass spectrometry (HPLC-ESI-MS/MS) analysis

Analyses of major individual phenolic compounds present in all extracts obtained from *A. macrosperma* fruit were performed as described [2].

2.9. Statistical analysis

All experimental measurements were conducted in triplicate and the results are expressed as mean±SD. The effects of the extraction solvent, and technique tested on the total phenol content, total flavonoid content, total flavanol content and antioxidant capacity values were analyzed by analysis of variance (ANOVA) using OriginPro8 software. Pairwise multiple comparisons were evaluated using Tukeyss' significance difference test used in Originpro8. Differences at $p = 0.05$ were considered significant.

3. Results and discussion

3.1. Extract yields

The yields of different solvent extracts obtained from *A. macrosperma* fruit varied from 39.3±1.3 to 51.3±1.5 g/100 g DW of the fruit (Table 1.). The yields from 80% methanol and 80% ethanol were not significantly different ($p = 0.05$) according to the Tukey test. It is reported that water and aqueous mixtures of ethanol, methanol, ethyl acetate, and acetone have been commonly used for extracting phenolics and influence the yields of phenolics extracted [21, 22, 23,24]. Variation in the yields of various solvent extracts obtained in this study could be attributed to differences in the polarity of compounds present, and these observations are in agreement with former studies reported in the literature [21, 22, 23, 24]. The use of only water as a solvent may yield an extract with a high content of impurities such as organic acids, sugars, and soluble proteins, which could interfere in the phenolic identification and quantification. However, it is reported that the use of water in combination with other organic solvents contributes to the creation of a moderately polar medium that ensures the extraction and solubility of polyphenols [21]. Our research findings are in accordance with a previous study reporting that solvents with high polarity, such as water, or very low solvent strength, such as chloroform or hexane, do not give good extraction results

for extracting phenolic compounds from *A.macrosperma* stems [13], mashua tubers [21], and pine sawdust [22].

Table 1: Effect of extraction solvent on percentage of Yield, TP, TFO, and TFA values for *A. macorsperma* fruit.

Solvent	Yield (%) a	TP[b]	TFO[c]	TFA[d]
70% aq. acetone	42.8±0.8[p]	823.1±14.4[p]	170.9±1.9[p]	82.6±0.6[p]
80% aq. methanol	39.9±1.0[q]	360.3±24.4[q]	61.9±5.0[q]	71.0±1.0[q]
80% aq. ethanol	39.3±1.3[q]	430.0±4.9[r]	75.8±0.8[r]	55.3±0.9[r]
100% methanol	46.6±1.9[r]	321.6±18.7[q]	96.3±0.6[s]	52.6±0.9[s]
100% water	51.3±1.5[s]	464.1±31.1[r]	14.2±6.0[t]	53.1±1.1[r,s]

[a] *Extraction yield is expressed as percentage g/100 g DW*
[b] *Total phenolic content is expressed as mg GAE/100 g DW*
[c] *Total flavonoid content is expressed as mg CAE/100 g DW*
[d] *Total flavanol content is expressed as mg CAE/100 g DW*
Results are expressed as mean±standard error. Means followed by the same letter in a column are not significantly different at p= 0.05

3.2. Total phenolic content

This study revealed that different solvent extracts from *A. macrosperma* fruit had total phenolic contents ranging from 322±18.7 to 823±14.4 mg GAE/100 g DW of the fruit (Table 1.). It is interesting to notice that 70 % aq. acetone extract had the highest (823±14.4 mg GAE/100 g DW) amount of total phenols, which is significantly different (p= 0.05) from other different extraction media tested. Research studies carried out by Xu and Chang reported that 70% acetone was the best solvent for extracting phenolics from lentils, black soybean, and common beans, while 50% acetone was best for yellow pea, green pea, and chickpea [25]. Research studies carried out showed that total phenolic content varied according to the solvent employed for extracting phenolics [21, 22, 23,24,26]. Furthermore, there was no significant difference in total phenolic content found between 100% methanol extract, and 80% methanol extract as well as between 80% ethanol and water extracts (Table 1.). It has been reported by several research studies that total phenolic content increases with the methanol content in the solvent mixture upto 80% but started decreasing after that [21, 22, 23, 24].

3.3. Total flavonoid content

This study showed that the total flavonoid contents of the solvent extracts tested were significantly different (p= 0.05) from each other and varied from 14.2±6.0 (100% water extract) to 171±1.9 mg CAE/100 g of DW of the fruit (70% aq. acetone extract) with the descending order of 70% acetone > 100% methanol > 80% aq. ethanol > 80% aq. methanol > water**.** Interestingly, it is noticed that 70% acetone extract had the highest, and this observation was well supported by the research carried out by Xu and Chang (2007), who revealed that 70% acetone extract obtained from peas had the highest total flavonoid content (TFO). The total flavonoid content of the water extract was significantly lowest, although it showed the highest yield of the dry weight and moderate total phenolic content. This is in agreement with the published literature. The total flavonoid content extracted from *Teucrium montamum* (medicinal plant) was high in acetone and methanolic extracts, while the lowest level was observed in water extract [27]. Our data are in agreement with other studies reporting flavonoid concentration in various solvent extracts from different plant parts such as potato peel, sugar beet pulp, sesame cake, mashua and pistachio hull [22, 23, 24].

3.4. Total flavanol content

The total flavanol contents varied from 52.6±0.9 to 82.6±0.6 mg CAE/100 g of DW of the fruit with the ranking order of 70% aq. acetone > 80% aq. methanol > 80% aq. ethanol > water > 100% methanol. It is interesting to notice that similarly to the total phenolic and flavonoid contents, 70% aq. acetone extract had the highest flavanol content (82.6±0.6 mg CAE/100 g of DW of the fruit) which was significantly different (p= 0.05) from the total flavanol contents (TFA) obtained from all other solvent extracts tested. The extract obtained from 100% methanol exhibited the lowest but not significantly different from that of 100% water extract.

There are no previous studies on the total flavanoid and total flavanol contents of *A. macrosperma* fruit reported in the literature for the comparison of our data obtained in this study. This is the first report on TFO and TFA of *A. macrosperma* fruit. However, similar as with the total phenolic and total flavonoid content, 70% aq. acetone extract from *A. macrosperma* fruit had the highest total flavanol content.

3.5. *In vitro* antioxidant capacity

This study showed that the FRAP values of the solvent extracts tested were significantly different (p= 0.05) and varied from 3.1±0.1 mmol Fe(II) equivalents/100 g DW (100% methanol extract) to 8.3±0.1 mmol Fe(II) equivalents/100 g DW of the fruit (70% acetone extract) with the descending order of 70% acetone > 80% methanol > water > 80% ethanol > 100% methanol (Table 1.). Antioxidant capacity measured by DPPH values for the different extraction solvents varied from 2.0±0.1 to 5.1±0.1 mmolTrolox equivalents/100 g DW of the fruit with the order of 70% acetone > 80% methanol > water > 80% ethanol = 100% methanol (Table1.). Interestingly, it is noticed that 70% acetone extract exhibited significantly highest antioxidant capacity at p= 0.05 by both of assays performed in this study. There are a few previous studies reported on the antioxidant capacity of *Actinidia* fruits but only one report on *A. macrosperma* fruit. The antioxidant activity previously reported of *A. macrosperma* fruit assessed by DPPH assay was 27.1 mg Ascorbic acid equivalent (AAE)/100 g DW fresh weight of the fruit [19]. 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.

3.6. HPLC-DAD and HPLC-ESI-MS/MS analysis of the extracts obtained from *A. macrosperma* fruit

The five different solvent extracts showed approximately a similar polyphenol profile but with differences in the concentration of various compounds extracted. HPLC-ESI-MS/MS analysis further confirmed that all extracts obtained from *A. macrosperma* fruit are rich in mainly flavonoids such as quercetin, and its glycosides namely, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-arabinoglucoside and phenolic acids namely, chlorogenic

acid and caffeic acid were identified in all extracts obtained from *A. macrosperma* fruit. Recent studies have reported that the fruit of *A. macrosperma* contains lutein, β-carotenes, zeaxanthin, violaxanthin, chlorophyll a and b, catechin, epicatechin, quercetin, tannic acid, gentisic acid, hydroxy benzoic acid, chlorogenic acid, *p*-coumaric acid and caffeic acid[19]. Quercetin-3-*O*-galactoside was identified as the most abundant among other phenolic compounds tested in all extracts in this study.

4. Conclusion

In this research work, it was aimed to study the effect of different solvents on extraction of polyphenol profiles and antioxidant capacity *in vitro* of a wild kiwi (*Actinidia macrosperma*) fruit. Phenolics were extracted from *A.macrosperma* fruit into different extraction solvents (70% acetone, 80% methanol, 80% ethanol, 100% methanol and 100% water) followed by evaluating yield, total phenolic, total flavonoid, total flavanol contents, radical scavenging activity, and antioxidant capacity. After the quantification of polyphenol profiles and antioxidant capacity in each solvent extract, 70% acetone was chosen as the best solvent for the extraction in this study. Among the solvents employed, 70% acetone of different extraction solvents on the antioxidant activities of biologically active compounds which were resulted in the highest values of total phenolic, total flavonoid, total flavanol contents, and antioxidant capacity. Analysis of each extract by HPLC-DAD and HPLC-ESI-MS/MS revealed that *A. macrosperma* fruit contains many flavonoids while quercetin-3-*O*-galactoside was the most abundant among them.

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