**Effects of ionic liquids and pulsed electric fields on the extraction of antioxidants from green asparagus roots**

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**Abstract**

*Asparagus officinalis* root (AR) contains valuable bioactive compounds that have beneficial health properties. The aim of this study was to optimize the extraction of polyphenols and flavonoids in green AR using two novel technologies; pulsed electric field (PEF) and ionic liquids (IL). This allowed the antioxidant activity of the obtained extracts to be reported. The total polyphenol content (TPC) , total flavonoid content (TFC), and antioxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH), Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing/Antioxidant Power (FRAP) assays) were determined. The PEF conditions (PEF strength of 1.6 kV/cm, frequency of 200 Hz and pulse width of 20 μs) resulted in a higher extraction yield as compared to conventional solvent extraction, but had lower antioxidant activities. The optimal conditions for IL extraction were by using 0.5% 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) at a solid: liquid (S:L) ratio of 1:10 for 4 minutes. The IL extraction resulted in a total of 120 mg RE/ mL TFC which was 70 to 80 folds more than the TFC obtained by PEF. Therefore IL extracts were characterised by a higher TFC and antioxidant activity than PEF.

Keywords: New Zealand asparagus roots; polyphenols; flavonoids; ionic liquids; pulsed electric field

## 1 Introduction

Asparagus (*Asparagus officinalis*) belongs to the *Asparagaceae* genus that is believed to be originated from the Eastern Mediterranean region and Asia (Aleventosa & Rojas, 2015). It is a versatile plant with a unique flavour, allowing it to be used in many dishes. Asparagus grows in a range of different soil types, and the plant has a range of health benefits such as improving the immune system and preventing cancer (Guo et al., 2020,Meng et al., 2021, Ghadimi et al., 2021). Under optimum climate conditions, asparagus can grow 1 cm/hour and are picked when they are at least 19 cm but less than 28 cm (Siomos, 2018). To re-plant asparagus, one-year-old crowns are established in soils with a pH range of 6.5-7.5. Old asparagus roots are considered as a waste and are commonly left to rot in fields; however, they are a potential source of bioactive compounds such as saponins, polyphenols, and flavonoids (Symes et al., 2018, Zhang et al., 2019). These bioactive compounds could be extracted from the roots, hence utilizing the waste and adding value to the production process. The roots also possess allelopathic properties which can prevent other asparagus plants from growing. Therefore, farmers could improve the re-growth of new asparagus by removing the allelopathic potential, and increase their income by selling the roots, allowing the recipients of the bioactive compounds to have access to a natural product with a wide range of health benefits. The extraction of bioactive compounds from asparagus has been predominately focused on the spears and to a lesser extent, the roots. In literature, different solvent extraction methods (water, ethanol and methanol) have been used to extract bioactive compounds from asparagus (Fuentes-Alventosa et al., 2009, Fuentes-Alventosa et al., 2013, Fan et al., 2015). Kobus-Cisowska et al. (2019) investigated the polyphenol content of asparagus spears by boiling them in water at 95$^{o}$C for 20 minutes. The DPPH activity was dependent on the variety and colour of asparagus, with the greatest activity shown in green asparagus. Solvent-based methods are notorious for not being environmentally friendly and more sustainable methods are urgently needed. Pulsed electric fields (PEF) or ionic liquid (IL) extraction methods are gaining much interest due to their compatibility with green chemistry extraction methods and sustainable food production systems (Ranjha et al., 2021, Khoo et al., 2021, Kumar et al., 2021). Currently, there is no information concerning the extraction of antioxidant compounds from asparagus roots using these technologies. Therefore, the objective of this research was to investigate the use of PEF and IL ([BMIM]$BF_4$, [BMIM]Cl and [BMIM]Br) extraction methods to determine the phenolic compounds and antioxidant activity of *A. officinalis* root extracts.

## 2 Materials and methods

### 2.1 Chemicals

Sodium carbonate, acetonitrile, trifluoroacetic acid and sodium acetate obtained from Fisher Scientific (Waltham, Massachusetts, United States). The 1-buyyl-3-methylimidazolium tetrafluroborate ([BMIN]$BF_4$), 1-butyl-3-methylimidazolium chloride ([BMIN]Cl), 1-butyl-3-methylimidazolium bromide ([BMIM]Br), gallic acid, sodium nitrite, rutin, 2,2-dihenyl-1-picrylhydrazyl (DPPH), fluorescein sodium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), caffeic acid, saponin and ferrous sulphate were obtained from Sigma-Aldrich (St. Louis, Missouri, United States). Folin-Ciocalteu reagent and di-sodium phosphate decahydrate were purchased from Merck Millipore Corporation (Billerica, Massachusetts, United States). Aluminium chloride was from Loba Chemie (Mumbai, Maharashtra, India). Sodium hydroxide and glacial acetic acid were from VWR International (VWR International, Randor, Pennsylvania, United States), sodium dihydrogen ortophosphate monohydrateand ferric chloride were from BDH Chemicals LTD (Poole, United Kingdom), and 2,2'-azobis-2-methyl-propanimidamide dichloride (AAPH) was from the Cayman Chemical Company (Ann Arbor, Michigan, United States). All chemicals used were of analytical grade.

### 2.2 Preparation of Asparagus Roots

New Zealand green *A. officinalis* (AR) roots were obtained from a commercial farm near Dunedin (Palmerston, New Zealand, 0.3523◦ S, 175.6082◦ E) from a 15-year-old crop. The AR samples were prepared as described previously (Symes et al., 2018) and dried powder samples from different crop plots (n = 3) were sieved through a 40 mm mesh and used in the experiments.

### 2.3 Extraction of Antioxidant Compounds

#### *2.3.1 Extraction using Pulsed Electric Field (PEF) assisted extraction method*

The study design was a two factor (PEF strength "E" and frequency) rotatable and orthogonally blocked central composite design and the experiment was conducted as described by Teh et al. (2014). The AR sample (10 g) was added to 200 mL of an aqueous 10%v/v ethanol for 10 minutes before the extraction with the PEF system, with samples kept on ice in the dark to allow for sample resolvatation. Then the samples were treated with PEF (ELCRACK®, German Institute of Food Technology, Quakenbrück, Germany), with various frequency and E

parameters (Table 1) according to the design and order generated by Minitab Statistical Software (Version 16). The treatment was carried out in a chamber with an electrode gap of 80 mm where treatment fixed parameters were the pulse width (20 µs) and the energy (10 kJ). The conductivity and temperature of the samples were recorded before and after the PEF treatment. All samples had a total treatment time of 60 seconds. A heat-control, which was not subjected to the PEF treatment but subjected to the highest heat temperature that occurred during PEF treatment, was run in parallel to examine if the heat generated during the treatment had an influence on the extraction of antioxidant compounds. This control sample was placed in a water bath (Biolab, Ontairo, Canada) at the highest temperature recorded from the PEF treatment (32°C), for two minutes to determine the effect of heat on the measured activities. The samples were cooled on ice immediately after treatment, centrifuged (3500 *xg*, 4°C), and the supernatants were frozen at -80$^{o}$C prior to being freeze dried. The dried extracts were weighed to determine the extract yield, vacuum packed and stored in the dark at room temperature until analysis.

### *2.3.2 Extraction using ionic liquids extraction system*

To determine the optimum IL extraction conditions, four different parameters were examined: the type of ionic liquid ([BMIM]$BF_4$, [BMIM]Cl, and [BMIM]Br); extraction time (2 and 4 minutes), ionic liquid concentration (1 and 0.5 M), and the solid: liquid (S:L) ratio (1:10 and 1:20). This was carried out using a shaking incubator at 70°C and 200 RPM based on preliminary trials. Accurately weighed samples of AR powder (1g) were mixed with either 10 mL or 20 mL of each of the IL assayed, and were subjected to shaking using a shaking incubator for the combination of investigated parameters. The samples were then centrifuged at 10,000 rpm for 10 minutes and then stored in the -80°C freezer until further analysis.

## 2.4 Analysis of Asparagus root extracts

The AR samples were analysed for their polyphenol content, flavonoid content and antioxidant activities. The samples were prepared at a concentration of 1 mg/mL in 50% aqueous methanol. These samples were prepared daily and kept on ice in the dark. All analyses were carried out in triplicate.

### *2.4.1 Total Polyphenol Content*

The total polyphenol content (TPC) of the samples was determined using the Folin-Ciocalteu colorimetric method and an Epoch spectrophotometer plate reader (Biotek, Winooski, United

States) as described by Farasat et al. (2014) using a 96-well microplate. A 20 µl sample of the extract was mixed with 100 µl of Folin-Ciocalteu reagent (diluted 1 to 10) and 80 µl of sodium carbonate (7,5%, m/v). The plate was incubated at room temperature in the dark for 30 minutes. Then, the absorbance of the mixture was read at 600 nm using the Epoch spectrophotometer. The TPC was determined as Gallic Acid Equivalents per milligram of extract (mg GAE/ g extract) using a Gallic acid standard curve (0-0.15 mg/mL).

### *2.4.2 Total Flavonoid Content*

The aluminium chloride colorimetric method of Herald et al. (2012) was used to determine the total flavonoid content (TFC) of the samples using a plate reader and a 96-well microplate. A mixture of 100 µl of distilled water, 10 µl of sodium nitrite (50 g/L) and 25 µl of the extract solution was pipetted in each well. The mixture was incubated in the dark for 5 minutes at room temperature. Then, 15 µl of aluminium chloride (100 g/L) was added to the mixture that was incubated for 6 minutes under the same conditions. Subsequently, 50 µl of sodium hydroxide (1 M) and 50 µl of distilled water was added to each well. The absorbance of the mixture was measured at 510 nm using the Epoch spectrophotometer and the TFC was determined as Rutin Equivalent per milligram of extract (mg RE/g extract) using a standard curve (1-0.05 mg/mL) constructed using a reference Rutin.

### *2.4.3 Antioxidant Activities*

The antioxidant activity of AR extracts were determined using DPPH radical scavenging activity, oxygen radical absorbance capacity (ORAC), and Ferric reducing ability power (FRAP) assays as described previously by Aid et al., (2015).

## 2.5 Statistical Analysis

The statistical analysis was run on Minitab Statistical Software (Version 16). The measured parameters investigated were the TPC, TFC, and the antioxidant activities (DPPH, ORAC and FRAP) of the obtained extracts.

For the PEF extraction method, a rotatable orthogonal central composite design with two factors (frequency and voltage) that consisted of 4 cube points, 3 centre points, 4 axial points and 3 centre points in axial with Alpha = 1.41421 was carried out to establish a model for the observed response using the Minitab Statistical Software. A full quadratic equation as described below was used for this experiment.

$$Y = \beta_o + \sum_{i=l}^{k} \beta_i X_i + \sum_{i=l}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j-i+1}^{k} \beta_{ij} X_i X_j$$

Where $Y$ was the predicted response, $\beta_o$, $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ were the coefficients of the intercept, linear, square and interaction terms, respectively. The $X_i$ and $X_j$ were the independent variables, and $k$ was the number of variables in the design (Teh et al., 2014).

For the ionic liquid extraction, a Multivariate Analysis of Variance (MANOVA) was used to investigate the effects of the type of IL, the concentration of IL, extraction time and the solid: liquid ratio and their interactions on the measured parameters. A post hoc Tukey test was carried out and the means were considered statistically different at $P < 0.05$. A Pearson's Correlation was also carried out to determine the correlation between the TPC, TFC and antioxidant activity measured (DPPH, ORAC or FRAP). One way analysis of variance was used to determine the effect of the joule heating (heated control vs run 11) and the means were compared at $P < 0.05$.

## 3 Results & Discussion

### 3.1 PEF Extraction Method

As there is no information regarding the potential to use PEF to extract compounds from the AR, the results were compared to literature where similar materials or antioxidant compounds were of interest. Teh et al. (Teh et al., 2014) investigated the effect of PEF treatment on TPC, TFC and antioxidant activity of hemp seed cake. The experiment design included four variables: the concentration of the extraction solvent, extraction time, PEF strength as indicated by the voltage used and PEF frequency. The authors concluded that optimal PEF conditions were 7.5 kV (equivalent to 0.94 kV/cm), 30 Hz, treatment time of 10 seconds and 10% ethanol as extraction solvent. The 10% ethanol was the maximum safe concentration to be used in PEF treatment since higher concentrations lead to an increased conductivity, causing a significant increase flashover or "electrical arcing" (Teh et al., 2014). However, small scale bench PEF systems appear to be able to use higher ethanol concentrations (Pappas et al., 2021). The highest temperature reached during the PEF treatment was 32°C (in experimental run 11), thus the heated-control was also 32°C under conventional heating to determine the effect of temperature on the extraction of antioxidant compounds. The TFC, FRAP and ORAC activities of run 11 and the heated control samples were not different ($P > 0.05$). However, higher yield, TPC and

DPPH activity were found in the run 11 samples compared to the heated-control ones ($P < 0.05$). These results suggested that PEF treatment improved the extraction process more than the mild heat caused by the joule effect.

### *3.1.1 PEF-Assisted Extraction Yield*

The yields obtained from the PEF treatments are shown in Table 1 and Figure 1A. There were no significant effects for the investigated parameters (Table S1). The lowest arithmetic yield (43.5%) was obtained by 1.1 kV/cm and 19 Hz, and by 1.6 kV/cm and 50 Hz treatments. PEF frequency appears to be an important factor for successful extraction of antioxidant compounds, however, the impact of frequency on the permeabilization of biological materials appear to vary depending on the material under investigation. For example, Asavasanti et al. (2011) found low frequency was ideal to increase permeabilization of onion tissue, but low frequency was not effective in extracting oil from sunflower seed (Shorstkii et al., 2017). The highest arithmetic yield (58.8 %) was achieved with 1.6 kV/cm and 200 Hz PEF treatment. This combination of a relatively high E and frequency appeared to facilitated extraction of compounds from the lyophilised roots. The yield from the treatments was not different from that obtained from the heated control. While differences in the cell structure and the material physiological properties (e.g., pH and conductivity) can affect the efficacy of antioxidant compounds extraction by PEF technology (Ranjha et al., 2021), the concentration of solvent used in PEF treatment could be crucial as demonstrated by Pappas et al. (Ranjha et al., 2021). These authors demonstrated that the yield from olive leaves in terms of phenolic content increased by increasing the ethanol concentration in a PEF extraction process with maximum yield obtained at similar ethanol concentration of 50% and 70%. In a previous study (Symes et al., 2018) from our laboratory that investigated conventional extraction (shaken at 80 RPM for 2 hours at 70°C) of antioxidants from AR using ethanol and methanol at concentrations of 50%, 70% and 90%, it was found that the ethanol concentration did not affect the yield. Considering the standard deviation, the yield obtained in the present study (43.5-58.8%) is similar or slightly higher than that reported for conventional extraction with ethanol and methanol (42.1 – 44.3%). However, PEF treatments and heated-control samples in the present study were more effective in terms of treatment time (60 s and 2 min for PEF and heat-control, respectively) and used less solvent (10% only) compared to the conventional solvent extraction method (Symes et al., 2018).

### *3.1.2 Effect of PEF Treatment on TPC and TFC*

The response surface regression models were formed from linear (E and frequency), square and the interaction components of these parameters (Table S1). The TPC was affected by the square of E (Table S1). Only the interaction of E and frequency had an effect on the TFC. Teh et al. (2014) found significant effects for frequency, voltage and their interaction on the extraction of phenolic compounds expressed as TPC and TFC from hemp seed cake. The highest arithmetic TPC was obtained at E of 0.4 kV/cm and frequency of 125 Hz. Pongkasamepornkul and Kamonpatana (2010) reported that a moderate frequency of 125 Hz extracted a higher content of antioxidants from rice-berry bran. Recently, Lakka et al. (Lakka et al., 2021) showed that the use of PEF for the extraction of TPC from dried *Vitis vinifera* fruit, *Crocus sativus*, and aerial part of *Sideritis scardica* was more effective at lower treatment intensity than higher PEF intensities. The AR extracts obtained by conventional extraction using ethanol and methanol had 40% to 60%, and 750-840% higher TPC and TFC, respectively, than that obtained in the present study (Symes et al., 2018), indicating that the qualitative aspects of the extracts from PEF treatment was low compared to conventional extraction.

Pappas et al. (Pappas et al., 2021) investigated the use of PEF treatment (1 kV/cm for 30 min at 10 and 100 μs pulse width) to extract phenolic compounds expressed as TPC from olive leaves using ethanol at various concentrations and compared the extraction efficacy to a solvent control treatment. The authors found that PEF treatment did not have any effect on the TPC whereas ethanol concentration was the most important parameter for the extraction of TPC from olive leaves. The use of higher ethanol concentrations in the present study was hindered by the system used (different from the simple bench top system used by Pappas et al. (Pappas et al., 2021)). Future studies should investigate higher solvent concentrations if the system is able to handle such solvent.

The lack-of-fit values in Table S1 demonstrated that the response surface models of TPC, DPPH and ORAC fitted the data well (Figure 1), while the models of the TFC and FRAP activity data were unsatisfactory since the lack of fit values were significant.

### *3.1.3 Effect of PEF Treatment on Antioxidant Activity of AR Extracts*

The DPPH radical scavenging activity was affected by the linear response of E and frequency, while the FRAP activity was affected by their interactions only (Table S1). However, the model

for FRAP was not successful in fitting the obtained data. Overall, the DPPH radical inhibition (ranged 1.6 to 8.0%) and ORAC (ranged from 25.5 to 30.9 mM TE/g extract) antioxidant activities were lower as compared to those obtained using conventional solvent extraction (ranged 10% to 26%, and 490 to 840 mM TE/g extract, for DPPH inhibition and ORAC activities, respectively) (Symes et al., 2018). Surprisingly, the FRAP activity in the present study and that reported by Symes et al. (Symes et al., 2018) did not differ substantially. Several studies documented that values of the parameters of antioxidant activity are linked to the specific composition of the extracted compounds in several systems (Llerena et al., 2020, Aroso et al., 2017) and various correlations among, TPC, TFC and antioxidant activities were reported. Regarding the AR extracts, conventional solvent extraction was found to exhibit differing correlations depending on the extraction time (2 h vs 10 h), but significant correlation between ORAC and TFC were found at both extraction times (Symes et al., 2018). Furthermore, the authors reported no correlations between TPC and DPPH inhibition at both extraction times and loss of correlation between TPC and TFC with FRAP in 10 h extracted samples. Given the low antioxidant activities, TPC and TFC obtained by PEF treatment under the described treatment conditions, PEF is not the best method for the extraction of antioxidants from AR.

### 3.2 Extraction of AR Antioxidants using Ionic Liquids

To determine the best IL extraction conditions, four parameters were investigated: the type of ionic liquid, S:L ratio, extraction time and IL concentration. The extraction of antioxidants by IL involved the use of a shaking incubator at a 70$^{o}$C for the specified times. Some studies reported the combined methods using both IL and microwave assisted extraction technique as it was found that they have a synergetic relationship (Ma et al., 2011, Lin et al., 2012). The IL helped to transfer the energy from the microwave to the sample, targeting the cell walls, thus leading to a shorter extraction time. A temperature-controlled microwave is unavailable to this research and thus the use of the shaking incubator.

There was a complication with the analysis of TPC for the samples obtained using IL. When the ionic liquid containing the AR extract were mixed with the Folin-Ciocalteu reagent, a blue colloidal fraction was formed, interfering with the absorption results. It was thought that the imidazole in the IL would not react with this reagent (Ikawa et al., 2003). However, the IL appeared to react with polyoxometalates, a cluster of anionic metal-oxygen compounds, creating a complex with the reagent that eventually formed a precipitate (Aid et al. (2015). This

precipitate formation was also found by Costa-Lopes et al. (2016) in their study on the solubilization of lignocellulose. The precipitate prevents the absorbance from being read by the plate reader and thus the TPC was unable to be determined for IL extracts. Aid et al. (2015) stated that polyphenols in IL solutions could be determined using a colorimetric paper microzone assay, however the reagents were not available in our laboratory. Other assays used in the present study were completed without any complications.

The extraction yield could not be determined for the IL as it was not possible to separate the IL from the extract before the freeze-drying step, hence a pure lyophilised sample was not achieved. A back extraction method described by Tan et al. (2016) was carried out using hexane. The extracted compounds were supposed to migrate into the organic phase while the ionic liquid should have remained in the aqueous phase, however this was not achieved. This could have been due to the extracted compounds having a higher affinity for the ionic liquid compared to the organic solvent. Hence, the results from the antioxidant compound analysis are reported per mL of ionic liquid and AR sample. Controls of IL at the same concentration were run in parallel to account for any contributions by the IL alone, and it was found that the IL solutions alone did not contribute towards antioxidant activity.

The toxicity of IL has been reported on pathogenic bacteria and cancer cells, however there is less literature on plant extraction and the effects on animals (Gonçalves et al., 2021). It is important to note IL have been considered as green solvents in literature (Xiao et al., 2018). However, IL environmental effects are also in question (Gonçalves et al., 2021). New IL compounds are being developed, included a new $CO_2$-based alkyl carbamate IL which has recently been reported and appears to have better environmental properties (Khoo et al., 2021). Future research should consider these new ILs for the extraction of AR.

### *3.2.1 Effects of Ionic Liquid Extraction Parameters on TFC and Antioxidant Activities*

The p-values obtained from MANOVA are shown in Table S2. The TFC, DPPH inhibition %, ORAC and FRAP activities were significantly affected by the type of IL used ($P < 0.05$). TFC, FRAP and ORAC were affected by the S:L ratio ($P < 0.05$). Only ORAC activity was affected ($P < 0.05$) by the extraction time. There were no effects for IL concentration used or for most of the factor interactions with the exception of S:L ratio and extraction time on ORAC activity.

### *3.2.2 Effect of Type of Ionic Liquid on the TFC and Antioxidant Activities of AR*

The TFC and antioxidant activities were affected by the type of IL ($P < 0.05$). As shown in Figure 2, [BMIM]Br and [BMIM]Cl treatments resulted in 20%-25% higher TFC (Figure 2a), ORAC (Figure 2c) and FRAP (Figure 2d) antioxidant activities compared to [BMIM]$BF_4$ treatment. [BMIM]Br is the most commonly reported IL in literature for the extraction of biological materials (Wang et al., 2021, Ahmad et al., 2017). While no differences were found between [BMIM]Br and [BMIM]Cl treatments on TFC and FRAP antioxidant activity, Higher ORAC and DPPH inhibition antioxidant activities were found with [BMIM]Cl than [BMIM]Br (Figure 2b and 2c). Surprisingly, [BMIM]$BF_4$ IL extraction had the highest DPPH inhibition (%) activity that was higher than both of the other IL (Figure 2b). This could be due to the different affinities the antioxidant compounds had for the ILs. As Br and Cl are on the same column in the periodic table, these ILs have similar extracting properties. However, [BMIM]Cl seemed to be more specific towards compounds with higher DPPH inhibition and ORAC antioxidant activities than the [BMIM]Br.

### *3.2.3 Effect of S:L Ratio on the Measured Parameters*

The S:L ratio significantly affected TFC, FRAP and ORAC activities ($p < 0.05$) (Table S2). A S:L ratio of 1:10 generated extracts that had higher TFC and ORAC and FRAP antioxidant activities compared with 1:20 S:L ratio (Figure 3). This was expected, as the concentration of the AR would have been higher in the 1:10 samples and the 1:20 samples would have had a diluting effect on the extraction of antioxidant compounds. Considering the total extraction volume, the 1:20 S:L extraction ratio appeared to provide a better economic value since total TFC and antioxidant activities in the total volume will be higher in 20 mL compared with the 10 mL extract volume obtained from the same AR weight (Figure 3).

The IL concentration acts as a driving force in the extraction of bioactive compounds (Passos et al., 2014). There are many different bond forces at play during IL extraction, including hydrogen-bonding, van der walls, pi-bond and electrostatic interactions (Passos et al., 2014). Therefore, the higher IL molecule presence, for example the 1:20 S:L ratio, provided more interactions that were able to break the cell walls in the AR sample, leading to more antioxidant compounds extracted overall.

### *3.2.4 Effect of Extraction Time on the TFC and Antioxidant Activities of AR*

The extraction time had a significant effect on ORAC activity only ($P < 0.05$). A four-minute extraction time resulted in a significantly higher (9.8%) ORAC activity compared to the 2 minute extraction time (data not shown). When long extraction times are used, a plateau can be reached where no more antioxidant compounds are extracted (Passos et al., 2014)a result of saturation of the extraction solvent, or it can indicate a completed extraction (Passos et al., 2014). Higher extraction times were not investigated in this research to help with the work logistics; therefore, a plateau was not seen in this research. Future research should consider longer extraction times to ensure a completed extraction occurs.

### *3.2.5 Pearson's Correlations for Ionic Liquid Extraction Results*

All of the correlations were found to be significant ($P < 0.05$) (Table S3). Positive highly significant correlations among the TFC, ORAC and FRAP antioxidant activities of the AR extract were found. Negative correlations between DPPH inhibition and the other measured parameters were found, suggesting that this assay was not influenced by flavonoids extracted under the stated experimental conditions. It is worth mentioning that the PEF AR extracts did not show any correlations between DPPH and TFC. Overall, the results suggested that these extraction systems targeted different biological compounds, and thus may offer tailored solutions for specific antioxidant formulations.

### *3.2.6 Optimal Ionic Liquid Extraction Conditions*

It was challenging to determine the optimal conditions required for the maximum extraction of AR antioxidant compounds using IL since the yield was not determined. As shown in Tables 2 and S2, there were common trends between the concentration, S:L ratio and time. The IL type appeared to be the most important factor. Therefore, the optimal conditions to extract antioxidant compounds were considered to be using the ionic liquid [BMIM]Cl, at a concentration of 0.5%, using the ratio 1:10 for 4 minutes.

## 3.3 Comparison of Antioxidant Compounds Extracted in the two Extraction Methods

From the optimal conditions described for the PEF and IL extraction methods, the amount of antioxidant extracts can be compared to each other and literature. IL extraction resulted in 1.2 mg RE/ mL and thus in the 10 mL (total volume of IL used to extract 1 g AR), a total of 120 mg RE/ g AR was obtained. This TFC is 70 to 80 folds higher than the TFC obtained by PEF

and about 2.2-fold higher than that could be extracted with conventional ethanol/methanol, giving that the yield is about 42% on average and the TFC on average was 130 mg RE/g extract (Symes et al., 2018). Examining the antioxidant activities of IL extracts as well as considering the high TFC generated by IL, it is clear that IL as an extraction system outperformed the PEF extraction system. The TPC found in this research (Table 4B) was much higher than that extracted by Fan et al. (2015), with 12.03 ± 0.22 mg GAE/g extracted from *A. officinalis* residues. The total flavonoids were also higher than that found in literature, as Fuentes-Alventosa et al. (2013) reported 0.28 ± 0.15 mg RE/g extract in *A. officinalis* spears. These differences in the TPC and TFC from literature and those from this study could have been due to the different species and part of the asparagus plant investigated. There is poor literature concerning the potential antioxidant of *A. officinalis* roots extracts. The antioxidant activity was commonly investigated through using only a DPPH assay in literature (Fan et al., 2015, Fuentes-Alventosa et al., 2009). In this study multiple antioxidant assays were carried out to allow a wider evaluation of the true antioxidant activity of AR. Nile and Park (2014) found that *A. racemosus* roots had a FRAP value of 380 ± 4.85 μM TE/100g dry weight. A direct comparison with the present study is not possible due to the units used for reporting.

## 4 Conclusions and implications

The TFC and antioxidant activities of AR were determined in extracts obtained through two novel extraction methods: PEF and IL. It was found that IL extracted compounds with better TFC and antioxidant activity than PEF. The optimal IL extraction conditions were found to be using 0.5% [BMIM]Cl at a S:L ratio of 1:10 for 4 minutes. The extraction methods developed in this study were simple and easy to carry out. They allowed the extraction of health benefiting antioxidant compounds from AR. Therefore, IL extraction may support the utilisation of AR as a valuable by-product and hence prevent them from being a waste. Future work should investigate methods that enable separation of AR antioxidants from environmentally friendly IL, and investigate animal models for robust evaluation on toxicity effects

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