Optimization of microwave-assisted extraction of bioactive compounds from New Zealand and Chinese *Asparagus officinalis* L. roots

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Abstract The extraction of total phenolics (TPC), total flavonoids content (TFC), total saponins content (TSC), and caffeic acid (AC) contents of asparagus roots extract (ARE) from New Zealand and Chinese AR cultivars was optimized following a microwave-assisted extraction combined with central composite design. The determination of AC was conducted by HPLC in samples extracted under the optimum extraction conditions. The optimal variables for ethanol extraction generated a maximum TPC, TFC and TSC of optimal results for 68.6 mg GAE/g, 11.9 mg RE/g and 0.7 mg SE/g as well as antioxidant power towards β -carotene bleaching assay ($\% \beta_{sc}$) (57.2%), superoxide anion radical ($\% O_{sc}^{2-}$) scavenging capacity (20.1%) and ferric reducing antioxidant power assay (FRAP) (1.63 μ mol/g). For methanol, optimum extraction conditions obtained maximum TPC (62.6 mg GAE/g) TFC (10.7 mg RE/g), TSC (0.68 mg SE/g) with $\% \beta_{sc}$ (53.9%), $\% O_{sc}^{2-}$ (19.1%) and FRAP (0.63 μ mol/g). The content of

caffeic acid from ARE ranged from 0.46 to 2.89 mg/g with ethanol and from 0.41 to 2.64 mg/g with methanol.

Keywords *Asparagus officinalis* L. · Roots · Antioxidants · Caffeic acid · MAE · Cultivars

Introduction

Asparagus officinalis L. (green asparagus) is widely consumed in the world because of its rich nutritional and bioactive compounds content (Fan et al. 2015). These bioactive compounds include inosine, quercetin, rutin, caffeic acid, ferulic acid (Huang et al. 2006), total phenolics (Fan et al. 2015), and vitamins C and E (Negi et al. 2010). Bioactive compounds in *Asparagus officinalis*. roots (AR) have been reported to exhibit cytotoxicity against human and mouse tumour cells (Huang et al. 2006) showing that they have an important medicinal uses. China is the largest asparagus grower and exporter, and has the most growing areas of *Asparagus officinalis* in the world. In China, asparagus has been used as a traditional medicine for treating cough, inflammation, fungal infection and cancer (Fan et al. 2015). In India, *Asparagus racemosus*, of the same genus, has very wide pharmacological properties, and used as an antitussive and anti-cough (Mandal et al. 2000), anti-inflammatory (Nwafor and Okwuasaba 2003), anti-diarrhoeal and antiulcerogenic remedy (Nwafor et al. 2000). Caffeic acid was found to be the main flavonoid in AR (Huang et al. 2006) and has been proposed as a bioactive for human health (Olthof et al. 2001). According to Chen and Ho (1997), caffeic acid is an important antioxidant and is recognised for its pharmacological properties that provide protection against chronic diseases such as coronary and heart diseases (Okutan et al. 2005).

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These diseases are generally considered to be caused by an imbalance between the oxidative stress and antioxidant capacity in the body (Chen and Ho 1997).

Modern extraction techniques, such as microwave-assisted extraction (MAE), have become more popular and common in the extraction process as “green techniques” (Singh et al. 2017). They offer reduced extraction time (ET), and savings in extraction power and solvent use (Li et al. 2012). In the MAE process, the extraction solvents are heated effectively by the microwave power (MP), which enables the rapid extraction of compounds into solvents at low power consumption and ET (Li et al. 2012). The extraction of bioactive compounds from AR using MAE has not been earlier investigated and the optimal conditions for maximum total antioxidant and other bioactive compounds yields are yet to be established.

In this study, MAE was employed to optimize the extraction of bioactive compounds (TPC, TFC, and TSC) and total antioxidant activities (FRAP, $O_2^{\cdot-}$ and β -carotene assays) from AR of several cultivars. A central composite rotatable design (CCD) combined with Response Surface Methodology (RSM) was used. Six AR cultivars from New Zealand and China were used in this study, and caffeic acid was determined by HPLC-UV. Ethanol and methanol were chosen as extraction solvents based on preliminary screening of several solvents.

Materials and methods

Chemicals and equipments

Phenazine methosulphate, nicotinamide-adenine dinucleotide phosphate (NADH), 2,4,6-tripryridyl-s-triazine (TPTZ), nitro blue tetrazolium, Tris-HCl, linoleic acid and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were from Sigma Chemical Company (St. Louis, MO, USA). *Trans*- β -carotene, butylated hydroxytoluene (BHT) and Folin–Ciocalteu reagent were from Beijing Sollebao Biotechnology Co. LTD (Beijing, China). Tween 80 emulsifier, aluminium chloride, sodium nitrite, sodium hydroxide, vanillic aldehyde, glacial acetic acid, ethanol, and methanol were from Tianjin Biotechnology Co. LTD (Tianjin, China). Gallic acid, rutin, saponin, and caffeic acid were from Lvyuan, Biotechnology Co. LTD (Shanghai, China). Micro-plant grinding machine (FZ102) was from Tianjin Shi Taisite Equipment Co. LTD (Tianjin, China). Freeze-dryer (ALPHA1–2) was from Martin Christ Gefriertrocknungsanlagen Co. LTD (Osterode, Germany). Power Wave HT -Microplate reader was from Biotech Co. LTD (Vermont, USA) and a high-performance liquid chromatography system (Agilent 1200 HPLC) was from Agilent Technologies (Palo Alto, USA). Microwave-

assisted extraction system MCR-3, was from Zhengzhou kechuang instrument Co. Ltd, Zhengzhou, China.

Materials

Purple and green *A. officinalis* roots were obtained from a commercial asparagus farm in the South Island of New Zealand (Palmerston, New Zealand). The plants were 15 years old. Yellow, green, purple and white *A. officinalis* roots were obtained from Heze city (Shandong province, China) that had been planted for 8–10 years.

Microwave-assisted extraction of bioactive compounds

AR extracts were obtained using MAE system with aqueous methanol and ethanol solutions that already shown to be the best solvents from trials in our laboratory. The extraction procedure was a modification from previous report (Li et al. 2012) and the design investigated the bioactive activities in Chinese green AR employing the CCD. In brief, 0.5–10 g freeze-dried green AR were weighed and then suspended in 100 mL of different concentrations of ethanol or methanol as extraction solvents in a Teflon perfluoroalkoxy-lined extraction vessel (CEM Corporation Inc., Matthews, NC, USA). The vessel was heated at 45 °C for 5 min, held at 55 °C for 5 min and then kept at a consistent temperature of 60 °C. A thermocouple was used to directly measure the temperature of the vessel. A magnetic stirrer was used to stir the mixture to allow for homogeneous heating. After treatment, the mixture was cooled down to room temperature and centrifuged at 3582 g for 5 min. and filtered using a 0.2 μ m PTFE membrane filter (VWR International, ON, Canada). All procedures were conducted under dim light and were performed in triplicate, and result is expressed as mean value (or Mean $\pm$ Standard Error, SE).

Experimental design

The RSM was employed to optimize the operating variables that affect the total antioxidant activities and bioactives yields in AR. Four independent microwave processing parameters [microwave power (MP), solid to liquid (S/L) ratio, the solvent concentration and extraction time (ET)] were investigated (Li et al. 2012) (Table 1). The maximum bioactive content in the AR extracts was obtained using a CCD. In this design, 30 treatments were run with three treatments as the centre point to estimate the reproducibility of the method (Tables 2, 3) using the Chinese green *A. officinalis* roots. The ET (X_1 : 15 s, 35 s, 55 s, 75 s, and 95 s), the concentration of methanol or ethanol

Table 1 Experimental factors for extraction of different cultivars of *Asparagus* root

Variable	Levels				
	− 2	− 1	0	1	2
X1 = Extraction time (min)	15	35	55	75	95
X2 = Solvent concentration (%) [*]	10	30	50	70	90
X3 = Power of microwave (W)	100	250	400	550	700
X4 = Solid to liquid ratio (g/mL)	1:4	1:28	1:52	1:76	1:100

^{*}Ethanol or methanol in water

(X₂: 10%, 30%, 50%, 70% and 90%), MP (X₃: 100 W, 250 W, 400 W, 550 W, and 700 W) and solid/liquid (S/L) ratio (X₄: 1:4, 1:28, 1:52, 1:76 and 1:100 g/mL) were chosen as the independent variables, which each included five levels as shown in Table 1. The TFC, TSC, TPC, %O_{sc}^{2−}, β-carotene and FRAP values were used as responses. Regression analysis of experimental data was performed to establish the empirical second order polynomial models as shown in Eq. (1):

Table 2 Central composite design with the observed responses and predicted values for TPC, TFC and TSC

Run	Coded variable levels				Observer (Y ₁) ^a						Predicted (Y ₀)					
	X ₁	X ₂	X ₃	X ₄	TPC (mg GAE/g)		TFC (mg RE/g)		TSC (mg SE/g)		TPC (mg GAE/g)		TFC (mg RE/g)		TSC (mg SE/g)	
					M	E	M	E	M	E	M	E	M	E	M	E
1	− 1	1	− 1	1	33.64	33.60	5.30	4.80	0.31	0.49	31.70	35.65	5.52	5.01	0.31	0.47
2	0	0	0	− 2	42.34	40.60	6.40	6.70	0.09	0.26	44.02	42.74	6.53	6.96	0.20	0.28
3	− 1	− 1	1	− 1	27.84	30.60	5.20	4.90	0.14	0.31	29.55	31.38	5.38	5.36	0.11	0.29
4	0	0	0	2	45.24	46.60	6.50	5.20	0.38	0.46	45.66	46.07	6.32	5.25	0.32	0.44
5	1	− 1	− 1	1	34.22	31.20	6.50	6.60	0.19	0.35	36.36	30.43	6.05	6.55	0.15	0.35
6	1	1	1	1	40.02	40.40	6.90	7.30	0.29	0.36	42.00	44.65	7.03	7.78	0.32	0.41
7	0	0	0	0	62.64	64.80	10.70	11.90	0.68	0.67	60.15	66.31	10.36	11.41	0.64	0.64
8	0	0	0	0	62.06	63.40	10.60	11.70	0.67	0.63	60.15	66.31	10.36	11.41	0.64	0.64
9	1	1	− 1	− 1	33.64	31.80	6.20	8.90	0.18	0.21	32.14	32.15	5.85	8.94	0.16	0.24
10	0	− 2	0	0	30.74	34.40	6.80	7.30	0.13	0.44	35.98	40.79	7.24	7.77	0.22	0.45
11	− 1	1	− 1	− 1	28.42	26.40	4.90	6.70	0.19	0.24	29.55	28.03	5.24	6.46	0.16	0.26
12	− 1	− 1	1	1	25.52	31.20	4.40	6.20	0.14	0.32	25.90	30.89	4.56	6.11	0.13	0.30
13	0	0	0	0	55.68	68.60	9.60	11.10	0.56	0.7	60.15	66.31	10.36	11.41	0.64	0.64
14	0	0	0	0	58.58	67.80	10.10	10.90	0.61	0.68	60.15	66.31	10.36	11.41	0.64	0.64
15	1	1	− 1	1	37.2	38.40	6.40	7.20	0.24	0.39	35.23	35.97	6.45	6.47	0.26	0.40
16	− 1	1	1	− 1	30.16	31.20	5.5	6.70	0.12	0.29	31.56	32.02	5.77	6.71	0.13	0.30
17	0	0	− 2	0	38.28	37.60	7.60	6.80	0.26	0.43	40.15	39.27	7.73	7.56	0.30	0.39
18	− 1	− 1	− 1	1	36.54	39.60	5.10	6.10	0.23	0.35	36.36	39.61	5.33	5.86	0.27	0.41
19	1	− 1	1	− 1	37.70	37.80	6.50	7.70	0.25	0.38	34.99	35.79	6.10	7.45	0.22	0.41
20	0	2	0	0	35.38	39.20	7.10	7.60	0.21	0.45	33.85	36.37	6.74	7.58	0.19	0.44
21	0	0	0	0	62.06	67.60	10.70	11.90	0.67	0.59	60.15	66.31	10.36	11.41	0.64	0.64
22	− 1	− 1	− 1	− 1	35.96	39.20	6.20	6.30	0.22	0.29	32.86	34.99	5.88	5.78	0.16	0.25
23	1	− 1	1	1	33.06	34.80	5.70	7.20	0.17	0.39	30.95	31.51	5.60	7.17	0.19	0.36
24	− 2	0	0	0	15.08	17.60	2.60	4.30	0.06	0.13	13.76	16.44	2.00	4.33	0.07	0.15
25	1	− 1	− 1	− 1	30.16	31.20	6.20	7.80	0.12	0.24	30.08	29.61	6.27	7.49	0.08	0.25
26	0	0	2	0	41.18	44.40	8.00	8.90	0.31	0.41	41.41	44.34	7.82	8.45	0.31	0.45
27	1	1	1	− 1	44.66	47.60	6.70	9.60	0.37	0.47	43.86	45.93	6.70	9.57	0.32	0.40
28	0	0	0	0	61.48	67.60	10.60	11.10	0.66	0.59	60.15	66.31	10.36	11.41	0.64	0.64
29	2	0	0	0	17.98	18.40	3.10	7.60	0.09	0.23	21.40	21.17	3.65	7.88	0.13	0.21
30	− 1	1	1	1	32.48	34.60	5.60	5.90	0.16	0.39	31.58	34.53	5.77	5.94	0.19	0.37

^aMean of triplicate samples, M methanol, E ethanol

Table 3 Central composite design with the observed responses and predicted values of FRAP and inhibition for β_{sc} , ABTS_{sc} and O_{sc}²⁻

Run	Coded variable levels				Observer (Y1) ^a								Predicted (Y0)			
	X ₁	X ₂	X ₃	X ₄	O _{sc} ²⁻ (%)		FRAP (mM)		%β _{sc}		O _{sc} ²⁻ (%)		FRAP (mM)		%β _{sc}	
					M	E	M	E	M	E	M	E	M	E	M	E
1	0	−1	−1	0	6.9	8.7	0.34	0.81	20.8	23.8	7.8	9.4	0.35	0.86	21.9	26.7
2	0	0	−1	1	9.2	10.6	0.42	1.04	28.3	39.3	9.3	10.6	0.44	1.05	28.5	37.7
3	1	0	0	−1	8.2	8.3	0.28	0.69	26.3	22.6	8.4	9.5	0.28	0.74	26.9	24.9
4	0	0	1	1	8.1	12.1	0.45	1.12	23.2	35.3	7.9	11.7	0.46	1.13	22.3	35.3
5	−1	1	0	0	8.3	9.3	0.34	0.75	23.8	22.3	8.4	9.8	0.32	0.74	23.9	20.1
6	0	0	0	0	11.2	11.4	0.40	0.97	32.3	33.3	11.7	11.6	0.42	1.07	31.5	32.1
7	0	0	0	0	19.1	20.1	0.63	1.56	53.1	57.1	18.6	19.8	0.60	1.56	53.3	55.5
8	0	−1	0	−1	18.9	19.9	0.62	1.60	53.9	56.8	18.6	19.8	0.60	1.56	53.3	55.5
9	0	0	0	0	11.4	11.2	0.34	0.72	32.9	31.4	11.6	11.5	0.32	0.76	33.1	29.3
10	0	−1	1	0	12.3	12.5	0.31	0.83	35.3	35.4	11.7	12.3	0.36	0.97	34.6	35.2
11	−1	0	1	0	10.8	9.3	0.28	0.64	31.1	25.2	10.0	9.5	0.29	0.68	29.8	26.0
12	1	0	0	1	9.3	10.8	0.26	0.75	26.9	22.3	9.5	10.4	0.26	0.72	26.8	23.8
13	1	0	1	0	18.2	19.2	0.56	1.63	53.2	56.9	18.6	19.8	0.60	1.56	53.3	55.5
14	0	1	0	1	18.5	19.3	0.59	1.46	53.4	57.2	18.6	19.8	0.60	1.56	53.3	55.5
15	0	−1	0	1	9.4	12.2	0.37	0.92	27.1	28.1	9.0	11.7	0.36	0.84	27.1	28.0
16	0	1	0	−1	10.8	11.0	0.31	0.75	31.1	22.5	11.0	10.4	0.32	0.78	31.1	24.1
17	0	0	−1	−1	12.2	12.9	0.38	0.91	35.2	24.4	12.1	12.6	0.40	0.94	35.5	24.4
18	−1	0	0	1	7.7	9.0	0.37	0.95	22.1	25.7	7.4	9.1	0.37	0.96	21.6	25.2
19	1	1	0	0	9.50	9.3	0.38	0.91	27.4	31.6	9.0	8.5	0.35	0.87	26.4	28.1
20	0	0	0	0	12.1	12.3	0.35	0.94	34.7	35.5	12.4	12.0	0.34	0.87	34.5	34.1
21	−1	0	0	−1	18.5	20.0	0.62	1.63	53.9	53.9	18.6	19.8	0.60	1.56	53.3	55.5
22	0	1	−1	0	8.70	8.9	0.36	0.94	25.1	25.5	8.60	8.6	0.33	0.85	25.9	26.0
23	−1	−1	0	0	9.1	9.2	0.33	0.84	26.1	23.6	9.7	9.7	0.31	0.76	28.1	25.0
24	−1	0	−1	0	4.8	4.9	0.15	0.42	13.7	13.4	5.2	4.6	0.14	0.38	13.5	9.8
25	1	−1	0	0	9.20	8.9	0.31	0.75	26.3	20.3	10.0	9.0	0.31	0.72	26.3	22.7
26	0	0	0	0	14.5	13.6	0.41	1.07	41.9	28.9	14.5	13.4	0.42	1.06	41.0	27.4
27	0	0	1	−1	11.9	10.5	0.45	1.15	32.3	30.9	11.9	11.0	0.44	1.11	33.4	33.7
28	1	0	−1	0	18.1	20.1	0.62	1.53	52.0	51.3	18.6	18.8	0.60	1.56	53.3	55.5
29	0	1	1	0	7.7	6.1	0.18	0.44	18.5	12.2	7.1	6.0	0.22	0.50	18.1	14.3
30	−1	1	1	1	12.1	10.2	0.33	0.83	26.7	24.5	11.1	10.6	0.32	0.83	27.3	24.3

^aMean of triplicate samples, *M* methanol, *E* ethanol

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{1 \leq i < j \leq k} \beta_{ij} X_i X_j \quad (1)$$

where *Y* is the measured response variable (TFC, TSC, TPC, %O_{sc}²⁻, β-carotene and FRAP value), *X_i* and *X_j* are input variables that influence the response *Y*; *k* is the number of variables; β₀ is a constant term; β_{*i*} is the linear coefficient (main effect), β_{*ii*} is the quadratic coefficient, β_{*ij*} is the two factor interaction coefficient.

HPLC analysis of caffeic acid compounds

The ARE with high TPC contents were used for the determination of caffeic acid content (Wang et al. 2004). The liquid extracts were centrifuged for 20 min at 3500 g at 4 °C and the liquid extracts were filtered through a 0.22 μm membrane filter before the HPLC-UV analysis. The HPLC system used (Agilent 1200, Palo Alto, USA) was equipped with a reversed-phase Agilent SB-C18 column (250 mm × 4.6 mm, 5 μm) connected with a quaternary pump, autosampler and a UV detector was used to determine the chromatographic validation of the root

extracts. The mobile phase used for separation was 0.01% formic acid in water (V/V, eluent A) and methanol (eluent B). The gradient programme was set at 0–6 min, linear gradient from 40–50% B, then 6–10 min, 50–65% B; maintain at 65% until 25 min. The flow rate was set at 0.25 mL/min; the injection volume was 10 μ L; the wavelength of the UV detector was 360 nm and the column temperature was at 30 °C. The chromatographic peak of caffeic acid was confirmed by comparing retention times and UV spectra with caffeic acid as the reference standard. Caffeic acid solutions (12.5–500 μ g/mL) by seven points standard series were used to produce the calibration curve.

Total phenolics content (TPC)

The Folin–Ciocalteu method was used to determine the TPC in the extracts of Chinese and New Zealand AR using the previous method (Rodríguez et al. 2005). The TPC was expressed as gallic acid equivalent per gram of dry AR (GAE/g).

Total flavonoids content (TFC)

TFC was determined using the aluminium chloride colourimetric method (Fan et al. 2015). A seven-point standard curve (0–60 μ g/mL) was constructed using rutin as the reference standard and the results were expressed as rutin equivalents (RE) mg per g dry powder (DP) using the following Eq. (2):

$$\text{TFC}(\text{mg RE/g}) = \frac{nCV}{m \times 1000} \quad (2)$$

where TFC (mg RE/g): total content of flavonoid compounds in mg/g dry powder, c : concentration of rutin established from the calibration curve in μ g/mL, the V : volume of extract in mL, m : weight of crude plant powder in g and n : dilution ratio.

Total saponins content (TSC)

TSC of the extracts was measured using the vanillin-glacial acid reagent method (Fang 2005). A seven-point standard curve (0–500 μ g/mL) was constructed using saponin as the reference standard and the results were calculated as TSC per gram of dried powder (mg SE/g) using the following Eq. (3):

$$\text{TSC}(\text{mg SE/g}) = \frac{C \times V_1 \times V_2}{m \times 1000} \quad (3)$$

where TSC = the total saponins content, C = the concentration of saponins, V_1 = sample volume (mL), V_2 = extract volume in each well (μ L) and m = weight of sample (g).

Antioxidant activity

Ferric-reducing antioxidant power (FRAP)

The FRAP assay was carried out using a microplate reader (Rodríguez et al. 2005). The standard curve was plotted by using $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ (10 μ L, 0.1 mM–1.0 mM) as the reference standard. Results were expressed in μ mol Fe (II)/g dry powder.

Superoxide anion radical scavenging($\%O_{sc}^{2-}$), and β -carotene bleaching activities ($\%\beta_{sc}$)

The O_2^{2-} and β -carotene bleaching activities were determined as described previously study (De Vargas et al. 2016).

Statistical analysis

Statistical analysis was conducted using Design Expert Statistical Software package 8.0.6.5 (Stat-Ease, Inc., Minneapolis, USA). The experimental data were analysed using multiple regressions and the significance of regression coefficients was evaluated by F test. The significant terms in the model were found by Pareto analysis of variance (ANOVA) for each response and ANOVA tables were generated. The regression coefficients were used to make statistical calculations to generate response surface plots from the regression models.

Results and discussion

Fitting the model

TPC

Response surface methodology (RSM) results are shown in Table S1. TPC and the extraction variables relationships were quadratic with good regression coefficient ($r_{\text{ethanol}}^2 = 0.9742$ and $r_{\text{methanol}}^2 = 0.9724$) (Eqs. 4, 5). The lack of fitting were 0.1012 and 0.4271 with ethanol and methanol extraction, respectively, and shows that the models fitted the parameters.

$$\begin{aligned} Y_{1-\text{Ethanol}} = & +66.31 + 1.18X_1 + 1.55X_2 + 1.27X_3 \\ & + 0.83X_4 + 2.38X_1X_2 + 2.45X_1X_3 \\ & - 0.95X_1X_4 + 1.90X_2X_3 + 0.75X_2X_4 \\ & - 1.27X_3X_4 - 11.88X_1^2 - 8.26X_2^2 - 6.13X_3^2 \\ & - 5.48X_4^2 \end{aligned} \quad (4)$$

$$\begin{aligned}
Y_{1-\text{Methanol}} = & +60.15 + 1.91X_1 + 1.93X_2 + 0.31X_3 \\
& + 0.41X_4 + 1.34X_1X_2 + 2.43X_1X_3 \\
& - 0.47X_1X_4 + 1.70X_2X_3 + 0.54X_2X_4 \\
& - 1.41X_3X_4 - 10.64X_1^2 - 7.54X_2^2 - 4.84X_3^2 \\
& - 3.83X_4^2
\end{aligned} \quad (5)$$

Significant effects were found for the quadratic terms X_1^2 , X_2^2 , X_3^2 , and X_4^2 for methanol and ethanol on the extraction of TPC (Table S1). Similarly, the linear terms of (X_1 , X_2 , X_3 , and X_4), and the interaction terms (X_1X_3 and X_2X_3) (Table S1) had significant effects ($p < 0.0001$) on TPC but not the interaction terms of (X_1X_4 , X_2X_4 and X_3X_4) and the linear terms of (X_1 , X_3 and X_4) for TPC with ethanol and the interaction terms of (X_1X_2 , X_1X_4 , X_2X_4 and X_3X_4) and linear terms of (X_3 and X_4) for TPC with methanol ($p > 0.05$).

TFC

The RSM analysis in Table S1 also indicates high regression values ($r_{\text{ethanol}}^2 = 0.9733$ and $r_{\text{methanol}}^2 = 0.9771$) and Eqs. (6) and (7) show the relationships between TFC and extraction variables. The lack of fitting values were 0.3881 and 0.5053 with ethanol and methanol extractions, respectively, meant that the models fitted the investigated parameters.

$$\begin{aligned}
Y_{2-\text{Ethanol}} = & +11.41 + 0.89X_1 + 0.32X_2 + 0.22X_3 \\
& - 0.43X_4 + 0.19X_1X_2 + 0.094X_1X_3 \\
& - 0.26X_1X_4 + 0.17X_2X_3 - 0.38X_2X_4 \\
& + 0.17X_3X_4 - 1.33X_1^2 - 1.12X_2^2 - 0.85X_3^2 \\
& - 1.33X_4^2
\end{aligned} \quad (6)$$

$$\begin{aligned}
Y_{2-\text{Methanol}} = & +10.36 + 0.41X_1 + 0.20X_2 + 0.021X_3 \\
& - 0.054X_4 + 0.056X_1X_2 + 0.081X_1X_3 \\
& + 0.081X_1X_4 + 0.26X_2X_3 + 0.21X_2X_4 \\
& - 0.069X_3X_4 - 1.88X_1^2 - 1.00X_2^2 - 0.65X_3^2 \\
& - 0.98X_4^2
\end{aligned} \quad (7)$$

The interaction terms of X_2X_4 for ethanol and X_2X_4 for methanol, the linear term of (X_1 , X_2 , X_3 , X_4) for ethanol and only X_1 for methanol and the quadratic term of X_1^2 , X_2^2 , X_3^2 and X_4^2 indicated their significant effects ($p < 0.0001$) on TFC in both methanol and ethanol extraction processes (Table S1). The interaction terms of X_1X_2 , X_1X_3 , X_1X_4 and X_3X_4 for both ethanol and methanol had insignificant ($p > 0.05$) effects on TFC.

TSC

The RSM analysis (Table S1) exhibits high regression values ($r_{\text{ethanol}}^2 = 0.9483$ and $r_{\text{methanol}}^2 = 0.9490$) for TSC in the extracts and Eqs. (8) and (9) show the models depicting the relationships between TSC and extraction parameters. The lack of fitting values were 0.5388 and 0.2196 with ethanol and methanol extractions confirmed the suitability of the models to describe the relationships.

$$\begin{aligned}
Y_{3-\text{Ethanol}} = & +6.43 + 0.13X_1 + 0.16X_2 + 0.13X_3 \\
& + 0.42X_4 - 0.044X_1X_2 + 0.29X_1X_3 \\
& - 0.14X_1X_4 + 6.25E - 003X_2X_3 + 0.14X_2X_4 \\
& - 0.37X_3X_4 - 1.16X_1^2 - 0.59X_2^2 - 0.56X_3^2 \\
& - 0.71X_4^2
\end{aligned} \quad (8)$$

$$\begin{aligned}
Y_{3-\text{Methanol}} = & +6.37 + 0.15X_1 + 0.36X_2 + 0.025X_3 \\
& + 0.30X_4 + 0.19X_1X_2 + 0.46X_1X_3 \\
& - 0.12X_1X_4 + 0.05X_2X_3 + 0.088X_2X_4 \\
& - 0.24X_3X_4 - 1.35X_1^2 - 1.29X_2^2 - 0.82X_3^2 \\
& - 0.95X_4^2
\end{aligned} \quad (9)$$

All the quadratic terms X_1^2 , X_2^2 , X_3^2 and X_4^2 , the linear term X_4 and the interactions X_1X_3 were significant ($p < 0.0001$). Similarly, the interaction term X_1X_3 for ethanol and the interaction term of X_3X_4 had significant effects on TSC. However, the methanol and ethanol extraction interaction parameters X_1X_2 , X_1X_4 , X_2X_3 , X_2X_4 and the linear term of X_1 and X_3 had no significant effect on the TSC ($p > 0.05$).

FRAP

The coefficients of determination (r^2) of the predicted models were $r_{\text{ethanol}}^2 = 0.9710$ and $r_{\text{methanol}}^2 = 0.9728$, and the lack of fit were 0.2856 and 0.3978 for ethanol and methanol extraction (Table S1). The mathematic models are shown in Eqs. (10) and (11).

$$\begin{aligned}
Y_{3-\text{Ethanol}} = & +1.56 + 0.029X_1 + 0.037X_2 + 0.03X_3 \\
& + 0.018X_4 + 0.051X_1X_2 + 0.066X_1X_3 \\
& - 0.023X_1X_4 + 0.051X_2X_3 + 0.017X_2X_4 \\
& - 0.031X_3X_4 - 0.28X_1^2 - 0.19X_2^2 - 0.14X_3^2 \\
& - 0.12X_4^2
\end{aligned} \quad (10)$$

$$\begin{aligned}
Y_{3-\text{Methanol}} = & +0.60 + 0.019X_1 + 0.019X_2 + 3.75^E \\
& - 0.003X_3 + 3.75^E - 0.003X_4 + 0.013X_1X_2 \\
& + 0.023X_1X_3 - 6.875^E - 0.003X_1X_4 \\
& + 0.018X_2X_3 + 5.625^E - 0.003X_2X_4 \\
& - 0.014X_3X_4 - 0.11X_1^2 - 0.076X_2^2 - 0.049X_3^2 \\
& - 0.039X_4^2
\end{aligned} \quad (11)$$

There were significant effects for the quadratic and linear terms of (X_1^2 , X_2^2 , X_3^2 , and X_4^2) on FRAP. Only X_1^2 , X_2^2 , X_3^2 , X_4^2 , X_2 , X_1X_3 , and X_2X_3 for ethanol and methanol extraction had significant effects on the response surface model. The terms X_3 , X_4 , X_1X_2 , X_1X_4 , X_2X_4 and X_3X_4 were not significant on the model ($p > 0.05$) with methanol extraction, and the extraction terms of X_1 , X_3 , X_4 and X_1X_4 , X_2X_4 and X_3X_4 had insignificant effects on this model ($p > 0.05$) with ethanol extraction.

O^{2-}

The RSA analysis (Table S1) indicated high regression value $r_{\text{ethanol}}^2 = 0.9891$ and $r_{\text{methanol}}^2 = 0.9875$ for $\%O_{\text{sc}}^{2-}$ in the extract and Eqs. (12) and (13) show the relationships between $\%O_{\text{sc}}^{2-}$ and extraction variables. The lack of fit, 0.1142 and 0.0920 for ethanol and methanol extractions, respectively, confirms the suitability of the models.

$$\begin{aligned}
Y_{3-\text{Ethanol}} = & +19.78 + 0.34X_1 + 0.69X_2 + 0.19X_3 \\
& + 0.27X_4 + 0.40X_1X_2 - 0.35X_1X_3 \\
& + 0.064X_1X_4 + 0.011X_2X_3 - 0.15X_2X_4 \\
& + 0.098X_3X_4 - 3.63X_1^2 - 2.29X_2^2 - 1.69X_3^2 \\
& - 2.17X_4^2
\end{aligned} \quad (12)$$

$$\begin{aligned}
Y_{3-\text{Methanol}} = & +18.58 + 0.47X_1 + 0.82X_2 + 0.60X_3 \\
& - 0.36X_4 + 0.069X_1X_2 - 0.18X_1X_3 \\
& - 0.094X_1X_4 + 0.33X_2X_3 - 0.26X_2X_4 \\
& + 0.57X_3X_4 - 3.10X_1^2 - 1.97X_2^2 - 1.32X_3^2 \\
& - 2.50X_4^2
\end{aligned} \quad (13)$$

There were significant effects for the quadratic and linear terms of (X_1^2 , X_2^2 , X_3^2 , and X_4^2) on $\%O_{\text{sc}}^{2-}$. Only X_1^2 , X_2^2 , X_3^2 , X_4^2 , X_1 , and X_2 for ethanol and methanol extraction had significant effects on the response surface model. The terms X_3 , X_4 , X_1X_4 , X_2X_3 , X_2X_4 and X_3X_4 were not significant on the model ($p > 0.05$) with ethanol extraction, and the extraction terms of X_3 , X_4 , X_1X_2 , X_1X_3 , X_1X_4 , X_2X_3 , X_2X_4 and X_3X_4 had insignificant effects on this model ($p > 0.05$) with ethanol extraction.

β -carotene

The RSA analysis (Table S1) indicates high regression values ($r_{\text{ethanol}}^2 = 0.9779$ and $r_{\text{methanol}}^2 = 0.9959$) for $\%\beta_{\text{sc}}$ in the extracts and Eqs. (14) and (15) show the relationship between $\%\beta_{\text{sc}}$ and extraction variables. The lack of fit were 0.3681 and 0.1332 with ethanol and methanol extraction confirms the suitability of the models.

$$\begin{aligned}
Y_{3-\text{Ethanol}} = & +55.54 + 1.12X_1 + 1.77X_2 + 0.75X_3 \\
& - 0.60X_4 + 1.63X_1X_2 + 1.60X_1X_3 \\
& - 0.46X_1X_4 - 0.22X_2X_3 + 0.36X_2X_4 \\
& - 0.087X_3X_4 - 10.88X_1^2 - 6.25X_2^2 - 7.42X_3^2 \\
& - 4.75X_4^2
\end{aligned} \quad (14)$$

$$\begin{aligned}
Y_{3-\text{Methanol}} = & +53.28 + 1.15X_1 + 1.84X_2 + 1.38X_3 \\
& - 1.54X_4 + 0.73X_1X_2 - 0.25X_1X_3 \\
& + 0.46X_1X_4 + 0.063X_2X_3 - 0.90X_2X_4 \\
& + 1.03X_3X_4 - 9.38X_1^2 - 5.62X_2^2 - 3.77X_3^2 \\
& - 6.97X_4^2
\end{aligned} \quad (15)$$

There were significant effects for the quadratic and linear terms of (X_1^2 , X_2^2 , X_3^2 , and X_4^2) on $\%\beta_{\text{sc}}$. Only X_1^2 , X_2^2 , X_3^2 , X_4^2 , X_2 , and X_1X_2 for ethanol and methanol had significant effect on the response surface model. The extraction terms of X_1 , X_3 , X_4 , X_1X_4 , X_2X_3 , X_2X_4 and X_3X_4 had insignificant effects on the model ($p > 0.05$) for ethanol extraction, and the extraction terms of X_1 , X_3 , X_4 , X_1X_3 , X_1X_4 , X_2X_3 and X_3X_4 had insignificant effects on this model ($p > 0.05$) for methanol extraction.

Optimization of extraction parameters

The extraction parameters for TPC, TFC, TSC, $\%O_{\text{sc}}^{2-}$, FRAP and $\%\beta_{\text{sc}}$ can be generated by enhancing the desirability function of the seven different responses. Once the optimum zone for the combination of the lowest factors was created, it will give an optimum yield for the other four dependent variables (ET, MP, solvent concentration and S/L ratio) because it represents the combination of extraction parameters (Zhang et al. 2016). The extraction of bioactive compounds was improved with the optimal concentration of ethanol in the range 62.6–64.1% (TPC), 60.7–63.9% ($\%O_{\text{sc}}^{2-}$), 61.4–64.3% (FRAP) and 62.5–65% ($\%\beta_{\text{sc}}$) at the constant ET of 57 s, which resulted in optimum TPC, $\%O_{\text{sc}}^{2-}$, FRAP and $\%\beta_{\text{sc}}$ (66.5 mg/g, 55.7%, 1.57 $\mu\text{mol/g}$ and 55.7%) values. Increasing the concentration of ethanol can enhance the breakage of the cell membranes (Gan and Latiff 2011). However, at a high level of ethanol concentration, a negative effect on

dispersion of bioactive compounds into the extraction solvent could occur (Al-Farsi and Lee 2008). This is because the coagulation of proteins at high levels of ethanol concentrations might increase diffusion resistance and hinder the diffusion of bioactives (Yang et al. 2009). The values of TPC, $\%O_{sc}^{2-}$, FRAP and $\%\beta_{sc}$ were increased gradually with the increase in S/L ratio in the range of 1:41.8 to 1:61.1. However, the range of S/L ratio did not have a significant effect on TPC and total antioxidant activities.

Based on the above findings, the optimal S/L ratio is approximated by 1:58.3, which is similar to the power of microwave when the optimal power of microwave is 436 W. It suggested that the mass transfer was slow at low power and the bioactive compounds in the raw materials required more power to dissolve into the extraction solvent (Jentzer et al. 2015). At higher power, however, the extraction of bioactive compounds reached equilibrium in a short time and had little change with the extension of the ET (Yang et al. 2009), which was similar to the findings with ET. Therefore, the maximum TPC, $\%O_{sc}^{2-}$, FRAP and $\%\beta_{sc}$ predicted by RSM analysis were 66.5 mg/g, 19.5%, 1.54 $\mu\text{mol/g}$ and 55.7% of AR under the following extraction condition for ethanol for TPC, $\%O_{sc}^{2-}$, FRAP and $\%\beta_{sc}$, extraction with 62.6% for 56.5 s and S/L ratio of 1:58.3 at 436 W as shown in Figs. S1a to S6a and S1b, S4b to S6b. Under the optimal conditions, the yield of TPC, $\%O_{sc}^{2-}$, FRAP and $\%\beta_{sc}$, was predicted by RSM models to be 66.5 mg/g, 19.8%, 1.57 $\mu\text{mol/g}$ and 55.7% of dried root powder of AR, respectively. Figs. S7a, S10a to S12a and S7b, S10b to S12b are contour plots of optimum four responses at time of 57.6 s and superimposed contour plots for the value of TPC, $\%O_{sc}^{2-}$, FRAP and $\%\beta_{sc}$, as a function of methanol concentration 64.9%, the power of microwave 480 W at the fixed S/L ratio 1:50 at 480 W.

Figures S2a and S2b show the contour and 3D plots for TFC with ethanol and methanol as the function of extraction parameters (ET, concentration of ethanol and methanol, MP and the S/L ratio). Results show S/L ratio (1:42.2 and 1:43.2) at an ET of 69 s gave highest TFC (11.6 mg/g), and a range of MP (403.9–484.3 W) along with ethanol concentration of 63.9–67.4% would give a high value of TFC (11.5 mg/g). From the contour plots, a big change with the increase in MP to reach the optimum TFC that was followed by a decrease with higher power, which is the same trend found with the S/L ratio. At MP of 440 W, ethanol concentration of 67.4% and S/L ratio 1:42, the highest value (11.6 mg/g) was obtained. Thus the optimum extraction parameters were 69 s, 67.4%, 440 W and 1:42. Similarly, for methanol extraction parameters, the optimal variables were 59 s, 72%, 400 W and 1:52 as shown in Figs. S8a and S8b.

Figures S3a and S3b show 3D response surfaces and contour plots that indicate the TSC was affected by the ET, MP, concentration of ethanol or methanol and S/L ratio. For ethanol extraction parameters; the maximum yield of TSC (0.65 mg/g) was achieved with an ET of 57 s, and then the yield decreased with the increasing of ET. When the concentration of ethanol was 63%, the yield of TSC obtained the highest yield of 0.64 mg/g. When the power of the microwave ranged from 402.4 W to 486.9 W, the yield of TSC gave the highest value of 0.64 mg/g at 460 W. The maximum value of TSC was 0.64 mg/g when the S/L ratio was 1: 50. The maximum value of TSC was maximum value of TSC was retained and remained unchanged when the S/L ratio continued to increase to 1: 68. Therefore, the maximum extraction variables were: ET 57 s, 63% of ethanol, extraction power 460 W and S/L ratio 1:68. Similarly, maximum methanol extraction parameters were ET = 58 s, 64% of methanol, extraction power: 436 W and S/L ratio of 1:62 resulted in the maximum value of 0.7 mg/g as indicated in Figs. S9a and S9b.

Verification of predictive models

Table 4 indicates the four optimum conditions depending on combinations of four responses. These optimal variables suggested extraction of TSC with 63% ethanol for 57 s at S/L ratio 1:68 at 460 W and extraction with 64% methanol for 58 s at S/L ratio 1:64 and 436 W. It suggested that the maximum value of TSC was 0.67–0.73 mg SE/g under ethanol extraction and 0.65–0.71 mg SE/g with methanol extraction under the optimum conditions. For optimal TFC, the optimum extraction parameters suggested extraction with 67.4% ethanol for 69 s, at S/L ratio 1:42 and 440 W and extraction with 72% ethanol for 59 s, at S/L ratio 1:52 and 400 W. The maximum value of TFC was 10.5–12.7 mg RE/g under ethanol extraction and 9.7–12.1 mg RE/g with methanol extraction as shown in Table 4.

Table 5 demonstrates the optimum conditions of TPC, $\%O_{sc}^{2-}$, FRAP and $\%\beta_{sc}$ suggested extraction with extraction with 62.6% ethanol for 56.5 s at S/L ratio 1:58.3 and 436 W and extraction with 64.9% methanol for 57.6 s at S/L ratio 1:50 and 480 W. These optimal parameters yielded TPC, $\%O_{sc}^{2-}$, $\%\beta_{sc}$ and FRAP of 67.6 mg GAE/g, 21.0%, 57.9% and 1.60 $\mu\text{mol/g}$, respectively. With the methanol extraction resulted in viz. 65.2 mg/g, 17.4–19.5%, 51.7% and 0.43 $\mu\text{mol/g}$, respectively. It could be observed that only small insignificant deviations ($p > 0.05$) were found between the experimental values and predicted values in both Tables 4 and 5. Thus, these models can be used for optimum extraction of bioactive compounds extraction from AR.

Table 4 Experimental and predicted values under optimum conditions based on combination of responses (TFC and TSC) with ethanol as the extraction solvent

Run	Variable levels				TFC (mg RE/g)		Variable levels				TSC (mg SE/g)	
	X ₁ (s)	X ₂ (%)	X ₃ (w)	X ₄ (%)	Obs (Y1) ^a	Pred (Y0)	X ₁ (s)	X ₂ (%)	X ₃ (w)	X ₄ (%)	Obs (Y1) ^a	Pred (Y0)
E-1	69	67.4	440	1:42	10.5 ns	11.4	57	63.0	460	1:68	0.73 ns	0.64
E-2	69	67.4	440	1:42	12.7 ns	11.4	57	63.0	460	1:68	0.67 ns	0.64
E-3	69	67.4	440	1:42	11.6 ns	11.4	57	63.0	460	1:68	0.68 ns	0.64
M-1	59	72.0	400	1:52	10.2 ns	10.5	58	64.0	436	1:62	0.71 ns	0.64
M-2	59	72.0	400	1:52	9.7 ns	10.5	58	64.0	436	1:62	0.68 ns	0.64
M-3	59	72.0	400	1:52	12.1 ns	10.5	58	64.0	436	1:62	0.65 ns	0.64

Obs observed, Pred predicted

(ns) = not significant ($p > 0.05$) between the experimental value and predicted values

^aMean of triplicate determinations

Table 5 Experimental value and predicted value under optimum conditions based on combination of responses (TPC, %O_{sc}²⁻, FRAP and %β_{sc}) with ethanol as the extraction solvent

Run	Variable levels				TPC (mg/g)		%O _{sc} ²⁻		%β _{sc}		FRAP (mM)	
	X ₁ (s)	X ₂ (%)	X ₃ (w)	X ₄ (%)	Obs (Y1) ^a	Pred (Y0)	Obs (Y1) ^a	Pred (Y0)	Obs (Y1) ^a	Pred (Y0)	Obs (Y1) ^a	Pred (Y0)
E-1	56.5	62.6	436	1:58.3	67.6 ns	66.31	20.2 ns	19.8	57.9 ns	55.5	1.60 ns	1.56
E-2	56.5	62.6	436	1:58.3	67.2 ns	66.31	21.0 ns	19.8	56.3 ns	55.5	1.61 ns	1.56
E-3	56.5	62.6	436	1:58.3	59.6 ns	66.31	19.5 ns	19.8	57.2 ns	55.5	1.48 ns	1.56
M-1	57.6	64.9	480	1:50	65.2 ns	60.6	17.4 ns	18.5	50.3 ns	53.3	0.43 ns	0.60
M-2	57.6	64.9	480	1:50	60.7 ns	60.6	19.5 ns	18.5	59.8 ns	53.3	0.43 ns	0.60
M-3	57.6	64.9	480	1:50	62.3 ns	60.6	19.0 ns	18.5	51.7 ns	53.3	0.43 ns	0.60

Obs observed, Pred predicted

(ns) = not significant ($p > 0.05$) between the experimental value and predicted values

^aMean of experiments triplicate determinations

TPC, TFC and TSC

In this study, TPC, TFC and TSC, which were sum of all phenolics, flavonoids and saponins evaluated, were analysed using the above chromogenic reaction for all samples. TPC, TFC and TSC were significantly different among all AR cultivars. The discrepancy is not surprising because plant source play an important role in total phenolic profile of AR, in addition to other factors such as ET, the S/L ratio, extraction solvent, the origin and species, which have been verified in previous studies (Rodríguez et al. 2005; Jain et al. 2011; Hossain et al. 2012; Guleria et al. 2013; Shah et al. 2013; Fan et al. 2015; Kulczyński et al. 2016). TFC of AR varieties measured by aluminum nitrate colorimetric method were between 11.6–19.1 mg RE/g with ethanol and 10.7–18.8 mg RE/g with methanol (Table 6). In previous study, TFC obtained from ethanol extracts was higher than methanol (Fan et al. 2015), but Hossain et al. (2012) study was totally opposite with Fan et al. (2015) study, which is

inconsistent with the finding of this study. It is likely that the plant root structure could contribute to these differing outcomes. The concentration of solvents can also affect the microwave extraction of flavonoids when the different concentrations of ethanol were used.

The TPC measured by the Folin–Ciocalteu method were 62.7–85.4 mg GAE/g the methanol and 64.8–91.8 mg GAE/g the ethanol extracts of the AR, which were higher than the TPC (Table 6). The greater TPC values as compared with the TFC and TSC values could be influenced by the microwave energy. Since polyphenols and ionic solutions strongly absorb microwave energy because of their permanent dipole moment, there will be an increase in the temperature and a transfer of energy to the plant materials and extraction solvent (Routray and Orsat 2012). Also, the interaction between microwave and solvent will cause the rupture of the plant cells which will then release the intracellular products into the solvent. The ethanol extraction of TPC was more efficient than methanol, which

Table 6 Effects of solvents on the total flavonoids content (TFC), total phenolics content (TPC), total saponins content (TSC), and total antioxidant activity (TAA) of different cultivars of asparagus roots

SAMPLE	Extraction solvent	TFC (mg RE/g)	TPC (mg RE/g)	TSC (mg SE/g)	O ²⁻ inhibition	mMFRAP mMFeSO ₄	%β _{sc} % inhibition	Caffeic acid (mg/g)
M-G-C	Ethanol	11.6 ± 0.3 ^{fg}	64.8 ± 2.0 ^{ef}	0.70 ± 0.02 ^{ef}	20.2 ± 2.3 ^{ef}	1.56 ± 0.17 ^c	57.1 ± 2.2 ^{de}	0.72 ± 0.03 ^{ef}
M-G-C	Methanol	10.7 ± 0.3 ^g	62.7 ± 2.0 ^f	0.69 ± 0.02 ^f	18.5 ± 2.3 ^f	0.43 ± 0.17 ^d	53.9 ± 2.2 ^e	0.55 ± 0.03 ^{fg}
M-G-NZ	Ethanol	18.4 ± 0.3 ^{ab}	81.2 ± 2.0 ^{bc}	0.93 ± 0.02 ^{ab}	67.8 ± 2.3 ^{abc}	5.13 ± 0.17 ^a	71.4 ± 2.2 ^{abc}	1.69 ± 0.03 ^c
M-G-NZ	Methanol	18.0 ± 0.3 ^{abc}	78.5 ± 2.0 ^{bc}	0.85 ± 0.02 ^{bcd}	67.3 ± 2.3 ^{bc}	5.03 ± 0.17 ^a	71.4 ± 2.2 ^{abc}	1.52 ± 0.03 ^c
M-P-C	Ethanol	15.3 ± 0.3 ^{de}	74.9 ± 2.0 ^{cde}	0.75 ± 0.02 ^{def}	31.2 ± 2.3 ^{de}	3.54 ± 0.17 ^b	67.4 ± 2.2 ^{bcd}	0.46 ± 0.03 ^g
M-P-C	Methanol	13.5 ± 0.3 ^{ef}	66.6 ± 2.0 ^{def}	0.70 ± 0.02 ^{ef}	22.2 ± 2.3 ^{ef}	1.35 ± 0.17 ^c	65.4 ± 2.2 ^{cd}	0.41 ± 0.03 ^g
M-P-NZ	Ethanol	17.5 ± 0.3 ^{abcd}	79.2 ± 2.0 ^{bc}	0.89 ± 0.02 ^{abc}	62.7 ± 2.3 ^c	3.89 ± 0.17 ^b	7.05 ± 2.2 ^{abc}	1.08 ± 0.03 ^d
M-P-NZ	Methanol	16.9 ± 0.3 ^{abcd}	78.1 ± 2.0 ^{bc}	0.83 ± 0.02 ^{bcd}	61.5 ± 2.3 ^c	3.54 ± 0.17 ^b	69.0 ± 2.2 ^{abc}	0.82 ± 0.03 ^e
M-W-C	Ethanol	16.1 ± 0.3 ^{bcd}	75.5 ± 2.0 ^{bcd}	0.84 ± 0.02 ^{bcd}	64.6 ± 2.3 ^c	3.87 ± 0.17 ^b	67.8 ± 2.2 ^{bcd}	0.50 ± 0.03 ^g
M-W-C	Methanol	15.8 ± 0.3 ^{cde}	67.3 ± 2.0 ^{def}	0.80 ± 0.02 ^{cde}	39.9 ± 2.3 ^d	1.60 ± 0.17 ^c	66.6 ± 2.2 ^{cd}	0.49 ± 0.03 ^g
M-Y-C	Ethanol	19.1 ± 0.3 ^a	91.8 ± 2.0 ^a	0.97 ± 0.02 ^a	79.4 ± 2.3 ^a	5.49 ± 0.17 ^a	79.6 ± 2.2 ^a	2.89 ± 0.03 ^a
M-Y-C	Methanol	18.8 ± 0.3 ^a	85.4 ± 2.0 ^{ab}	0.90 ± 0.02 ^{abc}	76.6 ± 2.3 ^{ab}	5.19 ± 0.17 ^a	78.0 ± 2.2 ^{ab}	2.64 ± 0.03 ^b

M-G-C microwave extraction of bioactives and antioxidant activity from Chinese green AR, *M-P-C* microwave extraction of bioactives and antioxidant activity from Chinese purple AR, *M-W-C* microwave extraction of bioactives and antioxidant activity from Chinese white AR, *M-Y-C* microwave extraction of bioactives and antioxidant activity from Chinese yellow AR, *M-P-NZ* microwave extraction of bioactives and antioxidant activity from New Zealand purple AR, *M-G-NZ* microwave extraction of bioactives and antioxidant activity from New Zealand green AR

The results were expressed as mean ± standard deviation (n = 3). Within each column, different letters indicates that difference was significant ($p < 0.05$)

is inconsistent with earlier studies on the residues of *A. officinalis* and *A. racemosus* roots (Hossain et al. 2012; Fan et al. 2015). Aqueous solvents have been reported to be more efficient in extracting total phenolics than absolute methanol (Jain et al. 2011; Guleria et al. 2013). TSC of AR varieties ranged from 0.7 to 0.97 mg SE/g with ethanol and 0.69 to 0.90 mg SE/g with methanol as measured by the vanillin colorimetric method. There are limited studies on TSC in *A. officinalis* roots, although a few Chinese studies have been investigated the Chinese green *A. officinalis* roots and stem. TSC in Chinese Green *A. officinalis* roots was compared with stem (104.9 and 102.1 mg saponin equivalent/g extract, respectively) with 75% ethanol extraction (Li et al. 2015).

The total antioxidant activity including FRAP, %O_{sc}²⁻, and %β_{sc} in six AR cultivars are shown in Table 6. All samples were extracted separately using three different conditions optimised for the FRAP, %O_{sc}²⁻ and %β_{sc} antioxidant activities. Results from the three antioxidant methods show no significant effect for the extraction solvent ($p > 0.05$), while there was a significant effect on the asparagus variety ($p < 0.05$) on the %O_{sc}²⁻, %β_{sc} and FRAP assays. These results show that Chinese yellow AR possesses the highest antioxidant activity followed by New Zealand green and the lowest activity is associated with the Chinese green variety, but some differences in the antioxidant activities suggest the antioxidant assays have

different sensitivities toward the bioactives in the extracts. In previous reports, ethanol was more efficient than methanol. However, those are inconsistent with the findings of this study (Ghimire et al. 2011; Jain et al. 2011). The results showed that the %O_{sc}²⁻ (64.6%) for the ethanol extraction was higher than that of methanol extract (39.9%) in the Chinese white AR, these are in contrast to previous studies (Subba and Mandal 2015). As previously reported, methanol is more efficient than ethanol for extraction of antioxidant capacity components (Subba and Mandal 2015). But for %β_{sc}, only one study has reported evaluation for β-carotene (336%) extracted from *A. officinalis* juice with absolute methanol (Sun et al. 2007). While FRAP values ranged from 1.56 to 5.49 μmol/g with ethanol and 0.43–5.19 μmol/g with methanol, indicating that the antioxidant activity as measured by the FRAP assay positively correlated with the TPC and TFC ($p < 0.05$). No effect was found for solvent on FRAP of New Zealand green, purple and Chinese yellow AR ($p > 0.05$). In previous study, the ethanol is more efficiency than methanol (Subba and Mandal 2015), influenced by the asparagus variety and origin (Guleria et al. 2013). In this study, different AR cultivars showed different antioxidant capacities, which is in agreement with the previous findings (Kulczyński et al. 2016). There was a strong positive correlation between the TPC and antioxidant activity. Lower correlation coefficients between the TSC and %β_{sc} may be related

to the kinetic action of antioxidants in the $\% \beta_{sc}$ assay, which might explain the discrepancy between the results obtained with the β -carotene assay.

Caffeic acid content

Several studies have reported that caffeic acid has an anti-diabetic effect in streptozotocin-induced diabetic rats (Celik et al. 2009) and suppresses lipid peroxidation (Son and Lewis 2002). Here, caffeic acid was extracted from the various cultivars with ethanol and methanol by MAE using the optimum variables through response surface methodology followed by HPLC-UV. The content of caffeic acid in AR cultivars ranged from 0.93 and 6.02 mg/g extract as indicated in Table 6. The ethanol and methanol extraction had a significant effect on caffeic acid obtained from the AR cultivars (Table 6), as the polarity of ethanol and methanol polarity can affect phenolic solubility (Subba and Mandal 2015). Ethanol extraction gave the highest yield of caffeic acid regardless of cultivar type and the concentration was in the following order; Chinese yellow AR > New Zealand green AR > New Zealand purple AR > TChinese green AR > Chinese white AR $\geq$ Chinese purple AR. A similar trend was found with methanol extraction. Huang et al. (2006) reported that 85 mg of pure caffeic acid was extracted from 710 g of AR extract. Extracts with higher caffeic acid content possessed stronger antioxidant capacity (Table 6). Yellow *A. officinalis* roots are a good source of caffeic acid. The study also showed that the activities of ARE are influenced by the environment and growing conditions since clearly higher bioactive levels were found in purple and green ARE from New Zealand compared with their counterparts from China.

Conclusion

RSM combined with a central composite design (CCD) was applied for MAE extraction to optimise extraction parameters of bioactive compounds from AR cultivars. In this study, four independent extraction parameters: ET, microwave powder, the S/L ratio and concentration of methanol and ethanol, had a significant effect on TPC, TFC, TSC and total antioxidant activities including FRAP, $\%O_{sc}^{2-}$ and $\% \beta_{sc}$ of AR cultivars. Generally, higher TPC, TFC and antioxidant activity were obtained with ethanol extraction in comparison to methanol extraction as compared to the conventional extraction methods. The Chinese yellow AR indicated the highest content of bioactive compounds including caffeic acid. Not only did the bioactive compounds extracted by the MAE have improved yield of total antioxidants, the extraction preparation also

required significantly lesser power and energy compared to the conventional methods.

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