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Optimization of extraction parameters of antioxidant activity of extracts from New Zealand and Chinese *Asparagus officinalis* L root cultivars

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ABSTRACT

Optimisation of the antioxidant activity of asparagus root extract (ARE) and inosine from New Zealand and Chinese asparagus root cultivars was carried out using a response surface methodology (RSM) using Box-Behnken design (BBD). The determination of inosine was carried out using HPLC under optimal extraction conditions. The independent parameters variables combination (extraction temperature 51 °C, extraction time 73.02 min, ethanol 75.23% and solid: liquid ratio 1:50) produced maximum total flavonoids content (TFC) (9.2–17.1 mg RE/g dry root), total phenolic content (TPC) (14.7–35.2 mg GAE/g dry root) and total saponin content (TSC) (9.2–17.1 mg RE/g dry root). Extraction at temperature 50 °C for 78.5 min, using 70% ethanol at solid: liquid ratio of 1:40 for maximum 2,2-diphenyl-1-picrylhydracyl (DPPH) (38.9–78.1%), 2,2-azinobis(3-ethylbenzo-thiazoline-6-sulfonate) (ABTS) (36.6–61.2%), ferric reducing antioxidant power assay (FRAP) (0.54–1.69 μmol/g), β-carotene bleaching assay (51.2–76.0%) and superoxide anion radical (O^{2-}) scavenging capacity (42.5–70.2%). For methanol, extraction conditions viz. extraction temperature at 51 °C for 75 min, using 75% methanol at solid: liquid ratio of 1:50 resulted in maximum TFC (12.0–13.4 mg RE/g dry root), TPC (25.1–26.2 mg GAE/g dry root) and TSC (5.9–6.4 mg SE/g dry root). Extraction temperature at 50 °C for 76.5 min using 80% methanol at solid: liquid ratio of 1:50 produced maximum%$DPPH_{sc}$ (55.8–69.9%), %$ABTS_{sc}$ (43.0–52.0%), FRAP (0.54–0.59 μmol/g dry root), %βsc (49.2%–71.2%) and%O^{2-}_{sc} (34.4–41.6%). The content of inosine from ARC ranged from 1.3 to 6.0 mg/g with ethanol and from 0.9 to 4.1 mg/g with methanol extraction.

1. Introduction

The use of herbal medicine for treatment of diseases dates back to ancient times (Velavan, 2015). Approximately 25% of all modern medicines produced contain plant-based active ingredients (Phillipson and Anderson, 1989). Moreover, 11% of the 252 drugs that are categorised as basic and essential drugs by WHO and 50% of the approved drugs used for the cancer treatment are synthesized from plant-derived active compounds (Himanshu et al., 2013; Veeresham, 2012). This is because the majority of these active compounds still cannot be synthesized economically (Himanshu et al., 2013). Due to ineffective therapy and side effects of synthetic drugs and lack of regular access to essential modern medicines by one-third of the world's population, herbal medicine plays an important role in treating illnesses (WHO, 2003). Over the past 30 years, the use of herbal medicine by the world's population has increased significantly (Veeresham, 2012). According to WHO (2003), the global market for herbal medicines has approached USD$60 billion, suggesting that the market for herbal medicines is substantial and will continue to grow steadily.

Asparagus officinalis L. (green asparagus), a member of the family Asparagaceae, has a long history of use as a herbal medicine (Fan et al., 2015). Green asparagus is known as the "king of vegetables" throughout the world (Fan et al., 2015). Green asparagus, which is native to Europe, Mediterranean and Western Asia, has been cultivated widely for more than 2000 years (McKinlay, 1992). The young edible shoots of green asparagus is considered a delicacy, while the roots of green asparagus can be used as a remedy for edema due to its diuretic effect (Negi et al., 2010). *Asparagus racemosus*, another asparagus species whose roots have been used for traditional medicines, has been receiving more interest as a result of its important pharmacological actions including anti-depressant, anti-hepatotoxic and anti-neoplastic (Muruganandan et al., 2000; Singh et al., 2009). Major bioactive compounds of *A. officinalis* L root (AR) include steroidal saponins, flavonoids (mainly rutin) and dietary fibre (Huang and Zhang, 2006;

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Huang and Kong, 2006; Huang et al., 2008; Negi et al., 2010). In addition, AR also has a high content of inosine (Huang and Zhang, 2006), which may have a potential therapeutic value. Inosine was reported to be 0.4% in AR (Huang and Zhang, 2006). The administration of inosine was associated with an improved behavioural outcome and axonal reorganization in rats with cortical strokes (Chen et al., 2002). Very little is known about bioactivity of the ARE, and none is available for New Zealand cultivars. Therefore, an effective technique to obtain bioactive compounds from AR, is highly desired to study the bioactivities of these material.

There are a few studies that investigated the effect of extraction parameters on bioactive compounds from Chinese AR using RSM and none is available for New Zealand (NZ) AR. The RSM is a collection of mathematical and statistical methods that is widely used in the optimization of extraction conditions in bioengineering and food processing (Ibrahim and Elkhidir, 2011). Compared to the traditional "single factor" technique, RSM is less laborious and time-consuming (Bezerra et al., 2008). In addition, RSM provides data on the interaction between variables and gives multiple responses at the same time (Bezerra et al., 2008). Therefore, RSM is a powerful statistical technique that is used in developing, improving and optimizing the extraction process variables (Bezerra et al., 2008). BBD was used in this study to optimize the experimental model. BBD requires fewer experiments, is efficient and easier to arrange and interpret than other RSM experimental designs (Ferreira et al., 2004).

In this study, conventional extraction technology was applied to extract TPC, TFC, TSC and total antioxidant activity of Chinese and NZ AR. Four factors (extraction temperature, solvent concentration, extraction time and solid: liquid ratio) were chosen for optimizing the extraction parameters of AR by employing four-factor, three-level BBD.

2. Materials and methods

2.1. Chemicals and equipment

Ultrapure water was prepared using a Millipore Direct®Q3 (Millipore Corp., MA, USA) and was used throughout the study. 2,2′-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid) (ABTS), DPPH(1,1-dipheny1-2-picrylhydrazyl), TPTZ (2,4,6, -tri(2-pyridyl)-s-triazane), nitrobue tetrazolium, phenazine methosulphate, dihydronicotinamide adenine dinucleotide, trans-β-carotene, butylated hydroxytoluene, tris-HCl and linoleic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was from TCL, company). Folin-Ciocalteu reagent (Beijing Sollebao Biotechnology Co., LTD, Beijing, China). Tween 80 emulsifier, chloroform, ethanol, methanol, $NaH_2PO_4{\cdot}H_2O$, Na_2HPO_4 (Tianjin Biotechnology Co., LTD, Tianjin, China), gallic acid, rutin and saponin were obtained from Lvyuan Biotechnology Co., LTD, (Shanghai, China). The shaker water bath (LSHZ-300) was purchased from Baowei Laboratory Equipment Co., LTD (Beijing, China). Micro plant grinding machine (FZ102) was from Tianjin Shi Taisite Equipment Co., LTD (Tianjing, China). Freeze-dryer (ALPHA1–2) was from Martin Christ Gefriertrocknungsanlagen Co., LTD (Osterode, Germany), Power Wave HT – Microplate reader was from Bioteck Co., LTD (Vermont, USA) and a High performance liquid chromatography system (Agilent 1200 HPLC) was from Agilent Technologies, (City, USA).

2.2. Materials

White, green, yellow and purple AR were obtained from a commercial asparagus farm (Haze City, Shandong Province, China). Green and purple AR were obtained from a commercial asparagus farm in the South Island of New Zealand (Palmerston, New Zealand).

2.3. Sample preparation

The samples were cleaned using running tap water and rinsed with distilled water prior to use (Himanshu et al., 2013). The AR were frozen at −20 °C and freeze-dried. The dried AR were powdered using the micro plant grinding machine and were screened through a 40-mesh sieve to obtain particles with a mean diameter ≤420 μm. These powders were flushed with nitrogen before being stored in sealed containers at −20 °C until analysis.

2.4. Extraction procedure

The optimization method was designed according to Zhao et al. (2011). A mathematical model was used for the description of the extraction conditions of AR. One-factor-at-a-time method was utilized to determine the range for the extraction conditions (solvent, time, temperature and solid: liquid ratio) and then a BBD was used for the optimization models, based on TPC, TFC and total antioxidant activities (TAC) (DPPH, ABTS, FRAP, O^{2-} and β-carotene) of AR samples (0.3, 0.5 and 1.5 g) added to 20%, 60% and 100% (v/v) of solvent (ethanol or methanol) in distilled water at solid: liquid ratio of 1:20, 1:60 and 1:100 in 50 mL flasks. The extraction flasks were placed in water baths maintained at 30 °C, 50 °C and 70 °C for 20, 60 or 100 min at shaking speed of 80 rpm. The experimental design model is described in detail in Section 2.10.

2.5. HPLC analysis of inosine in ARE

After extracting AR under optimal extraction variables, samples were centrifuged for 20 min at 4200 rpm and −4 °C (H1650R, Qimin Biotech Company, Shanghai, China), and then filtered using a membrane filter (0.22 μm) and analysed by HPLC-UV. The HPLC system was connected to a reversed-phase Agilent SB-C18 column (250 mm × 4.6 mm i.d.) using a quaternary pump, an auto sampler and a diode array detector for detection of eluted compounds. The injection volume was 10 μL for all standards and samples. The mobile phase was 0.01% formic acid solution in water (solvent A) and methanol (solvent B). The mobile phase consisted of 0.01% formic acid-water and methanol (10:90) and the flow rate was maintained at 0.25 mL/ min. The detector was set to a wavelength of 245 nm and the column temperature was maintained at 30 °C (Zhang 2011). Inosine (0–500 μg/ml) seven points standard series was used to produce the calibration curve.

2.6. Determination of total phenolic content (TPC)

Extraction of total phenols in the extracts of Chinese and NZ AR samples was carried out based on the Folin-Ciocalteu method of Hossain et al. (2012). The results of TPC were expressed as gallic acid equivalents per gram of dry weight (mg GAE/g).

2.7. Determination of total flavonoid content (TFC)

TFC was determined according to the aluminum chloride colorimetric method described previously by Fan et al. (2015). A seven point standard curve (0–60 μg/ml) was constructed using rutin as the reference standard and the results of TFC were expressed as rutin equivalents (RE) mg per g dry weight (DW).

$$\mathrm{C\ (mg\ RE/g)} = \frac{\mathrm{n}CV}{m \times 1000} \quad (1)$$

Where C = total content of flavonoid compounds in mg/g plant extract, C = concentration of rutin established from the calibration curve in mg/ml, V = volume of extract in ml, m = weight of crude plant extract in g and n = dilution ratio.