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The effects of different extraction methods on the physicochemical properties and antioxidant activity of *Amygdalus pedunculatus* seed oil

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Summary

The oil extracted from *Amygdalus pedunculatus* (*A. pedunculatus*) seeds is rich in nutrients. The method of oil extraction is very crucial for preserving its nutrients. The objective of the present study was to compare *A. pedunculatus* seed oil (APO) samples extracted by different techniques including aqueous enzymatic extraction (AEE), cold-press (CP), supercritical fluid extraction (SFE), and Soxhlet extraction (SE). Physicochemical properties and nutrients (fatty acids, triacylglycerol, polyphenol, tocopherol and phytosterol) of the oils were analyzed. Antioxidant activity was measured by DPPH, $ABTS^{\cdot+}$ radical scavenging capacity and reducing power assays. The results indicated that SFE was found to be the optimum method for APO extraction with higher nutrient contents as well as better DPPH, ABTS scavenging capacities and reducing power. APO is beneficial to human health, and it has potential to be used in nutraceutical industries.

Introduction

Amygdalus pedunculatus (*A. pedunculatus*), a member of the plant family *Rosaceae*, is a deciduous, sand-dune-stabilizing, and oil-bearing shrub. It is distributed in the arid region of Northwest China and Mongolia. The plant shows strong tolerance to cold and drought environments and good adaptability to different types of soil moisture. *A. pedunculatus* has been widely used to tackle afforestation for preventing desertification in China in recent years (Chu et al., 2013). It contributes significantly to local economy through its application in nutraceutical industry. However, most of *A. pedunculatus* seeds are wasted in the harvesting season due to lack of deep processing technique.

In the last decade, APO has drawn attention of many researchers because of its significant dietetic value. High contents of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) have been found in APO. On the other hand, APO is also rich in phenolic, tocopherol and phytosterol antioxidant compounds that have recently drawn attention of nutraceutical industry for their high functional properties (Maranz et al., 2003).

In the present scenario, it is important to design a suitable extraction method that not only extracts oil from *A. pedunculatus* seed but also preserves the nutrient content in oil. Aqueous enzymatic extraction (AEE) is an eco-friendly method based on simultaneous isolation process of oil and protein from seeds. The CP process has become a good substitute of solvent extraction for obtaining natural and healthy edible oil (Karaman et al., 2015). Supercritical fluid extraction (SFE) has gained a lot of attention because of carbon dioxide (CO_2) that is used as supercritical fluid in the method (Crowe and White, 2003); CO_2 is an environment friendly, inexpensive, non-toxic, and inert solvent which allows the extraction process to be performed at low temperature and pressure (Jung et al., 2012). Traditional Soxhlet extraction (SE) uses organic solvents, and it is considered as one of the most effective extraction methods for vegetable oil seeds.

Although there are many reports available on physicochemical properties and antioxidant activity of oils (Ni et al., 2015; Parry et al., 2005) very few investigations focus on the effects of extraction methods on those attributes of oils. Hence, we undertook this research work with the objective to investigate the effects of different extraction methods on physicochemical characteristics and valuable compounds (fatty acids, triacylglycerol, polyphenol, tocopherol, and phytosterol compounds) content of the extracted oil from *A. pedunculatus* seeds. Antioxidant activity was used to compare *A. pedunculatus* seed oil samples, extracted by four different extraction techniques.

Materials and methods

Materials

A. pedunculatus seeds were collected from Yulin, Shaanxi Province, China and stored at 4 °C prior to analysis. Alcalase2.4L was purchased from Novozymes (Novo, China). All reference substances for triacylglycerol (trilinolein, triolein, glycerol tripalmitate, glycerol trimyristate, and glycerol tristearate), tocopherols (α, β, γ, and δ isomers), phytosterols (cholesterol, botulin) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Aldrich.

Aqueous enzymatic extraction (AEE) process

A. pedunculatus seeds were crushed by roll crusher three times. A mixture of crushed *A. pedunculatus* seeds (100 g) and distilled water at 1:5 (wt/vol) were taken in a reaction by gentle stirring to make slurry, the slurry pH was adjusted to 8.00 by adding 0.20 mol/L NaOH and incubated at 60 °C for 0.5 h with continuous stir at 200 rpm. The protease (2 mL) was added after the pH and temperature of the slurry were adjusted to the optimal condition. Then the slurry was incubated for 6 h with continuous stirring, followed by centrifugation at 4350 rpm for 15 min to obtain maximum amount of free oil which was named AEEO.

Cold pressing (CP) process

A. pedunculatus seeds were wrapped inside four layers of filtration cloth and pressed using a laboratory hydraulic press (dimension: 650 L × 800 D× 1370 H mm) (National Eng Co., Ltd., Korea). The maximum pressure for mechanical hydraulic press extraction was 60 MPa and held at the pressure for 20 min. The oil was separated through centrifugation to remove any particles and was named CPO.

Supercritical fluid extraction (SFE) process

A. pedunculatus seeds were crushed by feed crusher three times. Crushed *A. pedunculatus* seeds were taken in a steel cylinder, equipped with mesh filters (100 μm) on both ends to protect particles from

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being flushed out. *A. pedunculatus* seeds (100 g) were transferred into a 300 mL extraction vessel. The equipment used for supercritical extraction (SCE) process with CO_2 was the SCE Screening System model manufactured by Autoclave Engineers (Applied Separations Inc., Newark, USA). The static extraction time was set for 0.5 h, the dynamic extraction time were 1.5 h with a CO_2 flow rate of 3 L/h. The extraction was carried out under the following conditions: pressure: 400 bar; temperature: 40 °C. The oil was separated by pressure reduction and collected in the flask and was named SFEO.

Soxhlet extraction (SE) process

The *A. pedunculatus* oil was extracted using the Foss Soxtec™ system 8000 extraction unit (Hilleroed, Denmark), following the method of Brkic et al. (2006). The oil was named SEO.

Physicochemical properties of A. pedunculatus seed oils

Standard methods made by American Oil Chemists' Society (AOCS, 1998) were used to determine phospholipid content, refractive index, color, density, free fatty acid (FFA), iodine value (IV), peroxide value (PV), and oxidative stability (OSI) (AOCS, 1998) of the extracted oils.

Fatty acid composition

Fatty acid composition were determined according to the method describe by Mandana et al. (2013). *A. pedunculatus* oil after methylation was determined using gas chromatography (GC) (6890N, Agilent, USA) with a capillary column (VF-23ms 30 m × 0.25 mm × 0.25 μm, Agilent, USA) and a flame ionization detector (FID).

Triglycerides composition

Triglycerides composition were determined according to the method describe by Shukla et al. (1983). A Waters high performance liquid chromatography (HPLC) column (Waters Corporation, USA), fitted with Symmetry 300TM C185 μm (4.6 mm × 250 mm), was used to carry out chromatographic separation.

Polyphenol content

Polyphenol content was determined according to the method by Parry et al. (2005). *A. pedunculatus* oil was determined by the Folin-Ciocalteu assay, the absorbances were recorded by a UV-visible spectrophotometer (Shimadzu, Japan) at 765 nm. We used milligram of gallic acid equivalent (GAE) per g (mg GAE/g) of extracted oil to express the results.

Tocopherol content

Tocopherol content was determined according to AOCS Method (AOCS, 1998) using a HPLC equipped with a fluorescence detector. The HPLC system was consisted of a LiChroCART @ 250-4 column (250 mm × 4.0 mm), 2695 pump and 2475 Multi λ Fluorescence Detector (Waters Corporation, USA). Excitation and emission wavelengths were set at 295 nm and 330 nm, respectively. The mobile phase was consisted of n-hexane/ tetrahydrofuran (1000/40 by vol.), and a flow rate of 1.0 mL/min was used. The oils were diluted in n-hexane before analysis. The tocopherol content was expressed in milligram of tocopherol per 1000 g of extracted oil.

Phytosterol content

The content and composition of phytosterol in *A. pedunculatus* seed oil samples were analyzed according to an ISO method (ISO, 1999). Agilent (6890N, Agilent, USA) GC loaded with a flame ionization detector and a HP-5MS capillary column (30 m × 320 μm × 0.25 μm) was used to confirm phytosterol peak identity. Betulin was used as an internal standard for quantification. Phytosterol from each analyzed oil sample was identified by the relative retention time (RRT). RRT was expressed as the ratio of retention time of phytosterol to be determined and betulin.

Antioxidant activity

Scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical

The antioxidant activity of *A. pedunculatus* seed oils extracted by four different methods was determined by a DPPH assay according to Brand et al. (1995). The decrease in absorbance of DPPH at 515 nm was determined using a UV-vis spectrophotometer (Shimadzu, Japan). The radical-scavenging ability of the experimental samples was measured based on the following formula:

Scavenging DPPH (%) = $[(A_{cont} - A_{sample})/A_{cont}] \times 100$,

where A_{cont} and A_{sample} were the values of absorbance of blank sample and test sample at particular times, respectively.

Scavenging activity of radical cation 2, 2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$)

The antioxidant activity of the extracted oils was determined using ABTS radical cation decolorization assay (Re et al., 1999). The radical-scavenging activity of the experimental oils was expressed as percentage inhibition of $ABTS^{\cdot+}$ and calculated based on the following formula:

Scavenging $ABTS^{\cdot+}$ (%) = $[(A_{cont} - A_{sample})/A_{cont}] \times 100$,

where A_{cont} and A_{sample} were the values of absorbance of blank sample and test sample at particular times, respectively.

Reducing power

Reducing power was determined according to a previously reported method by Hu et al. (2008).

Statistical Analysis

The data were expressed as mean ± standard deviation, and the analysis of variance (ANOVA) was performed using SPSS (version 17.0 for Windows 2007, SPSS Inc); results with $p \leq 0.05$ were considered as statistically significant.

Results and discussion

Physicochemical characteristics

Tab. 1 lists the oil yield and physicochemical properties of oils extracted using four different methods. As evident from the results presented in Tab. 1, the extraction yields of oil from the *A. pedunculatus* seed were 40, 41, 30, 50 g oil/100g seeds after AEE, CP, SFE, SE, respectively. The phospholipid contents in the oils were expressed as mg P/kg oil. The SEO exhibited the highest phosphorus content, followed by AEEO and CPO. Interestingly, SFEO did not have detectable quantity of phosphorus probably because of the low solubility of phospholipids. Our results agreed well with previous data obtained from the Perilla oil (Min et al., 2012). We did not find any significant differences in the values of refractive index and density of the extracted oils. The oils extracted by AEE and SE methods exhibit higher color attributes than those extracted via other two methods. The level of yellow color of oil obtained from AEEO, SEO, CPO and SFEO was at 31, 40, 17, and 20, respectively, while the level of red color was at 1.7 for all. This indicated that the higher the experimental temperature was, the deeper the yellowness of the

Tab. 1: Physicochemical characterization of *A. pedunculatus* seed oil extracted using four different methods

	Extraction method			
	AEE	CP	SFE	SE
Oil Yield (g/100 g seeds)	40±1.2 [b]	41±0.8 [b]	30±1.1 [a]	50±1.3 [c]
Phospholipid (mg/kg oil)	35 ± 0.03 [c]	14 ± 0.04[b]	ND [a]	215 ± 0.11[d]
Refractive index (25 °C)	1.5	1.5	1.5	1.5
Density, 25 °C (g/cm^3)	0.92	0.91	0.91	0.91
Color (yellow)	31	17	20	40
Color (red)	1.7	1.7	1.7	1.7
FFA (% oleic acid)	0.24 ± 0.01[b]	0.46 ± 0.01[d]	0.16 ± 0.01[a]	0.35 ± 0.02[c]
PV (mmol O_2/kg oil)	0.48 ± 0.04[ab]	0.76 ± 0.01[c]	0.42 ± 0.13[a]	0.62 ± 0.02[bc]
IV (g I_2/100 g oil)	102 ± 2.0[a]	98 ± 0.68[a]	113 ± 2.3[b]	103 ± 1.5[a]
OSI (120 °C, h)	7.0 ± 0.27[a]	7.5 ± 0.48[ab]	8.3 ± 0.42[c]	7.6 ± 0.25[b]

Values are means ± standard deviations for three preparations
Values given in rows followed by different superscript letters are significantly different at $p \leq 0.05$
FFA: Free fatty acid; IV: Iodine value; PV: Peroxide value; OSI: Oxidative stability; ND: not detect.

oils was. The FFA of APO extracted by CP was the highest among all. A low acidity value indicated higher stability of the oil extracted by SFE method compared to others. Among the four extraction methods, the oil obtained from SFE showed the highest IV, followed by the oils extracted by SE, AEE and CP. Compared to AEE and SE, oils extracted by CP exhibits the highest PV, while the oil extracted by SFE method showed the lowest PV (0.42 mmol O_2/kg). The reason for lowest PV could be attributed to the presence of large amount of natural antioxidants in the extracts of supercritical carbon dioxide. APOs could be preserved for a long period without deterioration because of their low PV values; oils became rancid when their peroxide value exceeds 10 mmol O_2/kg (AOCS, 1998). The lower the FFA and the PV value, the better.

The oxidative stability of seed oil is usually poor due to its high linoleic acid content. Here the stability of APO at 120 °C was expressed as induction time of oxidation that ranges from 7.0 h to 8.3 h for the studied oils. These values were greater than those measured for linseed oil (1.1 h) and olive oil (6.1 h) (WAGNER et al., 2000). The high oxidation induction time of APOs could be ascribed to the presence of large amount of natural antioxidants such as polyphenol, tocopherols, and phytosterols and Oleic acid. The OSI values had clearly revealed the differences in stability among the four studied oils. We found that the SFEO had the highest oxidation induction time. The higher the IV and the oxidation induction time, the better.

Fatty acid composition

Tab. 2 presents fatty acid composition of the oils extracted by four different methods. The oleic and linoleic acids were the most abundant unsaturated fatty acids present in the oils, while palmitic acid

Tab. 2: Fatty acid composition of *A. pedunculatus* seed oil obtained from different extraction methods (%)

	Extraction method			
	AEE	CP	SFE	SE
Palmitic ($C_{16:0}$)	1.5 ± 0.01[a]	2.4 ± 0.01[d]	1.6 ± 0.01[c]	1.6 ± 0.01[b]
Palmitoleic ($C_{16:1}$)	0.18 ± 0.01[a]	0.16 ± 0.01[a]	0.29 ± 0.03[b]	0.27 ± 0.01[b]
Stearic ($C_{18:0}$)	0.55 ± 0.01[b]	0.77 ± 0.02[c]	0.50 ± 0.01[a]	0.57 ± 0.01[b]
Oleic ($C_{18:1}$)	69 ± 0.05[c]	71 ± 0.04[d]	68 ± 0.15[a]	68 ± 0.07[b]
Linoleic ($C_{18:2}$)	28 ± 0.02[b]	25 ± 0.03[a]	29 ± 0.09[c]	28 ± 0.01[b]
Linolenic ($C_{18:3}$)	0.12 ± 0.00[a]	0.13 ± 0.01[a]	0.20 ± 0.01[b]	0.62 ± 0.02[c]
Eicosenoic ($C_{20:1}$)	0.22 ± 0.01[ab]	0.27 ± 0.01[b]	0.19 ± 0.01[a]	0.24 ± 0.02[b]
SFA	2.1 ± 0.01 [a]	3.2 ± 0.01[c]	2.1 ± 0.02 [ab]	2.1 ± 0.01[b]
USFA	98 ± 0.32[b]	97 ± 0.00[a]	98 ± 0.05[b]	98 ± 0.03[b]
MUFA	69 ± 0.30[b]	71 ± 0.04[c]	68 ± 0.15[a]	69 ± 0.05[bc]
PUFA	28 ± 0.02[b]	25 ± 0.04[a]	29 ± 0.09[d]	29 ± 0.03[c]
USFA/SFA	48 ± 0.20[d]	30 ± 0.08[a]	47 ± 0.05[c]	46 ± 0.12[b]

[1]SFA: saturated fatty acids; [2]USFA: unsaturated fatty acids; [3]MUFA: monounsaturated fatty acids; [4]PUFA: polyunsaturated fatty acids.
Values are presented as means ± standard deviations for three preparations
Values given in rows followed by different superscript letters are significantly different at $p \leq 0.05$

was the principal saturated fatty acid. The content of the essential unsaturated fatty acid (USFA) in APO samples extracted by different methods was high. We also identified some more fatty acids including palmitoleic, stearic, linolenic, and eicosenoic acids, though in less quantity. USFA was major component of the total fatty acid in AEEO (98%), SFEO (98%), SEO (98%), and CPO (97%). USFA exhibited excellent nutritional and physiological properties that help in preventing cancer and coronary heart disease (OOMAH et al., 2000). MUFA was the major component of total fatty acids in APOs, extracted by different methods, mainly because of the higher amount of oleic acid in APO. MUFA could lower "bad" cholesterol (low density lipoproteins or LDL) and retain "good" cholesterol (high density lipoproteins or HDL) (RAMADAN et al., 2010). The high MUFA content made APO a potential functional component of nutrition to be used in food industry. In addition, the fatty acid content and high PUFA content made APO an important component of nutrition. The ratio of unsaturated to saturated fatty acids obtained from AEEO, SFEO and SEO were 48, 47 and 46, respectively, while it was 30 for CPO. Therefore, the method of extraction had a significant ($p \leq 0.05$) effect on the fatty acid composition of the oils.

Triacylglycerol composition

Based on the total number of carbon in the acyl side chains and the equivalent carbon number (ECN), the triacylglycerol species were identified. Tab. 3 shows the triacylglycerol composition of *A. pedunculatus* seed oil. According to the data presented in Tab. 3, the oils had four types of triacylglycerol with ECN ranging from 42 to 48. Triacylglycerols with ECN 48 and 46 were dominant, followed by ECN 44 and 42. We found that SLO + OOO and OLO (O = Oleic acid, L = Linoleic acid, S = Stearic acid) were the major triacylglycerols representing approximately 65% of oils obtained from different extraction methods. Therefore, the major triacylglycerol molecular types were oleic and linoleic acids, indicating high unsaturation of APO. The CPO had lower contents of LLL, LOL + OLnO, LLP, OLO, OLP (P = Palmitic acid, Ln = Linolenic acid) compared to AEEO, SFEO, and SEO. The low triacylglycerol content of CPO could be due to the shortest extraction time involved in the process. In addition CPO had the lowest linoleic acid content as revealed from the fatty acid composition (Tab. 3).

Polyphenol composition

Polyphenol not only exhibit antioxidants but also have biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating action (PIETTA, 2000). Fig. 1 shows the total polyphenol contents of the oils obtained from different extraction methods. As evident from Fig. 1, SFEO exhibited the highest polyphenol content of 0.43 mg $GAEg^{-1}$ dry weight (DW), followed by AEEO (0.39 mg $GAEg^{-1}$ DW), SEO (0.34 mg $GAEg^{-1}$ DW), and CPO (0.26 mg $GAEg^{-1}$ DW). The polyphenol contents of our studied oils fall in the middle of those of olive oil (0.07 mg $GAEg^{-1}$ DW–1.3mg $GAEg^{-1}$ DW) (BELTAN, 2007). SEO, AEEO and SFEO exhibited higher total polyphenol content than CPO. This could be due to the reason that the pressure applied in CP sometimes (sporadically) failed to squeeze polyphenol out of the cell wall. The polyphenol content positively influenced oxidative stability, nutritional and health properties of APO.

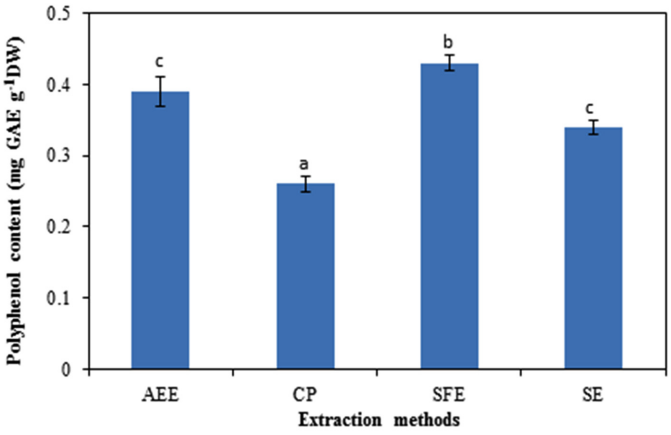

Fig. 1: Polyphenol content of *A. pedunculatus* seed oil obtained by different extraction methods [results with standard deviations shown by same letters (a-c) are not significantly different ($p < 0.05$)]

Tocopherol composition

Tocopherol, an essential nutrient to human, is the most significant lipid soluble antioxidant. It plays crucial role in scavenging free radicals and preventing lipid peroxidation in biological membranes. Fig. 2 depicts tocopherol contents of APOs obtained from four different extraction methods. SFEO had the highest total tocopherol content (1092 mg/kg) followed by AEEO (899 mg/kg), SEO (892 mg/kg), and CPO (793 mg/kg). The tocopherol content in *A. pedunculatus* seed oils obtained from different extraction methods

Tab. 3: Triglycerides content (%) found in *A. pedunculatus* seed oil obtained from different extraction methods

ECN	Triglyceride	Extraction method			
		AEE	CP	SFE	SE
42	LLL	0.75 ± 0.11^{a}	0.68 ± 0.03^{a}	0.97 ± 0.04^{a}	0.98 ± 0.13^{a}
44	LOL+ OLnO	10 ± 0.86^{ab}	9.4 ± 0.18^{a}	12 ± 0.76^{b}	11 ± 0.10^{ab}
44	LLP	3.5 ± 0.03^{b}	3.1 ± 0.01^{a}	4.1 ± 0.04^{b}	3.7 ± 0.01^{b}
46	OLO	29 ± 0.78^{b}	26 ± 0.13^{a}	30 ± 0.41^{b}	30 ± 0.74^{b}
46	OLP	10.9 ± 0.03^{b}	9.6 ± 0.01^{a}	11 ± 0.01^{c}	11 ± 0.01^{c}
48	SLO+OOO	37 ± 0.04^{b}	41 ± 0.05^{c}	33 ± 1.2^{a}	34 ± 0.52^{ab}
48	SLP	9.1 ± 0.01^{b}	10 ± 0.02^{c}	8.3 ± 0.02^{a}	8.6 ± 0.06^{ab}

ECN (equivalent carbon number) = CN (carbon number)-2DB (double bond number); L= Linoleic acid, O = Oleic acid, S = Stearic acid, P = Palmitic acid, Ln = Linolenic acid

Values are presented as means ± standard deviations for three preparations

Values given in rows followed by different superscript letters are significantly different at $p \leq 0.05$

was higher than that of *Sclerocarya birrea* seed oil (137 mg/kg) and olive oil (167 mg/kg - 463 mg/kg) (Mariod et al., 2004; Ceci and Carrelli, 2010). HPLC results revealed γ-tocopherol (followed by δ-tocopherol, β- and α-tocopherols) as the major tocopherol present in APO. SFEO contained the maximum amounts of γ-, δ- and β-tocopherols, followed by AEEO, SEO and CPO (Fig. 2). However, α-tocopherol was obtained as the lowest tocopherol isomer in solvent-extracted oils (23 mg/kg in SEO) as well as in solvent free oils (24 mg/kg in SFEO, 19 mg/kg in AEEO, and 26 mg/kg in CPO).

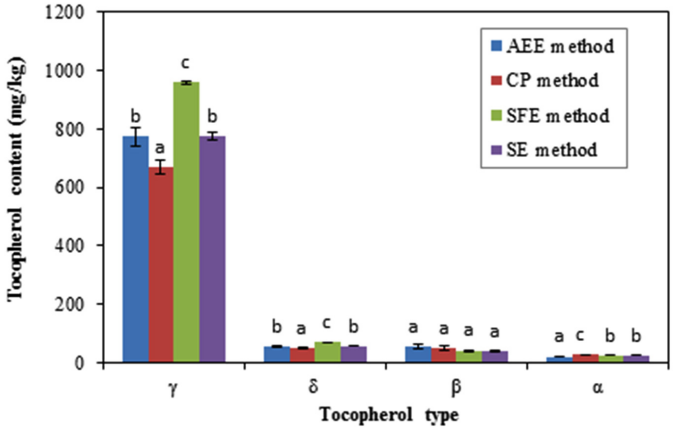

Fig. 2: Individual tocopherol contents of APO samples extracted by different methods [results with standard deviations shown by same letters (a-c) are not significantly different ($p < 0.05$)]

Phytosterols composition

The phytosterol level in seed oils is used to determine the quality of oil. Phytosterols are highly valued components in health products because of their ability of lowering blood cholesterol by blocking re-adsorption of cholesterol from the gut (Ceci and Carrelli, 2010). As evident from Tab. 4, individual as well as total phytosterols change depending on the extraction method. SFEO had a higher content of phytosterols than the oils obtained by other methods ($p < 0.05$). Sitosterol was the most abundant phytosterol found in all oil samples, followed by Δ5-avenasterol and campesterol. This confirmed SFE was an excellent method for accumulating phytosterol content in the extracted oil (SFEO). The high stability of SFEO and the low stability of CPO could be attributed to the fact that some of the phytosterols, such as Δ5-avenasterol, can function as antioxidants (Kamal et al., 1992).

Antioxidant activity

DPPH is a stable radical that is often used for evaluating radical-scavenging capacity of antioxidants. As shown in Fig. 3a, all the studied oils obtained from different extraction methods directly react with DPPH radicals and quench them. Among the studied oils, SFEO exhibited the least IC_{50} value (17 mg/mL), followed by CPO (25 mg/mL), SEO (39 mg/mL), and AEEO (44 mg/mL). Lower IC_{50} value indicated more intense activity of scavenging DPPH free radicals. Therefore, the DPPH radical scavenging activities of SFEO and CPO were higher than that of SEO and AEEO. Antioxidant activities of the APO might be due to the presence of phenolic and nonphenolic components. Our study suggested that SFEO had better correlation with DPPH radical scavenging capacity than others. Moreover, the intense antioxidant activity of SFEO could be attributed to its high content of tocopherols, especially γ-tocopherol.

ABTS radical cation was also suitable for evaluating radical-scavenging capacity of antioxidants. Fig. 3b shows the effective ABTS radical scavenging activity of the oils obtained from different extraction methods. SFEO showed the highest IC_{50} value (18 mg/mL), followed by SEO (18 mg/mL), AEEO (21 mg/mL), and CPO (22 mg/mL). However, we did not find any obvious differences ($p > 0.05$) in the prohibiting behaviors of the oils obtained from different extraction methods.

Natural antioxidants have been known for breaking free radical chain reactions by providing an electron or hydrogen atom to free radicals; the assays based on reducing power are often applied to estimate the capacity of natural antioxidants to provide an electron or hydrogen atom (Hu et al., 2008). Fig. 3c shows the reducing power of *A. pedunculatus* seed oil. The reducing power increased with increasing concentration of APO. The results indicated that APOs possess moderate electron donating ability that might have some connection with its antioxidant activity.

Tab. 4: Phytosterol content (mg/kg) in *A. pedunculatus* seed oil obtained from different extraction methods

	Extraction method			
	AEE	CP	SFE	SE
campesterol	216 ± 12^{ab}	178 ± 11^{a}	287 ± 14^{c}	268 ± 2.0^{bc}
campestanol	9.6 ± 0.54^{ab}	7.1 ± 0.77^{a}	8.1 ± 0.53^{a}	14 ± 0.68^{b}
[24R]-24-Methyl cholest-7-en-3β-ol	17.6 ± 0.84^{b}	10.5 ± 0.25^{a}	7.1 ± 0.12^{a}	tr
[24S]-24-Ethyl cholesta-5,25-dien-3β-ol	37 ± 0.64^{a}	29 ± 0.93^{a}	50 ± 0.83^{b}	48 ± 1.93^{b}
sitosterol	2706 ± 19^{a}	2274 ± 24^{a}	3766 ± 87^{b}	3510 ± 59^{b}
sitostanol	89 ± 9.00^{b}	49 ± 0.44^{a}	69 ± 4.82^{ab}	134 ± 3.34^{c}
Δ 5-avenasterol	534 ± 4.2^{ab}	422 ± 3.1^{a}	698 ± 5.9^{c}	651 ± 7.3^{bc}
stigmasta-5,24-dien-3-ol	52 ± 1.4^{a}	46 ± 0.07^{a}	68 ± 1.2^{b}	70 ± 1.3^{b}
stigmast-7-en-3β-ol	30 ± 0.52^{a}	84 ± 1.4^{b}	43 ± 0.41^{a}	43 ± 0.25^{a}
Δ 7-avenasterol	40 ± 1.3^{a}	46 ± 0.84^{a}	50 ± 0.82^{a}	50 ± 0.98^{a}
Total	3730 ± 26	3145 ± 30	5043 ± 93	4787 ± 71

[tr] Trace amount

Values are presented as means ± standard deviations for three preparations

Values given in rows followed by different superscript letters are significantly different at $p \leq 0.05$

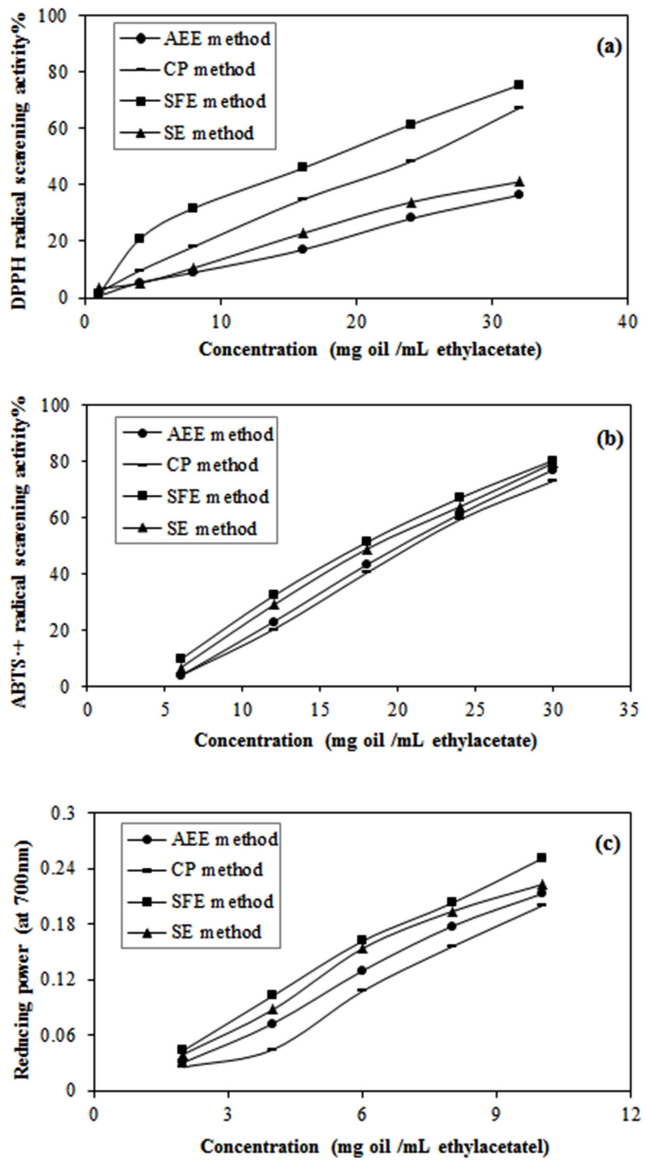

Fig. 3: Investigation of antioxidant activity of *A. pedunculatus* seed oil, obtained by different extraction methods, using the following in vitro assays: (a) DPPH radical scavenging assay, (b) ABTS radical scavenging assay, and (c) reducing power measurement.

Conclusions

This study clearly demonstrated that seed oil from *A. pedunculatus*, a wooden oil plant of deserts, was rich in fatty acid composition (especially, MUFA and PUFA), polyphenols, tocopherols and phytosterols. Those valuable compound were higher than the healthy oils (olive oil and camellia oil), which are beneficial for human health. The best extraction method with regard to oil yield, valuable compound and antioxidant activity: SE>CP>AEE>SFE, SFE>AEE>SE>CP and SFE>SE>CP>AEE, respectively. The SFE method extracted the highest content of valuable and health promoting compounds and the oil revealed the highest antioxidant activity compared to the oil obtained by the other extraction methods (AEE, CP and SE).

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References

AOCS, 1998: Official methods and recommended practices of the American Oil Chemists Society. Methods 5th ed. Champaign, IL, USA: AOCS Press.

Beltrán, G., Ruano, M.T., Jiménez, A., Uceda, M., Aguilera, M.P., 2007: Evaluation of virgin olive oil bitterness by total phenol content analysis. Eur. J. Lipid Sci. Tech. 109, 193-197.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995: Use of a free radical method to evaluate antioxidant activity. LWT – Food Sci. Technol. 28, 25-30.

Brkić, K., Radulović, M., Lukić, I., Setić, E., 2006: Application of soxtec apparatus for oil content determination in olive fruit. Riv. Ital. Sostanze Gr. 83, 115-119.

Ceci, L.N., Carelli, A.A., 2010: Relation between oxidative stability and composition in argentinian olive oils. J. Am. Oil Chem. Soc. 87, 1189-1197.

Chu, J., Xu, X., Zhang, Y., 2013: Production and properties of biodiesel produced from amygdalus pedunculata pall.. Bioresource Technol. 134, 374-376.

Crowe, T.D., White, P.J., 2003: Oxidative stability of walnut oils extracted with supercritical carbon dioxide. J. Am. Oil Chem. Soc. 80, 575-578.

Hu, C.C., Lin, J.T., Lu, F.J., Chou, F.P., Yang, D.J., 2008: Determination of carotenoids in dunaliella salina cultivated in taiwan and antioxidant capacity of the algal carotenoid extract. Food Chem. 109, 439-446.

ISO 12228, 1999: Animal and vegetable fats and oils-determination of individual and total sterols contents. In: Gas Chromatographic Method. ISO, Geneva.

Jung, D.M., Yoon, S.H., Jung, M.Y., 2012: Chemical properties and oxidative stability of perilla oils obtained from roasted perilla seeds as affected by extraction methods. J. Food Sci. 77, 1249-1255.

Kamal-Eldin, A., Appelqvist, L.Å., Yousif, G., Iskander, G.M., 1992: Seed lipids of sesamum indicum and related wild species in sudan. The sterols. J. Sci. Food Agr. 59, 327-334.

Karaman, S., Karasu, S., Tornuk, F., Toker, O.S., Geçgel, Ü., Sagdic, O., Ozcan, N., Gül, O., 2015: Recovery potential of cold press by-products obtained from oil industry: physicochemical, bioactive and antimicrobial properties. J. Agr. Food Chem. 63, 2305-2313.

Mandana, B., Russly Abdul, R., Farah Saleena, T., Noranizan Mohd, A., Sarker, M.Z.I., Ali, G., 2013: Supercritical carbon dioxide extraction of seed oil from winter melon (*Benincasa hispida*) and its antioxidant activity and fatty acid composition. Molecules. 18, 997-1014.

Maranz, S., Wiesman, Z., Garti, N., 2003: Phenolic constituents of shea (*Vitellaria paradoxa*) kernels. J. Agr. Food Chem. 51, 6268-6273.

Mariod, A., Matthäus, B., Eichner, K., 2004: Fatty acid, tocopherol and sterol composition as well as oxidative stability of three unusual sudanese oils. J. Food Lipids. 11, 179-189.

Min, J.D., Suk, Hoo, Y., Mun, Yhung, J., 2012: Chemical properties and oxidative stability of Perilla oils obtained from roasted Perilla seeds as affected by extraction methods. J. Food Sci. 77, 1249-1255.

Ni, Q., Gao, Q., Yu, W., Liu, X., Xu, G., Zhang, Y., 2015: Supercritical carbon dioxide extraction of oils from two torreya grandis varieties seeds and their physicochemical and antioxidant properties. LWT – Food Sci. and Technol. 60, 1226-1234.

Oomah, B.D., Ladet, S., Godfrey, D.V., Liang, J., Girard, B., 2000: Characteristics of raspberry (*Rubus idaeu* L.) seed oil. Food Chem. 69, 187-193.

Parry, J., Lan, S., Luther, M., Zhou, K., Peter, M., Whittaker, P., 2005: Fatty acid composition and antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils. J. Agr. Food Chem. 53, 566-573.

Pietta, P.G., 2000: Flavonoids as antioxidants. J. Nat. Prod. 63, 1035-1042.

RAMADAN, M.F., KINNI, S.G., SESHAGIRI, M., MÖRSEL, J.T., 2010: Fat-soluble bioactives, fatty acid profile and radical scavenging activity of semecarpus anacardium seed oil. J. Am. Oil Chem. Soc. 87, 885-894.

RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M., RICE-EVANS, C., 1999: Antioxidant activity applying an improved abts radical cation decolorization assay. Free Radical Bio. Med. 26, 1231-1237.

SHUKLA, V.K.S., NIELSEN, W.S., BATSBERG, W., 1983: A simple and direct procedure for the evaluation of triglyceride composition of cocoa butters by high performance liquid chromatography-acomparison with the existing TLC-GC method. Eur. J. Lipid Sci. Tech. 85, 274-278.

WAGNER, K., ELMADFA, I., 2000: Effects of tocopherols and their mixtures on the oxidative stability of olive oil and linseed oil under heating. Eur. J. Lipid Sci. Tech. 102, 624-629.

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