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PHYSICO-CHEMICAL COMPOSITION, ANTIOXIDATION PROPERTY AND SAFETY ASSESSMENT OF *AMYGDALUS PEDUNCULATA* PALL. SEED OIL

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Abstract

Characteristics of seeds and oil extracted from *Amygdalus pedunculata* Pall. are determined. The percentage composition of the *A. pedunculata* seeds was: ash 2.50, oil 48.00, protein 24.70, total sugar 4.85, crude fiber 15.21 and moisture 4.74%. Physico-chemical properties of the oil included: density, 0.919 g/cm^3; refractive index, 1.471; acid value, 1.40 mg KOH/g oil; iodine number, 109.00 g/100 g oil; peroxide value, 0.681 meq/kg; saponification number, 192.00 mg KOH/g oil; total carotenoid, 2.05 mg/kg and tocopherols 48.38 mg/100 g oil. Oleic acid (70.00%) was the most abundant, followed by linoleic (26.44%). The tested oil showed an appreciable free radical scavenging capacity and the acute oral toxicity on mice of the oil belongs to actually non-toxic grade.

Introduction

In the past a few decades, the global consumption of oils has increased, both for human and industrial use. This is mainly because of the growing population and improving living standards, particularly in China and India (Rosillo-Calle *et al.* 2009). Vegetable oils, one of the important oil sources, are generally more nutritious than animal fats owing to their less saturated fatty acids (SFAs) content and can be used as a renewable energy. However, the production of vegetable oils cannot meet the global demands of food and energy industries, thus leading to the price increase. Therefore, it is urgent to search for new sources of unconventional oils which can supply or replace the shortage of certain conventional vegetable oils. Recently, some novel seed oils from different plants, such as *Phoenix canariensis* (Hickman 1993), *Washingtonia filifera* (Ono *et al.* 1989), *Albizia julibrissin* (Braun 1961) and *Spartium junceum* (Hickman 1993) have been reported.

Amygdalus pedunculata, a member of the Rosaceae, is a type of perennial, sand dune-stabilizing, and oil-bearing shrub with notable tolerance to cold and drought. Because of above features, the species is regarded as a good desert reclamation plant. Based on its excellent desert control properties, it is of great importance to systematic study and develop products from *A. pedunculata* to add value to the plant. *A. pedunculata* seed is composed of 30% (w/w) of kernel and 70% (w/w) of shell. The kernel was proved to be rich in oil and protein. Recently, Li *et al*. (2010) have extracted oil from *A. pedunculata* seed kernels, afterwards, the cakes and shells were applied as raw materials for the preparation of protein powder and activated carbon accordingly (Zang *et al.* 2012, Li *et al.* 2010). In addition, methods for preparing biodiesel from *A. pedunculata* seed kernel oil (AO) and producing activated carbon from *A. pedunculata* seed shell, and application of the oil in cosmetics have been patented (Shen *et al.* 2009, Shen *et al.* 2010, Li *et al.* 2013).

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This work is aimed at determining the parameters of physico-chemical composition, antioxidant capacity and safety of the seed kernel oil from *A. pedunculata* to provide a theoretical basis for the further development of the oil as an edible oil.

Materials and Methods

Mature seeds of *Amygdalus pedunculata* fruit were collected from shrubs in June, 2010 in Shenmu County, Yulin, Shanxi Province, China (latitude 38°40' N. longitude 110°08' and elevation 1200 m). The seed kernels were removed from the hulls, dried in an oven at 60°C for 24 hrs, and stored at –15°C until analysis. All the chemicals and reagents used in this work were from Sigma Aldrich (Buchs, Switzerland).

BALB/C pure adult mice as test animal, weighing 22 ± 2 g, were randomly grouped in different cages with standard diet and acclimatized for a week.

The moisture of the seeds was measured according to the AOAC Official Method 930.15, and the result was shown in percentage. The total protein content was calculated by the Kjeldahl's method (Bradford MA 1976). The ash content was detected by incinerating 2 g powdered seed in a muffle furnace at 550°C for more than 2 hrs. The mineral constituents of the seed were analyzed with an atomic absorption spectrophotometer (Z-2000, Hitachi, Japan). The crude fiber was measured according to the AOAC official Method 991.43. Total sugars (TS) were calculated by the following Eq.:

$$TS = 100 - (\%\ \text{crude oil} + \%\ \text{moisture} + \%\ \text{protein} + \%\ \text{ash} + \%\ \text{fiber})$$

For extracting oil, the dried seeds ground into powder by a high speed grinder (WND-200, Wei neng da, China) were extracted with n-hexane in a Soxhlet extractor.

Refractive index of the oil was detected by an Abbe refractometer (NAR-1T, Atago Co. Ltd., Tokyo, Japan) at 20°C. Density of the oil was determined with respect to water density at 20°C. Standards ISO were used to measure acidity value (ISO 660), iodine value (ISO 3961), peroxide value (PV) (ISO 3960), saponification value (ISO 3657) and unsaponifiable matter (ISO 3596).

Carotenoid content was calculated as the description of Górnaś *et al.* (2013). The fatty acids in the seed kernel oil sample were converted to their corresponding fatty acid methyl esters as described by Nehdi (2011a). Then the fatty acid methyl ester (FAME) composition of the oil was measured using an Agilent 7890A GC equipped with a polar capillary column (BPX70, 60 m × 0.25 mm i.d., 0.25 μm film thickness).

The tocopherols were determined by HPLC according to the ISO 9936. The antioxidant activity of the oil was determined by a DPPH assay (Rezig *et al.* 2012) modified. Briefly, 2 ml of AO solution in ethyl acetate at different concentrations was added to 2 ml of DPPH solution. After 1 hr at room temperature in the dark, the absorbance was measured at 517 nm using a UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). BHT, a stable antioxidant, was used as a synthetic reference.

The acute oral toxicity test was developed by the procedures outlined by procedure and methods of food safety toxicological assessment, GB15193.3-2003.

Data were expressed as a mean ± standard deviation based on three replicates of each assay.

Results and Discussion

As listed in Table 1, moisture, ash, protein, fat, crude fiber, and total sugar levels of powdered *A. pedunculata* seeds are 4.74, 2.50, 24.70, 48.00, 15.21 and 4.85%, respectively. It is important to point out that *A. pedunculata* seed kernel contains 48.00% of oil. This content is close to that in

the woody plant *S. apricot* (50.18%) and almond seeds (51.00%) (Ahmad 2010), which belong to Rosaceae. Therefore, the high oil content combined with easy cultivation and seed collection make *A. pedunculata* a good candidate for commercial exploitation. Additionally, a high total protein content is noted. The result is comparable with that of almond (20.68 - 23.30%) (Ahmad 2010). The seeds show a crude fiber content of 15.21%, which is much higher than fiber in Indian almond (5.09%) (Agunbiade and Olanlokun 2006). The seeds also have varieties of important minerals (Table 1). Such a mineral composition and high amount of oil, protein and crude fiber confirm the nutritional value of *A. pedunculata* seeds, which may constitute a valuable source of mineral, protein and crude fiber in animal feed and food industries.

Table 1. Chemical composition, physico-chemical properties from seeds and seed oil of AO.

Component	
Chemical composition of seeds	
Moisture content (%)	4.74 ± 0.02
Oil[a]-dry mater (%)	48.00 ± 0.10
Ash-dry mater (%)	2.50 ± 0.05
Protein[b]	24.70 ± 0.13
Crude fibre (%)	15.21 ± 0.24
Total sugar[d]	48.5 ± 0.3
Potassium[d]	634.0 ± 0.1
Magnesium	183.0 ± 0.9
Calcium[d]	174.0 ± 1.0
Phosphorus[d]	512.0 ± 0.6
Sodium[d]	11.9 ± 0.8
Iron[d]	44.2 ± 0.7
Zinc[d]	47.9 ± 2.1
Manganese[d]	15.2 ± 0.3
Copper[d]	11.3 ± 0.1
Properties of seed oil	
Density (g/cm^3, at 20ºC)	0.919 ± 0.012
Index of refraction (20ºC)	1.471 ± 0.006
Total carotinoid (mg/kg)	2.05 ± 0.04
Acid value (mg KOH/g oil)	1.40 ± 0.04
Iodine value (mg/kg oil)	1090.0 ± 6.3
Peroxide value (mEq O_2/kg oil)	0.68 ± 0.01
Saponification value (mg KOH/g oil)	192.00 ± 0.74
Unsaponifiable matter (%)	0.81 ± 0.09
Phosphorus (mg/kg)	11.00 ± 0.51

[a] Oil = Weight of extracted oil × 100/weight of seed; [b] Protein = (N (%) × 6.25);
[c] Carbohydrate obtained by difference; [d] In mg/kg of dry matter.

Physio-chemical properties of AO were shown in Table 1, a yellow liquid at room temperature with refractive index and density of 1.471 and 0.919 g/cm^3, respectively. The oil's phosphorus content is 11.00 ppm, indicating lower phospholipid content. The peroxide value is used to measure the primary oxidation level of oils and fats. The peroxide value of AO is much lower than that of canola oil (2.39 mequiv O_2/kg) (Teh and Birch 2013) which is the most commonly consumed vegetable oil.

The saponification value of AO is higher than that of soybean (179.45 mg KOH/g) (Nehdi 2011b), however, comparable to that of olive oil (191.93 mg KOH/g) (Cerchiara *et al.* 2010). A high saponification value obtained from AO indicates high amount of low-molecular-weight fatty acids in this oil because saponification value indicates the average molecular weight of oil (Egan H *et al.* 1981). In addition, a high saponification value is also an indicator that the oils are normal triglycerides and very useful in the liquid soap and shampoo industries (Akbar *et al.* 2009).

The iodine value of AO is higher than that of olive oil (828.4 g/kg) (Cerchiara *et al.* 2010). The iodine value, an indicator of the degree of unsaturation in oils, states AO has a large amount of unsaturated fatty acids (UFAs), as listed in Table 2.

Carotenoid is the most important provitamin A source and it presents antioxidant capability to inhibit singlet oxygen, thus protecting the lipids from oxidizing (Samaniego-Sánchez *et al.* 2010). The concentration of carotene pigments detected in AO (Table 1) is comparable with that in canola oil (2.20 mg/kg) (Teh and Birch 2013) but lower than in olive oils (6.90 mg/kg) (Tuberoso *et al.* 2007).

The fatty acid profile of AO was shown in Table 2. Among the 13 fatty acids detected, oleic acid (C18:1) and linoleic acid (C18:2) are the major fatty acids. These values are close to those in canola oil (Teh and Birch 2013), oleic acid (C18:1) and linoleic acid (C18:2). AO can be characterized by a high degree of monounsaturated fatty acids (MUFAs), which accounts for 70.58% of total fatty acids, and polyunsaturated fatty acids (PUFAs), accounting for 26.61% of total fatty acids. The AO can be considered as an oleic-linoleic oil since oleic acid is the most affluent, followed by linoleic acid. Oleic acid plays an important role in preventing cardiovascular diseases, constructing the nervous cell, and lowering cholesterol in blood (Nehdi *et al.* 2010). Since the heat stability of oleic acid equals to that of saturated fats, thus high-oleic-acid oils are suitable to replace saturated fats in commercial food requiring long shelflife. Linoleic acid has an essential role in the healthy growing of human skin and can be converted by human organism into a series of long fatty acid chains, which are the precursors of eicosanoids, a family of compounds with 20 carbons containing prostaglandins and leucotriens in particular (Nasri *et al.* 2005). In terms of fatty acid profile and nutritional value of the AO, it may be applied as edible oil in food industries and as a material for the preparation of biodiesel.

The tocopherols in oils are important due to their capability of preventing the oxidative deterioration of PUFA in plant material, and they are natural lipophilic antioxidants in plant oils. As listed in Table 2, the total tocopherol content is 484.0 mg/kg. The tocopherols composition of this oil includes three species: γ-, δ- and α-tocopherol, with the majority being γ-tocopherol followed by δ- and α-tocopherol. The result obtained is in conformity with previous study (Lei *et al.* 2009). The level of tocopherols in AO is close to that in extra olive oil (455.0 mg/kg) (Miraliakbari and Shahidi 2008).

To evaluate the antioxidant potential of AO, DPPH scavenging activity was measured, in comparison with a known antioxidant BHT. The DPPH radical-scavenging activities of seed kernel oil and the reference BHT are shown in Fig. 1. It can be observed that AO exhibited notable DPPH radical-scavenging activity, with an efficacy lower than that of BHT. The values for AO ranged from 67.30 to 2.01%, and for BHT varied from 98.20 to 15.83%, accordingly with the same concentration range from 16.00 to 0.50 mg/ml. The concentration of sample required to scavenge 50% of DPPH radical (IC_{50}), was also calculated. The IC_{50} value of seed kernel oil was 12.36 mg/ml. Moreover, the decrease in the concentration of seed kernel oil resulted in the reduction of its antioxidant ability. The same behavior was observed in BHT.

The acute oral toxicity of AO was detected by animal experiments. The test mice were monitored in the short term (within 14 days). If infected, the mice will be lethargic and anorectic,

but they will gradually return to normal after 4 days. What is worse, the mice will be depressive, no eating or even die (Lu *et al.* 2014). The experimental results (Tables 3 and 4) illustrated that: all

Table 2. Fatty acid, tocopherol and sterol composition of AO.

Fatty acid	Composition (%)
Saturated	
$C_{16:0}$	2.06 ± 0.09
$C_{18:0}$	0.66 ± 0.03
$C_{20:0}$	0.06 ± 0.06
Monounsaturated	
$C_{16:1}$	0.26 ± 0.07
$C_{17:1}$	0.10 ± 0.04
$C_{18:1}$	70.00 ± 0.19
$C_{20:1}$	0.14 ± 0.16
$C_{22:1}$	0.08 ± 0.13
Polyunsaturated	
$C_{18:2}$	26.44 ± 0.04
$C_{18:3}$	0.17 ± 0.06
SAFA	2.78 ± 0.09
MUFA	70.58 ± 0.2
PUFA	26.61 ± 0.14
UFA	97.19 ± 0.22
U/S	34.96
Tocopherol	Composition (mg/kg)
α-tocopherol	17.7 ± 0.1
γ-tocopherol	445.0 ± 0.3
δ-tocopherol	21.1 ± 0.5

SAFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acid.

Table 3. The effect of AO on the body weight of mice.

Sex	Number	Body weight (g) $\overline{X}$ ± (s)		
		1st	7st	14st (day)
Female	10	18.8 ± 1.3	27.6 ± 1.6	31.5 ± 1.4
Male	10	20.3 ± 1.6	34.0 ± 1.4	38.6 ± 2.5

animals survived during the observation period and their diets were normal, their weights were growing steadily and had no toxic clinical signs. According to acute oral toxicity classification standard in the procedure and methods of food safety toxicological assessment, GB15193.3-2003, the acute oral toxicity on mice of AO belongs to actually non-toxic grade.

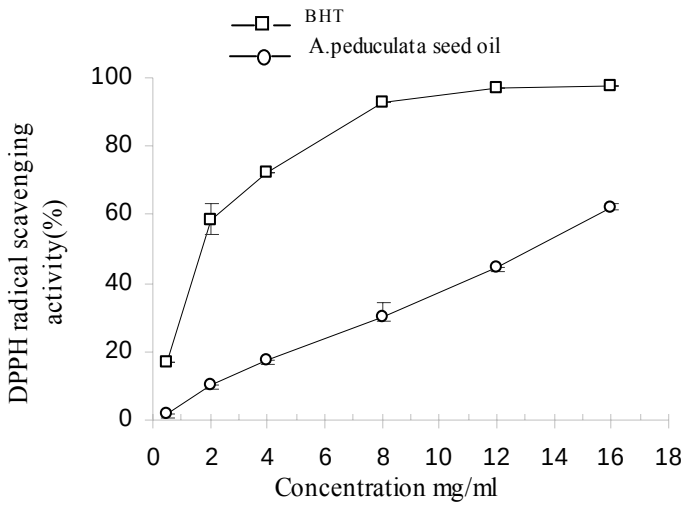

Fig. 1. DPPH radical scavenging activity of AO and BHT.

Table 4. The results of oral acute toxicity test of AO on mice.

Sex	Number	Dosage (g/kg.bw)	The number of death	Death rate (%)
Female	10	18.31	0	0
Male	10	18.31	0	0

This study showed that *A. pedunculata* seeds are rich in many beneficial nutrients that appear to play an important role in human health and give a considerable yield of oil. The oil seems to contain essential fatty acids and lipid-soluble bioactives. The unique fatty acid, tocopherol composition, and antioxidant property make the oil nutritionally valuable. The oil can protect against UV light, which assists its use in the cosmetic industry. And the acute oral toxicity on mice of AO belongs to actually non-toxic grade, which advocates the potential use of this oil as feed stock for edible and/or oleochemical industries. Production of oil from *A. pedunculata* seeds provides a new edible oil resource, adds value to the plant which can improve local farmer's enthusiasm for the soil conservation, and can potentially create employment opportunities.

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