Comparative Study on Functional Components, Physicochemical Properties and Antioxidant Activity of *Amaranthus Caudatus* L. Oils Obtained by Different Solvents Extraction

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Abstract: Functional compositions, physicochemical properties and antioxidant activities of *Amaranthus caudatus* L. oils (ACO) obtained by different solvents were comparatively investigated. All the resulted ACO were enrich in 75% unsaturated fatty acid and in squalene of about 4 g/100 g. Different solvents showed varying in oil extraction, where acetone results a highest yield of 6.80 g/100 g. ACO extracted by ethanol showed a highest tocopherol (1351.26 mg/kg), polyphenols (211.28 mg/kg) and squalene (42519.13 mg/kg). However, phytosterols in ACO extracted by hexane (27571.20 mg/kg) was higher than that by acetone (19789.91 mg/kg), ethanol (22015.73 mg/kg) and petroleum ether (24763.30 mg/kg). Furthermore, antioxidant activity of ACO was also measured by DPPH, ABTS and FRAP assay. According to principal component and correlation analysis, squalene was correlated with the DPPH scavenging ability, but phytosterols and tocopherols was correlated with the ABTS and ferric reducing ability of the oils, respectively. This study provides a promising excellent source of functional oil for food industries.

Key words: *Amaranthus caudatus* L. oil, antioxidant activity, physicochemical characterization, principal component analysis, squalene

1 Introduction

Increasing interest was generated by research on the functional special oils for their efficacy in obesity, cardiovascular disease, and many cancers¹. *Amaranthus caudatus* L., an ancient crop, has good nutritional profiles as health-promoting foods in a fascinating history, including anti-cancerous, antiviral, hepatoprotective, neuroprotective, cardioprotective and antidiabetic². Moreover, many studies have shown that *Amaranthus caudatus* L. have the effect of reducing cholesterol³. However, it has not been fully developed and utilized in China, and there are few reports on *Amaranthus caudatus* L. because of the research on *Amaranthus caudatus* L. oil is even less. With the increasing number of chronic diseases such as cancer, cardiovascular disease, aging and neurodegenerative disease, natural dietary antioxidant has been widely concerned as a preventive and therapeutic drug in reducing the risk of oxidative stress and certain diseases⁴,⁵. Although *Amaranthus caudatus* L. contain only about 6 g/100 g crude fat, it is found that *Amaranthus caudatus* L. oil has unique nutritional characteristics (e.g., vitamin E, polyphenols, sterols and other trace active components) with a potential stronger antioxidant activity than ordinary oils⁶,⁷. *Amaranthus caudatus* L. oil contains among others linoleic acid (up to 50%), tocopherols (~802 mg/kg), total sterols (~2460 mg/100 g) and squalene (~4.16 g/kg seed)⁸,⁹. These components have shown remarkable antioxidant activity, benefits to nutrition and cardiovascular system health¹⁰. Efficient techniques had been used to recover oil from the plant resources, such as solvent extraction, pressing, subcritical extraction, supercritical fluid extraction, ultrasound- and enzyme-assisted extraction. Comparatively, solvent extraction provides efficient oil recovery, low-cost and low-energy consumption. In addition, both the extraction efficiency and oils activities were depended on the methods as well as the solvent used¹¹,¹². As we known, various compounds with different chemical characteristics and polarities have different solubility in a...
particular solvent. There are several solvents such as water, ethanol, methanol, ethyl acetate, or hexane, among others for extracting compounds from fresh vegetable foods. Conventional solvents as hexane are recognized for providing high extraction yields, but their use raises some safety, environmental and health issues; however, ethanol represents a safer alternative. Furthermore, it was found that oil yield by ethanol extraction from avocado pulp were significantly lower than that obtained by petroleum ether, resulting in a high oxidative stability yet.

Consolidation of sustainable technologies for Amaranthus caudatus L. oil extraction might increase the utilization of this high-quality oil. The objective of this study was to determine the physical and chemical properties, the fatty acids profile and the functional components of the oils extracted from Amaranthus caudatus L. seeds, obtained by four different extraction solvents. Moreover, the antioxidant capacity of Amaranthus caudatus L. oil was also systematically investigated, including DPPH radical scavenging ability, ABTS free radical scavenging ability and ferric reducing ability, to provide a theoretical basis for the development and research of functional Amaranthus caudatus L. oil in the future.

2 Materials and Methods

2.1 Materials

Amaranthus caudatus L. was provided by Zhengzhou Linno Industrial Co., Ltd. Standards including fatty acid methyl ester (FAME), phytosterols, tocopherols, 5α-cholestanol, gallic acid (GA), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA). L. oil in the future.

2.2 Physicochemical analysis of Amaranthus caudatus L.

Protein content (N × 6.25) was determined using a Kjeldahl nitrogen analyzer (Kjeltec 8400, Foss, Sweden). Lipids, starch, crude fiber, moisture, and ash contents were examined according to official AOAC methods.

2.3 Amaranthus caudatus L. oil extraction

Amaranthus caudatus L. seeds were firstly ground and then their oil was extracted according to the Soxhlet method (AOAC 992.23). The thimble containing the dried sample was placed in the Soxhlet device supporting with glass beads. The dehydrating beaker (pre-dried for 1 h at 105°C and weighed) was rinsed with 150 mL of extractants (e.g., petroleum ether, hexane, ethanol and acetone) to the thimble. The samples were soaked overnight and refluxed for 8 h with a heat adjusted so the extractor siphoned at least 30 times. When the extraction was complete, the samples were air-dried at 70°C to constant weight (~1 h). The oils obtained were cooled in desiccators and stored at -50°C for less than two weeks until further analysis.

2.4 Physical and chemical properties of Amaranthus caudatus L. oil

Physical and chemical properties (including acid value (AV), peroxide value, and sensory characteristics) of Amaranthus caudatus L. oil obtained with the different extraction solvents were characterized according to the standard AOCS procedures.

2.5 Fatty acid composition analysis

The fatty acid composition was determined according to the method proposed by Shi et al., after the conversion of fatty acids into fatty acid methyl esters (FAME). The FAMEs analyses are carried out a 7890B Agilent gas chromatography interfaced with an Agilent 5977A mass selective detector (Agilent Technologies, Palo Alto, CA, USA) and a HP-88 MS column (100 m × 0.25 mm × 0.20 μm). Oven temperature was set initially at 140°C for 5 min, programmed to increase at 4°C/min to 240°C with final holding time of 20 min. Helium (99.999% purity) was used as carrier gas at a flow rate of 1 mL/min. The injection volume was 1 μL with an autosampler split ratio of 1:50. The MS conditions were: transfer line: 260°C; ion source: 250°C; collision energy: 35 eV; positive ionization mode. FAMEs were identified and quantified according to their retention times to both the standards and mass spectrometry (MS).

2.6 High-performance liquid chromatography (HPLC) analysis of tocopherols

Tocopherols were analyzed with the high-performance liquid chromatography (HPLC) according to the method of He et al., with some modifications. 500 mg of oils was mixed with 10 mL of hexane and syringe filtered (0.45 μm PTFE filter) into a 2 mL of amber HPLC vials. Analysis of the tocopherols was performed on a Waters e2695 HPLC (Waters, USA) equipped with a Waters Spherisorb® NH2 analytical column (4.6 mm × 250 mm, i.d. = 5 μm) and a 996 fluorescence detector (Shimadzu). The injection volume was 20 μL and the oven temperature was 30°C. The mobile phase was hexane/isooctane (98.5:1.5 v/v) solution at a flow rate of 0.8 mL/min. Tocopherols were detected and quantified at 290 nm excitation wavelength and 330 nm emission wavelength, and identified by comparison of their retention factors with those of standards.

2.7 Determination of total phenolic compounds (TPC)

The TPC content was analyzed according to the Folin-Ciocalteu colorimetric method described by Can-Cauich et al., with some modifications. Briefly, 2 g of oil was mixed...
with 2 mL of an aqueous ethanolic solution (80%, v/v in distilled water). After shaking for 10 min, the mixture was centrifuged at 8,000 g for 5 min. Recovering the supernatant, the residue was subjected to further extraction in the same manner for additional three times. The supernatants obtained were pooled and quantitated in a 10 mL flat volumetric flask. 10 μL of extracts were mixed with 150 μL of 6% Folin-Ciocalteau reagent and equilibrium for 3 min. Then, 50 μL of Na₂CO₃ (0.6 M) was added to the extracts. The mixtures were mixed (600 rpm for 1 min) and kept for 30 min in the dark. The absorbance readings of the mixtures were measured at 765 nm using an ultraviolet-visible (UV-Vis) spectrometer Pye Unicam (Spectronic Camspec Ltd., Leeds, UK). The TPC content was expressed as mg gallic acid equivalents (GAE)/kg of oil.

2.8 Quantification of phytosterols and squalene by gas chromatography-mass spectrometry (GC-MS)

Phytosterols and squalene were simultaneously identified and quantified with a GC-MS technology. 0.25 g of oil sample was mixed with 3 mL of 2 mol/L KOH ethanol and 0.5 mL of 0.1 mg/mL internal standard (5α-cholestanol) in hexane. After the saponification, the mixture was mixed using a vortex mixer and incubated at 85°C for 1 h in water bath. After cooling, 2 mL of distilled water and 5 mL of hexane were added, respectively. Subsequently, the clear upper layer was collected into a 50 mL centrifuge tube and washed twice with 5 mL of hexane each. All of the upper layers of extracts were combined and dried with nitrogen. After silylation with 0.2 mL of BSTFA + TMCS at 70°C for 40 min, the solution was filtered through a 0.45 μm filter. 1 μL of the filtered solution was injected into a Thermo Scientific gas chromatograph (Thermo Fisher Scientific, CA, USA) equipped with a HP-5MS column (30 m × 250 mm × 0.25 μm) and a single quadrupole mass spectrometer (ISQ; Thermo Fisher Scientific, CA, USA). Oven temperature was set at 285°C. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The split ratio was 20:1. The mass spectrometer was operated in the electron impact mode at 70 eV with a scan range of 50–700 m/z. The temperatures of injector, transfer line and ion source were set at 280, 280 and 250°C, respectively.

2.9 Antioxidant activity

The antioxidant activities of ACO were evaluated in terms of free-radical scavenging activity (e.g., 2,2-diphenyl-1-picrylhydrazil (DPPH) radical, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and ferric reducing ability (FRAP).

2.9.1 DPPH radical scavenging capacity

DPPH radical scavenging capacity was determined, following the procedure described by Chen et al., with some modifications. Briefly, 200 μL of sample were added into 3.8 mL of DPPH reagent (1.75 × 10⁻⁴ M in methanol) and the mixture was placed in a dark room for 60 min. Subsequently, the absorbance was determined by UV spectrophotometer at 517 nm. The DPPH radical scavenging capacity was calculated and expressed as μmol Trolox equivalent (TE) per g in the sample.

2.9.2 ABTS radical scavenging assay

ABTS scavenging activity was evaluated according to Can-Cauich et al. In brief, ABTS working solution (7 mM) was prepared by dissolving 20 mg ABTS powder in 5.2 mL 2.45 mM potassium persulfate solution. The mixture was incubated in darkness at room temperature for 12 h, and then diluted with ethanol until an absorbance of 0.700 ± 0.020 at 734 nm before use. Then, an aliquot of 20 μL samples was mixed with 280 μL diluted ABTS solution and incubated in the dark at room temperature for 6 min. The ABTS concentration in the mixture was measured at 734 nm by UV spectrophotometer. The ABTS free radical activity was computed and expressed as trolox equivalent antioxidant capacity (TEAC).

2.9.3 Ferric reducing antioxidant power (FRAP) assay

FRAP assay was evaluated measured according to Deetee et al. with minor modification. Specifically, FRAP reagent contained 25 mL of acetate buffer (0.1 mol/L, pH 3.6), 2.5 mL of TPTZ solution (10 mmol/L) dissolved in 40 mmol/L HCl and 2.5 mL of FeCl₃ solution (20 mmol/L) was daily prepared and warmed at 50°C. The reactions were started by mixing 0.3 mL of methanolic extracts with 2.7 mL of FRAP reagent in a 10 mL volumetric flask. The blue solution was kept at room temperature for 10 min and then centrifuged at 3,000 rpm for 10 min. The absorbance was measured at 593 nm against a reagent blank using UV spectrophotometer and the reducing power of the sample was calculated, expressed as μmol Trolox equivalent (TE) per g of sample.

2.10 Statistical analysis

All analysis were performed in triplicate and results were reported as means ± standard deviations (SD). Statistical analysis was performed using SPSS 20.0 by one-way analysis of variance (ANOVA), and assessed by Duncan’s multiple-range tests at p<0.05.

3 Results and Discussion

As a kind of medicinal and edible plant, Amaranthus caudatus L. is widely cultivated in China because of its high nutritional value. The composition of Amaranthus caudatus L. seeds were as follows: 11.13 ± 0.06 g/100 g moisture, 5.573 ± 0.407 g/100 g fat, 43.440 ± 0.085 g/100 g carbohydrates, 22.70 ± 0.10 g/100 g protein, 0.6472 ± 0.053 g/100 g ash, and 14.390 ± 0.441 g/100 g crude fiber (Table S1). Although the oil content in amaranth seed is only ~ 5.6 g/100 g, Amaranthus caudatus L. oil (ACO) has been
widely reported in deceasing blood cholesterol levels and used as traditional medicine to improve eyesight, facilitate excretion and dispel chills during the thousand’s years in China\textsuperscript{[20]}. Moreover, the \textit{Amaranthus caudatus} L. contain more fat with grain amaranth that containing 4.37g oil per 100 g seed\textsuperscript{[22]}, implying that it is a good resource for functional edible oil.

3.1 \textit{Amaranthus caudatus} L. oil extraction yields

For the different solvents, the extraction yields were obtained by comparing the amount of extracted oil to the previously determined value of 5.57% fat. From Fig. 1, the highest extraction yield obtained with ethanol and acetone extraction solvents was 6.43 g/100 g and 6.80 g/100 g, respectively. This extraction yield was significantly \((p<0.05)\) higher than that of petroleum ether (5.30 g/100 g) and hexane (4.36 g/100 g). In addition, according to the analysis of significance, there is no significant \((p>0.05)\) difference in the oil yield between acetone and ethanol, but there is a significant difference \((p<0.05)\) between the oil yield of petroleum ether or hexane and that of other solvents. This may be due to the fact that the former can dissolve polar substances with a large amount of lipid accompanying microcomponents (e.g., glycolipids and phospholipids, polyphenols, etc.), whereas petroleum ether and hexane do not dissolve polar substances, as can be seen from a higher clarity of the extracted \textit{ACO}\textsuperscript{[20]}.

3.2 Physical and chemical properties of ACO

The physical properties of the oils extracted with different solvents were shown in Table 1. According to the acid value \((AV)\), all the oils showed significant \((p<0.05)\) difference. Moreover, the oils extracted with ethanol and acetone showed a higher acid value; the oil extracted with hexane and petroleum ether had the lower values. These results agree with those reported by Moreno \textit{et al.}\textsuperscript{[12]}, who concluded that the acid value was related to the solvent polarity. Peroxide value \((POV)\) is another important quality index because it is mandated by legislature and reflected appropriate processing conditions\textsuperscript{[23]}. From the Table 1, such value was 200% of \textit{ACO} obtained by the extraction of polarity either ethanol or acetone higher than of the samples obtained by hexane and petroleum ether. These values agree with the ones reported by Miraalakbari and Shahidi\textsuperscript{[24]}. In this case, the oil extracted by petroleum ether had the lowest peroxide value of 1.07 \(\pm\) 0.07 mmol/kg. On the senses, the oil extracted by hexane had a clearly golden color. Additionally, the oils extracted by hexane and petroleum ether are golden and clear, while the oils extracted by acetone and ethanol are dark green and turbid, and the oil extracted by ethanol is pasty. In addition to the sedimentation and turbidity observed in the oil extracted with acetone and ethanol, there is a possible presence of a greater amount of unsaponifiable material\textsuperscript{[10,24]}.

3.3 Fatty acid profile of ACO

Fatty acid composition is an important identity characteristic of fat and oil, that is closely related to the stability and nutritional quality. The fatty acid composition of \textit{Amaranthus caudatus} L. oils extracted from different solvents was identified through gas chromatography-mass spectrometer (GC-MS), as also presented in Table 1. Seven types of fatty acids were identified through gas chromatography-mass spectrometer (GC-MS). Linoleic (18:2) acid of \(~50\)% is the major fatty acid of \textit{ACO}, followed by oleic acid (18:1) of \(~25\)%, palmitic (16:0) of \(~19\)%, and stearic (18:0) of \(~5\)%. Other fatty acids such as arachidic (20:0) and linolenic (18:3) acid were presented at levels below 1% while myristic acid (14:0) was only detected in trace amounts \((<0.2\%)\). The ACO also contains oleic acid (omega-9) which helps overcome cancer and also speeds wound healing\textsuperscript{[25]}. In general, differences of the fatty acid proportions among the extracted oils were not significant \((p>0.05)\), proving the types of solvent nonsignificant effect on the fatty acid composition. Significant differences \((p<0.05)\) were only found in the fatty acid content of C18:0 and C18:1. It was also found that the ACO followed the order: PUFA>MUFA = SFA. The fatty acid profiles of the oils extracted by hexane and acetone are very similar to the reported by Do \textit{et al.}\textsuperscript{[11]}. This is also in agreement with the results reported by others that the similar fatty acid profiles of lipid extracted from the same source material by using varying extractants (e.g., petroleum ether, hexane and isopropanol) was identical with the Soxhlet technique\textsuperscript{[26]}. These results demonstrated that \textit{ACO} should be encouraged because of its high amounts of functional fatty acids such as C18:1 and C18:2, which stimulated as a source of nutrients with benefits to consumers.
Amaranthus Caudatus L. Oils Obtained by Different Solvents Extraction

J. Oleo Sci. 70, (2) 155-164 (2021)

ethanol were higher than that extracted by hexane and petroleum ether. The oil extracted by four solvents were as follows: ethanol > petroleum ether > acetone > hexane. According to the literature, α-tocopherol is the most active ingredient in vitamin E, the biological activity was 100-folds than that of other tocopherols. The content of α-tocopherol in the oil extracted by four solvents was ethanol > petroleum ether > acetone > hexane. This was especially the case for α-tocopherol, which is more effective in protecting vegetable oils against lipid oxidation.

Six phytosterols, including campesterol, stigmasterol, Δ7-campesterol, β-sitosterol, Δ7-stigmasterol and Δ7-avenasterol, were found in the ACO, as shown the ion diagrams in Fig. S2. Total phytosterol content was ranged from 19789.91 ± 41.61 to 27571.20 ± 45.73 mg/kg. The total phytosterol is higher than those common vegetable oils such as soybean oil (250-362 mg/100 g), peanut oil (163-207 mg/100 g), sesame oil (595-865 mg/100 g) and sunflower oil (298-451 mg/100 g) reported in literatures.

Moreover, β-sitosterol was the dominating component in concentrations ranged from 8744.56 ± 20.45 to 9996.92 ± 9.48 mg/kg. The overall ranges of campesterol and stigmasterol was 595-865 mg/100 g and 26, 27 28-451 mg/100 g, respectively (Fig. S1). Meanwhile, the amounts of α-tocopherol, β-tocopherol and δ-tocopherol extracted by acetone and ethanol were higher than that extracted by hexane and petroleum ether. However, α-tocopherol was only detected in the oil with the concentrations of 21.16-50.50 mg/kg, indicating that α-tocopherol may be worked as a characteristic component in the ACO. The sum of tocopherol was 791.76 ± 10.53 mg/kg (hexane), 952.00 ± 20.70 mg/kg (petroleum ether), 1050.05 ± 13.06 mg/kg (acetone) and 1351.26 ± 6.79 mg/kg (ethanol). So, the contents of total tocopherol in the oil extracted by four solvents were as follows: ethanol > acetone > petroleum ether > hexane.

### 3.4 Effect of extraction solvent on the active components in ACO

Active substances, such as tocopherols, polyphenols, sterols and squalene, were natural biologically-active substances to delay the oxidation and affect the quality of oils. The contents of the trace active components (vitamin E, sterol, squalene and polyphenol) in ACO were determined respectively (Table 2). In the ACO, α-, β-, δ-tocopherol and α-tocotrienol were identified with the contents of 415.39-619.88, 303.76-575.98 and 51.35-104.89 mg/kg, respectively (Fig. S1). Meanwhile, the amounts of α-tocopherol, β-tocopherol and δ-tocopherol extracted by acetone and ethanol were higher than that extracted by hexane and petroleum ether. However, α-tocotrienol was only detected in the oil with the concentrations of 21.16-50.50 mg/kg, indicating that α-tocotrienol may be worked as a characteristic component in the ACO. The sum of tocopherol was 791.76 ± 10.53 mg/kg (hexane), 952.00 ± 20.70 mg/kg (petroleum ether), 1050.05 ± 13.06 mg/kg (acetone) and 1351.26 ± 6.79 mg/kg (ethanol). So, the contents of total tocopherol in the oil extracted by four solvents were as follows: ethanol > acetone > petroleum ether > hexane. According to the literature, α-tocopherol is the most active ingredient in vitamin E, the biological activity was 100-folds than that of other tocopherols. The content of α-tocopherol in the oil extracted by four solvents was ethanol > petroleum ether > acetone > hexane. This was especially the case for α-tocopherol, which is more effective in protecting vegetable oils against lipid oxidation.

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ly. Δ⁷-campesterol, Δ⁷-stigmastenol and Δ⁷-avenasterol were detected in the ACO with the amounts of 4506.36-7026.01 mg/kg, 3523.46-5697.92 mg/kg and 2365.19-3760.15 mg/kg, respectively. The comparison of the total phytosterol contents showed that the hexane and petroleum ether extraction system afforded oils with significance (p<0.05) higher content. However, acetone-extracted ACO contained the lowest amount of phytosterols. The results about phytosterols were in agreement with previous reports⁹, 29. Total polyphenol concentrations in the ACO ranged from 11.31 ± 2.87 to 211.28 ± 6.26 mg/kg. Moreover, the polyphenol content in the ACO extracted by ethanol and acetone was higher than that extracted by petroleum ether and hexane. This may be due to the high polar of formers, resulting in a higher affinity to polar polyphenol. Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,20-tetracosahexane) is a triterpenic hydrocarbon precursor of vitamin D and cholesterol, and enriched in shark liver oil and some plant oils. As shown in Table 2, high squalene levels (identified by GC-MS as shown in Fig. S3) in the ACO ranged from 39659.77 ± 193.4 to 42519.13 ± 69.61 mg/kg, which was remarkably higher that of olive oil (1100-8890 mg/kg) and other edible oil (e.g., walnut oil of 3.17-5.90 mg/kg, grapeseed oil of 88.17 ± 4.61 mg/kg), avocado oil of 42.93 ± 4.21 mg/kg, pequi oil of 118.04 ± 14.06 mg/kg and palm oil 119.24 ± 10.81 mg/kg⁹.²⁹. Significance analysis found that there was no significant (p>0.05) difference in squalene contents of ACO extracted by hexane and acetone; and there were significant (p<0.05) differences among the others. One study demonstrated the ability of squalene to “quench” free radical oxygen molecules to revert to an unexcited state and thereby effectively prevent lipid oxidation⁹.¹. Hence, ACO enrich in squalene would be an important foodstuff for a high nutritional III. Moreover, high squalene and phytosterols would also cause a good pull-in behavior and a good spreading ability to the skin’s own lipids⁹.³. In summary, these plentiful trace active components of ACO may have significant benefit for antioxidation ability and oxidation stability.

### 3.5 Evaluating the antioxidant activity of ACO

Antioxidant capacity of ACO was also determined by free radical scavenging capacity assay using DPPH free radicals, ABTS radical scavenging assay and iron(III) reducing power ability. The results are presented in Fig. 2.

As shown in Fig. 2a, all of the ACO exhibited a very strong DPPH radical scavenging activity was remarkably (>95%). Moreover, the results of DPPH scavenging capacity were also showed difference under different extraction methods. Specifically, the scavenging ability of the ACO extracted by petroleum ether, hexane and acetone was significantly (p<0.05) higher than that of ethanol. It
Amaranthus Caudatus L. Oils Obtained by Different Solvents Extraction

J. Oleo Sci. 70, (2) 155-164 (2021)

may be due to the trace active components (Table 2) in the oils. However, in the ABTS assay (Fig. 2b), the highest value was found for ACO extracted by acetone and ethanol (495.45 ± 0.45 μmol/L Trolox) and the lowest value was found for ACO extracted by petroleum ether and hexane (288.33 ± 5.00 and 393.18 ± 2.73 μmol/L Trolox, respectively).

In comparison, higher DPPH and ABTS free radical scavenging capacity and ferric reducing ability were observed in the oils extracted by petroleum ether, hexane and acetone. The reason of the difference of the antioxidative ability was due to the difference of the trace amounts of natural antioxidants in these crude oils. Moreover, the difference antioxidant activities among the evaluated methods were due to their underlying different chemical mechanism and reflect different aspect of antioxidant properties. For instance, DPPH method is more suitable for an assessment of the antioxidant capacity of lipophilic antioxidants instead of hydrophilic antioxidants. In contrast, ferric ion reducing method is more suitable for an assessment of the antioxidant capacity of hydrophilic substances instead of lipophilic antioxidants. These results were also indicated the importance of using more radical scavenging capacity assays in the investigation of oil samples.

3.6 Correlations among antioxidant activity and phytochemical composition

Correlations between phytochemical compositions and antioxidant activity were evaluated and the results are showed in Table 3. It was noted that there is a significant (r = −0.981, p < 0.05) negative correlation between the DPPH scavenging ability and squalene for ACO. The correlation between the ABTS scavenging ability and Δ7-avenasterol was negative significant (r = −0.963, p < 0.05). Furthermore, there was a significant correlation between ferric reducing ability and α-tocotrienol of ACO (r = 0.998, p < 0.01), followed by β-tocopherol and δ-tocopherol with a lower correlation of 0.970 and 0.968, respectively.

To quantify the relationships between antioxidant capacity in various antioxidant assays and minor components among different resulted oils, multivariate analysis of principal component analyses (PCA) were further performed. As illustrated in Fig. 3a, they could be reduced into two components (PC1 and PC2) explaining 93.074 % of total variance. Each component was responsible for 71.309 % and 21.765 %, respectively. The greater the absolute value of the load, the greater the influence. Therefore, PC1 was highly contributed by acid value, tocopherols, polyphenols and the ferric reducing ability, whereas PC2 was mainly positively correlated to phytosterols and squalene. The results agreed with the study on the relationship between radical scavenging activity and antioxidant activity reported by Bubonja-Sonje et al. and Do et al., where reported that the minor components were related to the antioxidative activity.
It is noteworthy that the ACO extracted by four solvents processing were divided into three main clusters from a result obtained by a hierarchical cluster analysis (HCA), as shown in Fig. 3b. The ACO extracted by petroleum ether and hexane were arranged into one group characterized by similarly low values of AV and POV with high phytosterol contents and DPPH scavenging ability. And the ACO extracted by acetone was divided into a cluster because of its high scavenging ability of DPPH and ABTS free radicals. However, the ACO extracted by ethanol was divided into dant activities in edible oils.

Table 3  Correlations between phytochemicals and free radical scavenging capacity.a.

|                | α-T | α-T3 | β-T | δ-T | Cam | Sti | Δ7-Cam | Sit | Δ7-Sti | Δ7-Ave | Squ | Pol |
|----------------|-----|------|-----|-----|-----|-----|--------|-----|--------|--------|-----|-----|
| DPPH scavenging ability | -0.920 | -0.889 | -0.843 | -0.823 | -0.107 | 0.366 | 0.389 | -0.590 | 0.384 | 0.422 | -0.981* | 0.358 |
| ABTS scavenging ability   | 0.630 | 0.811 | 0.824 | 0.846 | -0.411 | -0.664 | -0.922 | -0.093 | -0.895 | -0.963* | 0.419 | 0.646 |
| Ferric reducing ability    | 0.913 | 0.998** | 0.970* | 0.968* | -0.124 | -0.564 | -0.727 | 0.349 | -0.707 | -0.733 | 0.843 | 0.108 |

a Values represent the mean ± standard deviation of three independent replicates (n=3). Sample codes: α-T, α-Tocopherol; α-T3, α-Tocotrienol; β-T, β-Tocopherol; δ-T, δ-Tocopherol; Cam, Campesterol; Sti, Stigmasterol; Δ7-Cam, Δ7-Campesterol; Sit, β-Sitosterol; Δ7-Sti, Δ7-Stigmastenol; Δ7-Ave, Δ7-Avenasterol; Squ, Squalene; Pol, Polyphenols. Data were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple comparison procedures (Tukey post hoc test, p < 0.05). * Significant at p < 0.05, respectively. ** Significant at p < 0.01, respectively.
the third cluster. Therefore, the sample has the high AV, POV, tocopherol, polyphenol and squalene with high scavenging ability of ABTS free radicals and ferric reducing ability. These results were consistent with the data observed in PCA (Fig. 3a).

4 Conclusion

Oils extracted by different solvents extraction from the seeds of *Amaranthus caudatus* L. native to China were systematic characterized. It was found that *Amaranthus caudatus* L. oil (ACO) was rich in 75% unsaturated fatty acid. Acetone resulted in a highest extraction yield of 6.80 g/100 g. Meanwhile, ACO extracted by ethanol showed significantly higher tocopherol, polyphenols and squalene than that of extracted from hexane, petroleum ether and acetone. Phytosterol content of the oils extracted by hexane was higher than that of acetone, ethyl alcohol and petroleum ether. Furthermore, antioxidant activity of oil was also measured by DPPH, ABTS and FRAP assay. According to both principal component and correlation analyses, squalene was correlated with the DPPH scavenging ability, phytosterols and tocopherols were correlated with the ABTS and ferric reducing ability of the oils, respectively. These results indicated that ACO had good quality and controllable functional from different solvents extraction, and highlighted the high potential resource in application of foods, medicines and cosmetics.

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Supporting Information

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