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ORIGINAL PAPER

Phenolic compounds, tocochromanols profile and antioxidant properties of winter melon [*Benincasa hispida* (Thunb.) Cogn.] seed oils

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Abstract

The seed oils from two different cultivars of winter melon were evaluated for their phenolics, tocochromanols and antioxidant properties. The oils contained 961.8 to 1027.6 µg/g of total tocochromanols, including α-tocopherol (2–3%), β-tocopherol (46.6–61.7%), γ-tocopherol (23–24.9%), γ-tocotrienol (6.8–8.2%), and δ-tocopherol (4.6–19.2%). Gallic acid, protocatechuic acid, 3,4-dihydroxybenzaldehyde, vanillic acid, vanillin, *para*-coumaric acid, *trans*-cinnamic acid and ferulic acid were found to be in the range between 12.27 and 17.25 µg/g oil. As far as antioxidant properties is concerned, 80% ethanol seed oil extract (SOE) from cultivar 1 (round) and 100% methanol SOE from cultivar 2 (hybrid round) showed the highest DPPH radical scavenging activity (IC_{50} value 7.03 and 6.88 mg/mL, respectively) while 80% methanol SOE from cultivar 2 exhibited the least (IC_{50} value 64.71 mg/mL). Trolox equivalent antioxidant capacity of the SOE ranged from 9.82 to 24.61 µg TE/g (cultivar 1) and 12.17–26.83 TE/g (cultivar 2), with 80% ethanol extract having the highest antioxidant potential. The extracted total phenolic compounds were 19.37 to 203.93 µg GAE/g oil for cultivar 1, whereas 30.77 to 190.07 µg GAE/g oil for cultivar 2, with 80% isopropanol recovering the maximum amounts. These results conclude that aqueous alcoholic solvents were a better choice for extraction of potent antioxidants from winter melon seed oils, nevertheless, the antioxidant properties considerably ($p < 0.05$) varied in relation to extraction solvents and cultivars selected. Data of this study support that the seed oils of the tested cultivars of winter melon are potential dietary source of tocochromanol, phenolic compounds, and natural antioxidants.

Keywords Antioxidant capacity · HPLC · Melon seed oil · Phenolic compounds · Radical scavenging · Tocotrienol

Introduction

Conventional sources of vegetable oils such as cotton seed, olive, palm, peanut or groundnut, rapeseed, soybean and sunflower, due to their limited yield and production, can hardly meet the world's continuingly increasing demand of vegetable oils with current estimates as high as 145 million metric tons per annum [1]. Consequently, several under-utilized and non-conventional oil seeds have been investigated as new and emerging sources of vegetable oils [2, 3]. In line with the modern optimal nutrition trends that advocate utilization of functional foods due to their medicinal benefits, now it has become important to characterize vegetable oils for the bioactives and antioxidant profile so as to explore their uses as high-value oils [2–5]. Many species in *Cucurbitaceae* family, being valued as an economically important

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vegetable and fruit crops, are widely consumed for nutrition and folk medicinal uses [3–6].

One of the important members of *Cucurbitaceae* namely winter melon [*Benincasa hispida* (Thunb.) Cogn.], is widely grown in Asian regions and medium dry areas at lowland tropics [7]. In addition to its English names that includes winter melon, white gourd, ash gourd or pumpkin, the plant is also known by different local names such as *Kundur* by Malay, *Dong Gua* by Chinese and Korean, *Petha* by Hindi, *Tougan* by Japanese, and *Kondol* by Philipines [6]. A number of medicinal properties have been ascribed to winter melon fruits including its therapeutic effectiveness against dropsy, diseases related to liver, leucorrhea, detoxication, fever, strengthen bladder function as well as the small and large intestines [6]. A broad range of biological activities such as anti-obesity, anti-inflammatory, anti-diarrheal, antioxidant, antipyretic, anti-compulsive and anti- ulcer have been ascribed to different parts, especially the fruit of this nutritious vegetable [6–8]. A recent review article reveals that winter melon fruit is a potential source of valuable nutrients such as natural sugars, organic acids, phenolics, vegetable protein, essential minerals and vitamins and possesses multiple medicinal benefits [6].

Currently, winter melon fruits are widely utilized for cuisine purposes in Asia and /or incorporated into food preparations such as soup, sweet, Jam and beverage [6]. Interestingly, large-sized winter melon fruits yield considerably high amount of seeds (200–300 kg/ha) which are often discarded as agro-waste [9] rather than utilized for value-addition by extracting valuable vegetable oil for preparation of high-value nutraceutical or functional food products. According to our preliminary study, winter melon seed is a potential source of oil which contains high proportion of an essential fatty acid, linoleic acid [10].

The bioactives profile of several under-utilized seed oils has been studied in order to explore their potential uses as new and alternative source of high-value oils for the nutraceutical industry [5]. In Malaysia, winter melon is popularly known by two cultivars namely round and oval, however, a hybrid-type round is also grown which is developed through breeding of the fuzzy white gourd genotype and green winter melon genotype [6]. As such no significant efforts have yet been made towards investigating the composition of valuable bioactives and antioxidant principles of the fruit seed oils from different cultivars of Malaysian winter melon. In the context of recent trends of optimal nutrition and functional foods industry, there is prompt need to explore nutraceutical potential of winter melon seed oil by appraising the profile of its high-value components and antioxidant nutrients. The present study therefore was undertaken to evaluate and quantify tocochromanols and phenolic compounds as well as antioxidant properties of winter melon seed oils of different cultivars so as to explore their functional food and nutra-pharmaceutical prospective. The composition of oil tocols (tocopherols and tocootrienols) and phenolics was elucidated using HPLC, whereas, the oil antioxidant components, extracted by various solvents, were evaluated using colorimetric assays.

Materials and methods

Materials

The fruits of two different cultivars namely round (cultivar 1) and hybrid round (cultivar 2) of winter melon were harvested from the winter melon farms in Sungai Lang, Sabak Bernam, Selangor, Malaysia and Temerloh, Pahang, Malaysia, respectively. The seeds were manually separated from the fruits, dried by placing in an oven at 45 °C for 24 h, and then ground using a Waring blender. The powdered seed material that passed through 500 µ mesh size sieve was used for extraction purposes. All the chemical reagents used were of analytical grade purchased from Merck, Germany and Fisher Scientific, Malaysia. DPPH (1,1-diphenyl-2-picrylhydrazyl), tocopherol, tocotrienol, and phenolic compound standard used in this study were from Sigma-Aldrich, (St Louis, MO). All chemicals and reagents were analytical grade and used as such without any further treatment.

Oil extraction

The powdered seed material was extracted by petroleum ether using conventional Soxhlet extractor. The extraction was performed for 6 h on water bath. After extraction, the solvent was removed at 40 °C under reduced pressure using rotary vacuum evaporator (Eyela Co. Ltd., Tokyo, Japan). The recovered oil was filtered using filter paper (Whatman No. 1) with a small amount of sodium sulphate anhydrous on it to absorb any traces of moisture. The oil was capped in a dark brown bottle and stored below 5 °C until used for further analyses.

Tocochromanols profile

Analysis of tocochromanols in the oil was carried by Normal Phase HPLC (NP-HPLC). For sample preparation, 250 mg of winter melon seed oil was dissolved in 1 mL *n*-hexane (HPLC) and filtered through 0.45 µm nylon membrane filter. The filtered oils solution was analyzed to evaluate α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, and γ-tocotrienol according to a previously described method with minor modifications [11]. A Water HPLC system equipped with waters 2487 dual wavelength absorbance detector and waters 600 pump was used. Briefly, a 20 µL sample was injected onto ACA 5Sil column (250×4.6 mm)

at room temperature (28 ± 1 °C) using 1.0 mL/min flow rate of mobile phase.

The tocochromanols were eluted isocratically using a mobile phase of *n*-hexane and isopropanol (98:2, v/v). The data were acquired by Water Empower2 software (Waters, Milford, MA). The peaks were identified by matching their retention time with those of pure standards of α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, and γ-tocotrienol, detected at wavelength of 295 nm. The quantification of compounds was based on standard calibration curve, constructed by analyzing each of the standard solutions over concentration range of 10 to 1000 mg/L. The concentration of tocochromanols was reported as µg/g of oil.

Phenolic compounds extraction

The winter melon seed oil phenolic extract was prepared by phase partitioning following the procedure described by Christophoridou et al. [12] with some modifications. Five grams of oil was dissolved in *n*-hexane (1:1, w/v) and extracted using 3 × 3 mL of methanol/water (80:20, v/v). The mixture was stirred for 2 min using vortex apparatus and centrifuged for 15 min at 3000 rpm. The extracts were combined and washed with 3 × 3 mL of *n*-hexane. The methanolic solution was evaporated under vacuum after the *n*-hexane was discarded; giving a residue (10–30 mg) that was dissolved in 0.3 mL of methanol. The solution was filtered by 0.45 µm nylon membrane filter prior to HPLC analysis. The phenolics standard solutions were prepared by dissolving respective pure compounds; gallic acid, 3,4-dihydroxybenzoic acid, 3,4-dihydroxybenzaldehyde, vanillic acid, vanillin, *p*-coumaric acid, trans-cinnamic acid, and ferulic acid in methanol/water (1:1, v/v).

Chromatographic analysis of phenolic compounds

The separation of phenolic compounds was carried out on Thermo Fisher Scientific C18 column (150 × 3.9 mm, 5 µm) at room temperature (28 ± 1 °C). The mobile phase consisted of 0.02% of trifluoroacetic acid (TFA) in deionized water as solvent A and absolute methanol (99.99%) as solvent B. The gradient conditions used were as follows: 100–25% solvent B (0–3 min), 25–30% solvent B (3–8 min), 30–45% solvent B (8–13 min), 45% solvent B (13–15 min), and 45–100% solvent B (15–30 min).

The flow rate of mobile phase was set at 0.6 mL/min, and a 20-µL sample was injected. The detection of phenolic compound was monitored at 254 nm. Identification of the phenols was made by comparing their retention times with those of pure standards. For quantification, standard curves were plotted using peak area against known concentrations of standards phenolic compounds. Mean values of two replicates were expressed as µg/g sample.

Antioxidant analysis

Preparation of extracts for evaluation of antioxidant activity

A modified extraction method as described by Parry et al. [4] was used for the preparation of oil extracts. The oil were independently extracted with 80% and 100% of each of the three solvents; ethanol, methanol, and isopropanol. Briefly, 1 g of oil was weighed in a test tube and mixed with 3 mL of solvent. The mixture was vortexed for 2 min and centrifuged for 5 min at 6000 rpm. Subsequently, the supernatant was collected.

The dried extracts were obtained after the solvents is evaporated under reduce pressure at 40 °C. A series of concentration of, the dried extracts were prepared in ethanolic solution and were evaluated for total phenolic content (TPC), DPPH and ABTS radical scavenging activity. The ethanolic solutions were subjected to filtration using 0.45 µm nylon membrane filter before the absorbance was measured by microplate reader.

Total phenolics content

The amount of total phenols was determined by Folin–Ciocalteu method based on a procedure described by Parry et al. [4] with minor modifications. The different solvent extracts of oil (0.5 mL) were mixed with 0.5 mL of the Folin–Ciocalteu reagent, and the reaction was terminated using 10 mL of 7% sodium carbonate. After 1 h incubation at room temperature (28 ± 1 °C), the absorbance was read at 750 nm. The standard curve was prepared using gallic acid standard solution of known concentrations (7.8, 15.6, 31.25, 62.5, 125, 250, 500 µg/ mL) and the results were expressed as µg gallic acid equivalent/g oil (µg GAE/g oil).

DPPH radical scavenging activity

DPPH scavenging capacity of the oil extracts was assessed according to the previously reported protocol using the stable 2,2-diphenyl-1-picryhydrazil radical (DPPH) [4]. Freshly made 25 mg/L DPPH–EtOH solution was mixed with a winter melon seed oil extracts at various concentrations of 0, 0.34, 0.69, 1.38, 2.75, 5.50, 11, 22, 44, and 88 mg oil equivalents/ mL. The absorbance at 517 nm determined after 60 min was used to compare the IC_{50} against DPPH scavenging capacities of individual oil extracts. The dose-dependencies of winter melon seed oil extracts and DPPH reactions were expressed by plotting the percent of DPPH inhibition against a series of concentration of oil extracts tested.

$$\%\ \text{inhibition of DPPH} = \left[(\text{Absorbance of control} - \text{Absorbance of sample})/\text{Absorbance of control}\right] \times 100$$

IC_{50} value = The effective concentration to obtain 50% of a maximum scavenging capacity by DPPH radical. A linear regression analysis of dose response curve were plotted to measure the value.

TEAC (Trolox equivalent antioxidant capacity)

TEAC was examined based on 2,20-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) assay which was determined according to the method of Re et al. [13]. Five milliliters of 7 mM ABTS and 88 µL of 140 mM potassium persulfate were incubated in the dark at room temperature for 12–16 h. It was diluted with 50% (v/v) ethanol to obtain absorbance of 0.700 ± 0.005 at 734 nm. A 280 µL of $ABTS^{\cdot+}$ solution was mixed with 20 µL of oil extracts vigorously. The blank samples were prepared with respective solvents but without extract addition and their absorbances were recorded at 734 nm. Various concentrations of Trolox were prepared to obtain standard calibration curve. The data were compared at 60 min of reaction and expressed as µg trolox equivalent/ g oil.

Statistical analysis

The results were expressed as mean values and standard deviation of three replicates for various parameters unless otherwise stated. The significant variations were assessed by computing the mean data using one way ANOVA followed by Duncan's test through The SAS System for Windows 9.0 (2004, NC, USA) at a 5% significance level.

Results and discussion

Tocochromanols profile

The tocopherols and tocotrienols composition of winter melon seed oils are shown in (Table 1). Total tocochromanols amounts were found to be in the range between 961.8 and 1027.6 µg/g seed oil. The highest tocopherols detected in both cultivars were *β*-tocopherol (593.3 µg/g and 478.98 µg/g oil) followed by γ-tocopherol (239.2 µg/g g and 236.41 µg/g oil), and the least *α*-tocopherol (19.3 µg/g and 30.87 µg/g oil). However, cultivar 2 contained significantly higher α-tocopherol, *δ*-tocopherol, and *γ*-tocotrienol contents than cultivar (1) contrarily, cultivar 1 exhibited significantly higher *β*-tocopherol content than cultivar (2) there was no significant difference found for *γ*-tocopherol content within those cultivars. According to Nyam et al. [5], Malaysian Cucurbits (bitter melon, Kalahari melon and pumpkin) seed oils were found to be a good source of tocopherols and had 40%–75% of γ-tocopherol, followed by 18%–33% of α-tocopherol, 0%–14% of *β*-tocopherol, and 0.05%–12% of δ-tocopherol. Sudanese *Citrullus lanatus, Cucumis prophetarum, Cucumis sativus, Luffa echinata* and *Cucumis melo* seed oils were comprised primarily of γ-tocopherol which was up to 90% of the total amount of analyzed tocopherols (14 to 433 µg/g) [14]. Stevenson et al. [15] reported that pumpkin seed oils of 12 cultivars had between 589.4 and 1234.2 µg/g of total tocopherols including *α*-tocopherol (27.1–75.1 µg/g), *γ*-tocopherol (74.9–492.8 µg/g), and *δ*-tocopherol (35.3–1109.7 µg/g).

Table 1 Tocopherols and tocotrienols content (µg/g) of winter melon seed oil

Seed oil	Cultivar 1	Cultivar 2
α-T	19.30 ± 2.31^{b}	30.87 ± 1.73^{a}
β-T	593.30 ± 34.87^{a}	478.98 ± 34.95^{b}
γ-T	239.20 ± 17.13^{a}	236.41 ± 24.70^{a}
γ-T3	65.80 ± 3.55^{b}	84.38 ± 7.77^{a}
δ-T	44.20 ± 1.95^{b}	196.96 ± 29.30^{a}
Total	961.80 ± 14.65^{b}	1027.60 ± 29.36^{a}

Values are mean ± SD of triplicate analysis; Means with different letters within the same row denote significant differences among cultivars ($p < 0.05$)

The antioxidant activity of *α*-tocopherol is usually lower than other tocopherols; nevertheless, it has greater Vitamin E potency [2, 16]. In general, vegetable oils, nuts and nut oil seeds, soya beans, and wheat, are among the good sources of tocopherols with concentration between 60 and 3720 µg/g. Moreover, it is well known that tocotrienols are also present in palm oil, corn oil, rice bran oil, grape seed oil, and barley [17, 18]. The order of the distribution was as follows; in palm olein γ-T3 > γ-T > α-T3 ≥ α-T, in soybean oil γ-T3 > γ-T > α-T3 > α-T and in corn oil γ-T3 > γ-T > α-T3 ≈ α-T [19]. The promising health benefit of γ-tocopherol is towards lowering the risk of cancer and cardiovascular disease whereas α-tocopherol is noted to be effective in cellular signalling, preventing lipid and polyunsaturated fatty acids peroxidation [20]. Tocotrienols have been reported in neuroprotection and stroke prevention with the latter being attributed to the antiproliferative, cholesterol-lowering properties, suppresses growth of human breast cancer cells and reduces oxidative protein damage [21, 22].

Phenolic compounds

The composition of individual phenolic compounds in winter melon seed oils is given in (Table 2). The results revealed that winter melon seed oils contained phenolic components in variable amounts. Vanillin, *para*-coumaric acid, ferulic acid, and *trans*-cinnamic acid were the most abundant in the oils of both cultivars. On the other hand, gallic acid, 3.4-dihydroxybenzoic acid (protocatechuic

Table 2 Individual phenolics content (μg/g) of winter melon seed oils

Phenolic compound	Cultivar 1	Cultivar 2
Gallic acid	0.65 ± 0.02^{a}	0.65 ± 0.08^{a}
3,4-Dihydroxybenzoic acid	0.56 ± 0.04^{a}	0.56 ± 0.01^{a}
3,4-Dihydroxybenzaldehyde	0.45 ± 0.07^{a}	0.44 ± 0.14^{a}
Vanillic acid	0.68 ± 0.09^{a}	0.87 ± 0.28^{a}
Vanilin	4.78 ± 1.80^{a}	8.86 ± 2.32^{a}
p-Coumaric acid	1.92 ± 0.24^{a}	2.02 ± 0.44^{a}
trans-Cinnamic acid	1.35 ± 0.06^{a}	1.58 ± 0.13^{a}
Ferulic acid	1.88 ± 0.38^{a}	2.26 ± 0.50^{a}
Total	12.27 ± 2.11^{a}	17.24 ± 2.08^{a}

Values are mean ± SD of duplicate analysis. Means with same letters within the same row denote non-significant differences among cultivars ($p > 0.05$)

acid), 3,4-dihydroxybenzaldehyde, and vanillic acid were found in small amounts. The phenolic compounds identified showed minute differences within these two cultivars. Van Hoed [23] summarized that the main compounds for most seed oils are phenolic acids, such as protocatechuic acid, *p*-(or 4-) hydroxyl benzoic acid, vanillic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, sinapic acid, elenolic acid, 3,4-dihydroxybenzoic acid, ellagic acid and gallic acid while vanillin and 3,4-dihydroxybenzaldehyde are detected in specific seed oils. Siger et al. [24] quantified the total phenolic acid (0.221 μg/g) in pumpkin seed oil, in which vanillic acid being the predominant (0.114 μg/g). Furthermore, Andjelkovic et al. [25] presented that Slovenian and Belgian pumpkin seed oils had 1.58–20.92 μg/g of total phenolic compound in which vanillic acid, vanillin and ferulic acid were the main phenols.

Conversely, Nyam et al. [5] found a higher level of phenolic acids in pumpkin seed oils which included vanillic acid (6.0 μg/g), caffeic acid (4.1 μg/g), gallic acid (2.6 μg/g), *p*-hydroxybenzoic acid (2.0 μg/g), *p*-coumaric acid (1.7 μg/g), ferulic acid (1.5 μg/g), and protocatechuic acid (0.8 μg/g). Virgin olive oil is known to be very rich in various phenols, in which *p*-coumaric acid being the main phenolic compound [26]. However, winter melon seed oil had a higher amount of total phenolic acid (7.042–7.938 μg/g) than the extra virgin olive oil (2.36–6.00 μg/g) [27]. The distribution of phenolics in plants foods vary depending upon several genetic/varietal as well as agro climatic factors [26, 27]. The levels of *p*-coumaric acid, ferulic acid, *trans*-cinnamic acid, gallic acid, and protocatechuic acid from winter melon seed oils were 90%, 88%, 58%, 30%, and 87%, higher than the average levels of extra virgin olive oils reported thus far. Nergiz and Ünal [26] depicted a higher content of total phenolic acids in only one out of eleven samples of virgin olive oil sample when compared to winter melon seed oils. A lower level of phenolic acids (0.004–2.566 μg/g) of cold-pressed oil from soybean, sunflower, rapeseed, corn, grapeseed, hemp, flax, and rice bran were found by Siger et al. [24]. It is now well recognized that phenolic compounds delay the initiation of the oxidative process, preserving the endogenous antioxidant pool. Tripoli et al. [28] reviewed the beneficial effects of phenolic compound on human health including the prevention of cardiovascular diseases, prevention of tumoral diseases, anti-inflammatory activity and antimicrobial activity.

Total phenolics content (TPC)

Figure 1 shows the total phenolics content (TPC) of the tested winter melon seed oil extracts measured using Folin–Ciocalteu colorimetric method. The TPC of winter melon seed oils ranged from 19.37 to 203.93 μg GAE/g oil for cultivar 1, and 30.77 to 190.07 μg GAE/g oil for cultivar 2. In comparison, the content of total phenolics at a level of 980 μg GAE/g oil was discovered in cold-pressed pumpkin seed oil [29]. Meanwhile, Parry et al. [4] reported a higher level of total phenolics in the fruit seed oils of blueberry, red raspberry, marionberry, and boysenberry, 90–2000 μg GAE/g oil. In the present analysis, the seed oils from cultivar 1 and cultivar 2 expressed higher amount of total phenolics than black raspberry seed oil which is detected between 35 and 93 μg GAE/g oil.

Oils extracted from both the selected cultivars possessed significant amounts of TPC except for 100% ethanol and isopropanol. Moreover, cultivar 1 oil, extracted by 80% isopropanol, recovered the highest value of TPC among all oil extracts. However, cultivar 2 oils extracted by 100% methanol and 80% methanol and ethanol obtained a higher level of TPC than cultivar 1. According to literature, solvent polarity plays a significant role in increasing phenolic solubility and their subsequent recovery from a plant matrix [30]. Typically, the least polar solvents are observed to be suitable for the extraction of lipophilic phenols except when very high pressure is applied. On the contrary to our present data, Bimakr et al. [31] revealed that ethanol used in the Soxhlet extraction technique gave the highest TPC value for the winter melon seed rather than ethyl acetate and *n*-hexane. Hence, it could be assumed that there is no standard extraction procedure suitable for the extraction of all plant phenols. Organic solvents such as methanol, ethanol, acetone, *n*-butanol, ethyl acetate and n-hexane, have been commonly used for the extraction of phenolics from plant materials [32].

Antioxidant activity

In this study, two methods have been used to evaluate the antioxidant capacity of the oil extracts obtained from two cultivars of winter melon seed oil by different solvent

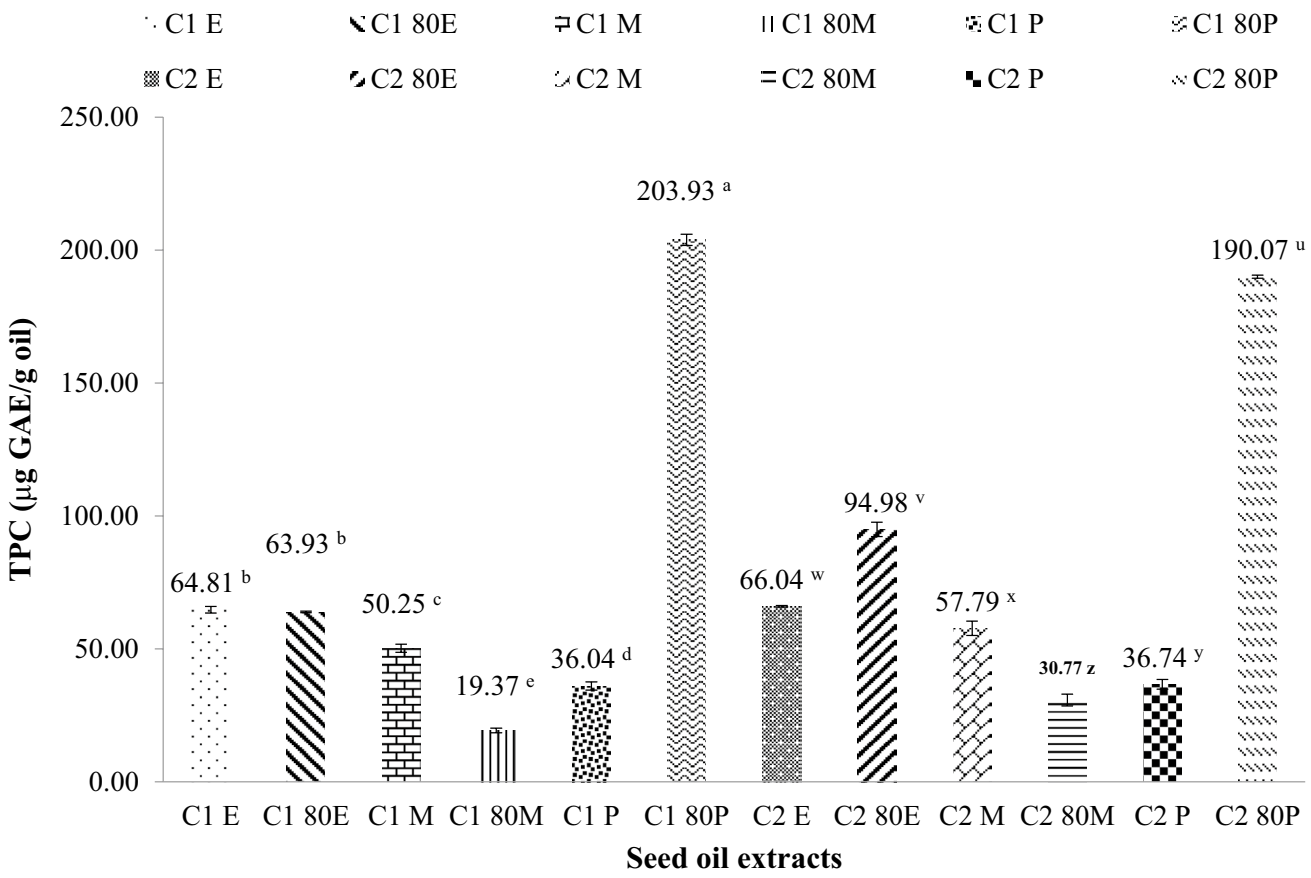

Fig. 1 Total phenolics content (TPC) of winter melon seed oil extracts from two cultivars. Values are mean ± SD of triplicate analysis. Means with different letters within the same cultivar denote significant differences among oil extracts ($p < 0.05$). *C1* cultivar 1, *C2* cultivar 2, *E* 100% ethanol, *M* 100% methanol, *P* 100% isopropanol, *80E* 80% ethanol, *80M* 80% methanol, *80P* 80% isopropanol

extraction systems; 1,1-diphenyl-2-picrylhydrazyl (DPPH·) and TEAC using 2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) free radical scavenging assay. DPPH scavenging activity of winter melon seed oils was dose-dependent.

Figure 2 shows the inhibition percentages of DPPH radical plotted against the concentrations of oil extracts. The antioxidant activity of all ethanolic (100%), methanolic (100%) and isopropanolic (100%) oil extracts of cultivar 1 and cultivar 2 seed oils are presented in Fig. 2a, while ethanolic (80%), methanolic (80%) and isopropanolic (80%) oil extracts from both cultivars are illustrated in Fig. 2b. DPPH scavenging capacities of oil extracts were expressed as IC_{50} values (Table 3). A lower value of IC_{50} indicates a strong antioxidant activity. In agreement with the TEAC assay, the oil extracts from both cultivars prepared by 80% ethanol and isopropanol exhibited a significantly higher antioxidant activity than that of 100% ethanol and isopropanol, whereas 100% methanolic oil extract had significantly pronounced antioxidant activity compared to 80% methanolic oil extract Fig. 3.

Of the two cultivars, oils from 80% ethanol extracts offered the strongest antioxidant activity in both IC_{50} and TEAC determination. Ethanol (80%) extracts of cultivar 1 seed oil (7.03 mg/mL) and methanol (100%) extracts of cultivar 2 seed oil (6.88 mg/mL) gave the strongest antioxidant activity against DPPH in term of IC_{50} values and did not show any significant differences. For 80% methanol extract, cultivar 1 and cultivar 2 showed the weakest IC_{50} value.

Cultivar 1 seed oil extracted by 80% ethanol, 80% isopropanol and 100% methanol and cultivar 2 seed oil extracted by 100% methanol showed almost twofolds higher DPPH radical scavenging activity with contribution of 81–89% inhibition percentage at 22 mg/mL instead of 47.56% of inhibition of kapok seed oil at 100 mg/mL [33]. Previously, Hu et al. [34] used hexane/acetone/ethanol (2/1/2, v/v/v) for carotenoid extraction from algal and the extract showed higher antioxidant activity than each carotenoid standard including all-*trans* forms of zeaxanthin, lutein, β-carotene and α-carotene. Nevertheless, the carotenoid extract from algal exhibited lower radical scavenging activity in the range of 22.80 to 24.5 mg/mL compared to 100% methanol and

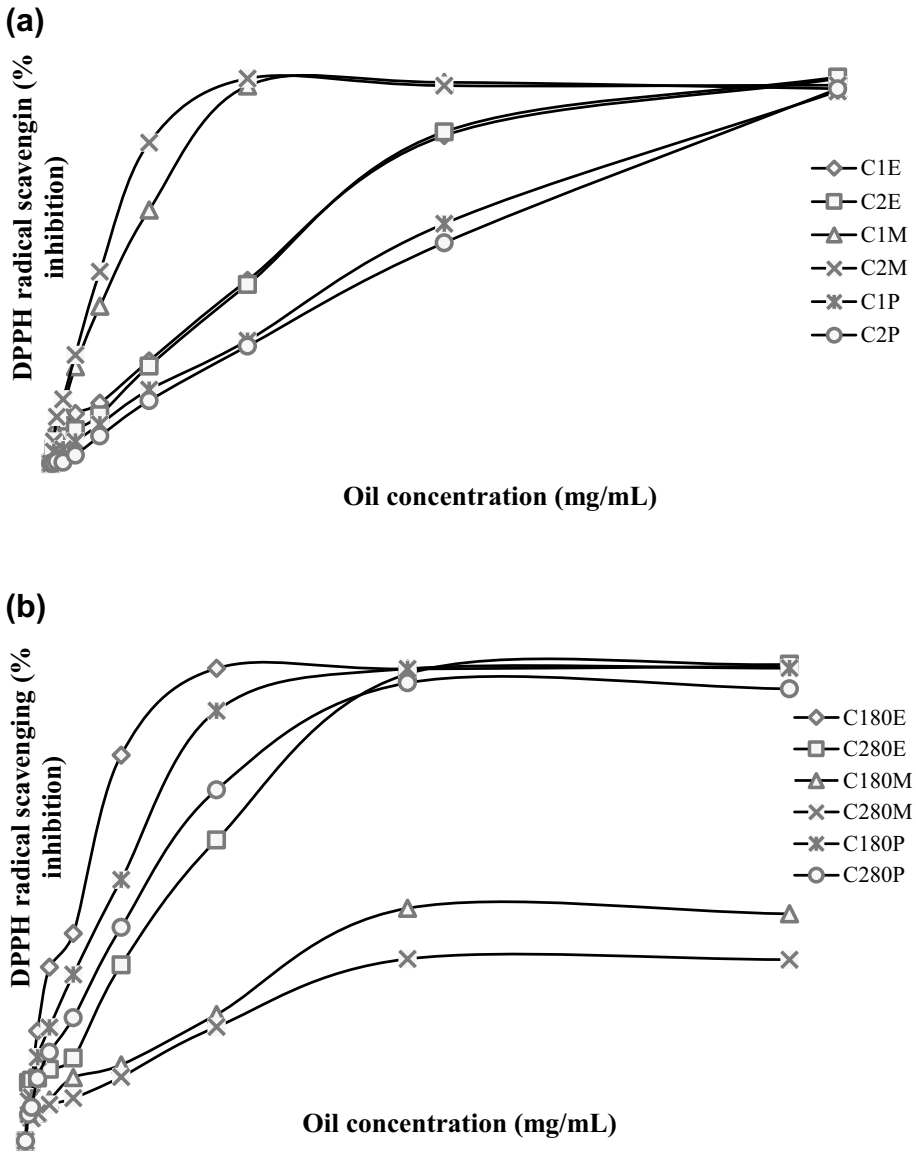

Fig. 2 Dose effect of winter melon seed oil extracts obtained from two cultivars by different extraction solvents (**a** 100% and **b** 80% solvents) against DPPH free radicals (inhibition of DPPH radical). *C1* cultivar 1, *C2* cultivar 2, *E* 100% ethanol, *M* 100% methanol, *P* 100% isopropanol, *80E* 80% ethanol, *80M* 80% methanol, *80P* 80% isopropanol

Table 3 DPPH radical scavenging capacity (IC_{50}) of winter melon seed oil extracts from two cultivars

Solvent	IC_{50} (mg/mL)	
	Cultivar 1	Cultivar 2
100%		
Ethanol	27.39 ± 0.10^{c}	27.67 ± 0.31^{c}
Methanol	10.84 ± 0.03^{e}	6.88 ± 0.09^{f}
Isopropanol	46.46 ± 0.34^{b}	47.98 ± 0.33^{b}
80%		
Ethanol	7.03 ± 0.10^{f}	22.11 ± 0.33^{d}
Methanol	50.84 ± 1.02^{a}	64.71 ± 0.66^{a}
Isopropanol	12.22 ± 0.05^{d}	15.60 ± 0.07^{e}

Values are mean ± SD of triplicate analysis. Means with different letters within the same cultivar denote significant differences among oil extracts ($p < 0.05$)

80% ethanol and isopropanol of winter melon oil extracts in the present analysis.

In addition, seed oil of boysenberry exhibited highest antioxidant activity among seed oil of red raspberry, blueberry, and marionberry when extracted using 100% methanol but showed the lowest antioxidant activity against 80% methanol extracts [4]. The recovery of antioxidant from plant materials is influenced by the solubility of the antioxidant compound in the solvent used for the extraction purposes. This indicates that the solvent polarity may affect extraction efficiencies for different antioxidant compounds. Oilseeds and other sources of edible oils contain less polar antioxidants soluble in the oil phase while more polar antioxidants remain in seed meal [35]. Oxidative damage to cellular components can lead to numerous diseases, including cancer, cardiovascular disease, and other chronic

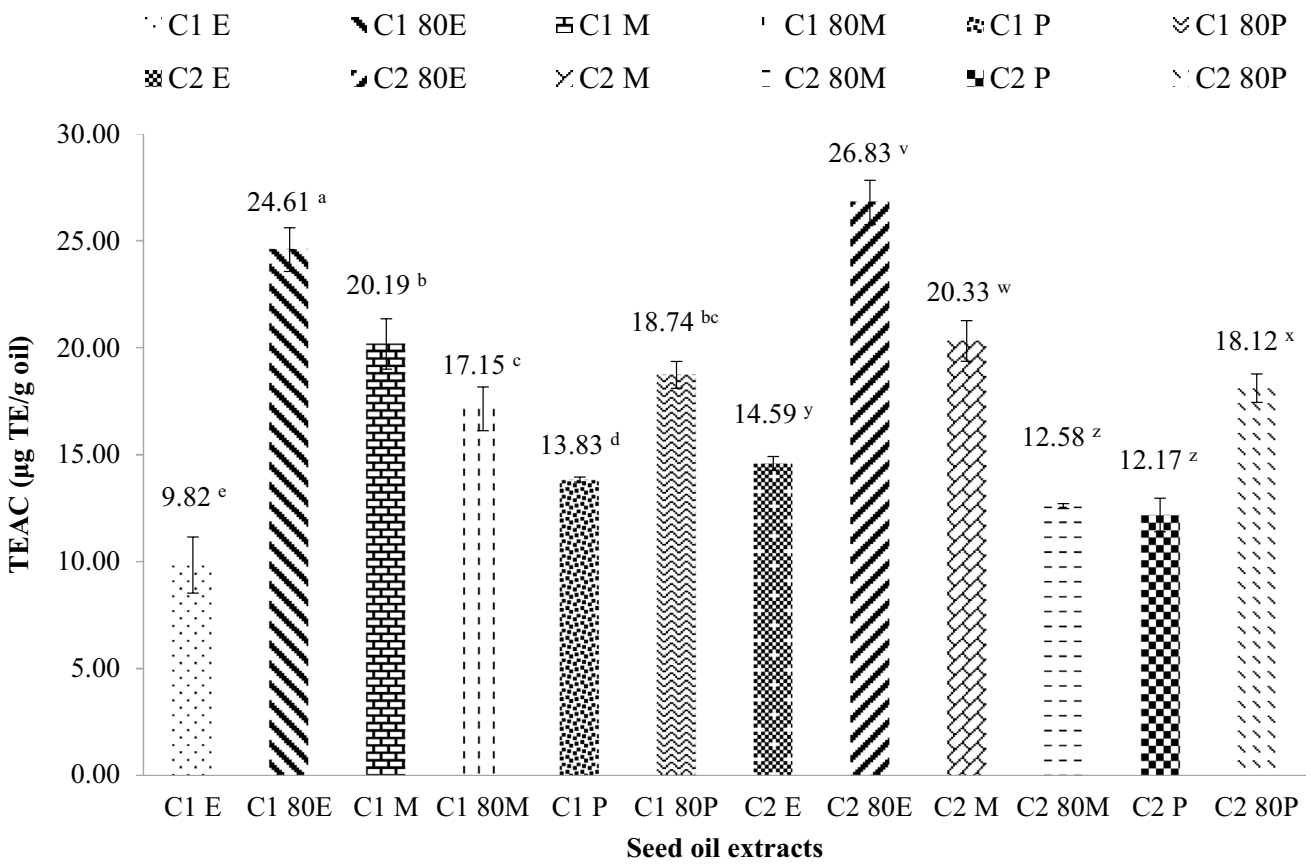

Fig. 3 TEAC (trolox equivalent antioxidant capacity) of winter melon seed oil extracts from two cultivars. Values are mean ± SD of triplicate analysis. Means with different letters within the same cultivar denote significant differences among oil extracts ($p < 0.05$). *C1* cultivar 1, *C2* cultivar 2, *E* 100% ethanol, *M* 100% methanol, *P* 100% isopropanol, *80E* 80% ethanol, *80M* 80% methanol, *80P* 80% isopropanol

diseases. An exposure to other oxidative stressors (oxidants) will cause the damage on biomolecules (DNA, lipids, and proteins), tissues, and organs. Vegetable seed oils are one of the natural antioxidants sources, not only used as food ingredients but also have health beneficial phytochemicals and nutraceuticals with potential as potent inhibitor of these oxidative stressors [36]. Hence, consumption of vegetable oils with greater natural antioxidants content can reduce the risk of various diseases including cancers [36, 37].

Conclusions

The recovery of phenolics and other natural free radical scavenging agents from the winter melon seed oils was found to be dependent on the nature of oil, cultivar as well as the polarity of extraction solvent used. Aqueous ethanol (80%) was found to be the most efficient solvent for extraction of antioxidant phenols among other solvents for ABTS assay. Aqueous ethanol also extracted maximum amount of DPPH radical scavengers for cultivar 1, nevertheless, methanolic (100%) oil extract of cultivar 2 displayed the strongest radical scavenging activity. Meanwhile, aqueous isopropanol (80%) extracted greater contents of total phenolics from both the cultivars. These results advocate that not a single assay is sufficient to authenticate the antioxidant principles of winter melon seed oil, rather, multiple antioxidant assays coupled with the selection of an appropriate solvent need to be employed for the extraction and subsequent assessment of antioxidant potential of such high-value vegetable oils. Overall, the results of this study reveal that an appreciable amount of dietary antioxidants including phenolic compounds and tocols is present in the seed oils of both tested winter melon cultivars supporting that these oils, being a high-value commodity, might serve as a potential ingredient for functional food and nutraceutical industry.

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