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Phenolic content and antioxidant activity of cantaloupe (*cucumis melo*) methanolic extracts

Hajar Iqbal Ismail [a], Kim Wei Chan [b], Abdalbasit Adam Mariod [b], Maznah Ismail [a,b,*]

[a] Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia
[b] Nutraceuticals and Nutrigenomics Programme, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia

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ABSTRACT

The objectives of this study were to determine phenolic content and antioxidant activity of methanolic extracts from different parts of cantaloupe (leaf, stem, skin, seed and flesh). The flesh extract afforded the highest yield (89.6 ± 0.3%) whilst the lowest yield was obtained from the seed (13.7 ± 0.5%) ($p < 0.05$). The leaf extract showed the highest total phenolic content (26.4 ± 0.3 mg GAE/g extract) and total flavonoid content (69.7 ± 3.37 μg RE/g extract) accompanied with best antioxidant activity through all antioxidant assays ($p < 0.05$). In addition, the stem extract also exhibited good antioxidant activity. Thus, these results suggest that methanolic extracts of cantaloupe leaf and stem may serve as a potential source of natural antioxidant for food and nutraceutical application.

1. Introduction

Living organisms require ample amount of oxygen for their metabolism and energy production. However, free radicals are produced during the energy production process (Packer, 1999), as the unavoidable consequence of respiration in aerobic organisms. Free radicals are unstable species that react rapidly and destructively with biomolecules such as protein, lipid, DNA and RNA in the body. Uncontrolled generation of free radicals is associated with lipid and protein peroxidation, resulting in cell structural damage, tissue injury or gene mutation and ultimately lead to the development of various health disorders such as Alzheimer's disease, cancer, atherosclerosis, diabetes mellitus, hypertension and ageing (Mantle, Eddeb, & Pickering, 2000). On the other hand, lipid auto -oxidation that is initiated by free radicals also results in food quality deterioration, (Kanner, 1994).

Antioxidants play an important role in defending the body against free radicals damage. Antioxidants refer to a group of compounds that are able to delay or inhibit the oxidation of lipids or other biomolecules and thus, prevent or repair the damage of the body cells that is caused by oxygen (Shahidi & Naczk, 2004; Tachakittirungrod, Okonogi, & Chowwanapoonpohn, 2007). They work by preventing the formation of new free radical species, converting existing free radicals into less harmful molecules and preventing radical-chained reactions (Rodriguez et al., 2007). For instance, phenolic compounds such as, quercetin and ellagic acid, are good antioxidants that able to protect body cells from injuries caused by reactive oxygen and nitrogen species (Sroka & Cisowski, 2003).

The Cucurbitaceae family includes several species of cultivated plants of great economic importance, including watermelon (*Citrullus lanatus* L.), squash (*Cucurbita maxima* L.), cucumber (*Cucumis sativus* L.) and cantaloupe (*Cucumis melo* L.) (Ritschel et al., 2004). Cantaloupe is one of the most consumed fruit crops worldwide due to its pleasant flavour and nutritional value. Cantaloupes are a diverse group of fresh, dessert fruits that includes the orange flesh cantaloupes, green flesh honeydew, and mixed melons. A previous study showed that cantaloupe pulp extract possesses high antioxidant and anti-inflammatory properties (Vouldoukis et al., 2004). However, antioxidant assessment on different parts of *C. melo* is very limited (Mariod & Matthaus, 2008). Thus, the main objectives of the study were to determine the phenolic content and antioxidant activities of methanolic extracts from different parts of cantaloupe.

2. Materials and methods

2.1. Materials and chemicals

Cantaloupe plant was harvested in Taman Agroteknologi (A), Bukit Changgang, Banting, Selangor, Malaysia. The chemicals used in this study were of analytical reagent grade that include:

* Corresponding author. Address: Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia. Tel.: +60 3 89472115; fax: +60 3 89472116.
E-mail address: maznah@medic.upm.edu.my (M. Ismail).

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methanol, chloroform, Tween 20 and trichloroacetic acid (Fisher Scientific, Loughborough, Leicestershire, UK); ammonium thiocyanate (99.99%) and linoleic acid (Sigma Chemical Co., St. Louis, MO, USA); gallic acid (Sigma–Aldrich, Madrid, Spain), β-carotene (Type I synthetic, 95%) and (+) α-tocopherol (Sigma–Aldrich Co., St. Louis, MO, USA), iron (II) chloride-80 mesh 98%, ethylenediaminetetra acetic acid (EDTA), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and L-ascorbic acid 99% (Sigma–Aldrich, Deutschland, Germany); Folin–Ciocalteu's phenol reagent (Fluka Biochemica, Buchs, Switzerland); hydrochloric acid (37%), aluminium trichloride ($AlCl_3$) and dimethyl sulphoxide (DMSO) (Merck, Darmstadt, Germany); thiobarbituric acid (AppliChem GmbH, Deutschland, Germany) and buffer solution (phosphates) pH 7.00 ± 0.02, 20 °C (R&M Chemicals, Essex, UK).

2.2. Sample preparation

Cantaloupe (*C. melo*) plants were cleaned and separated into five different parts namely, skin, leaf, stem, flesh and seed. All parts were individually freeze-dried (VirTis benchtop K, Bieleveld, Germany).

2.3. Methanol extraction

Different parts of cantaloupe were respectively pulverised into fine powder using a stainless steel blender (Waring Commercial, Torrington, CT, USA) and mixed with methanol at the ratio of 1:10 (w/v). Then these mixtures were manually swirled for 15 min and left in a sonicator (Power sonic 505, HwaShin Technology Co., Seoul, Korea) for 60 min. After that, these mixtures were individually filtered through Whatman filter paper No. 1 and the entire extraction process was repeated twice on the residue obtained from the filtration process. The filtrates were individually pooled and methanol was removed from the filtrates under reduced pressure (Rotavapor R210, Buchi, Postfach, Flawil, Switzerland). Finally, cantaloupe extracts were cooled in a dessicator for 30 min before the yield of each extract was calculated. The methanolic extracts from different parts of cantaloupe were kept at −80 °C prior to further analyses.

2.4. Total phenolic content (TPC)

Total phenolic contents of cantaloupe extracts were determined using Folin–Ciocalteu assay (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). Briefly, 100 mg of cantaloupe methanolic extracts were individually dissolved in 10 ml of methanol. Then, 0.1 ml of these solutions was mixed with 2.5 ml of 10-fold diluted Folin–Ciocalteau reagent, and 2.0 ml of 7.5% sodium carbonate (Na_2CO_3). After incubation at 40 °C for 30 min, the absorbance of the reaction mixtures were measured at 760 nm by using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). Gallic acid was used as a standard and TPC of cantaloupe extracts were expressed in milligram gallic acid equivalents (mg GAE/g extract).

2.5. Total flavonoid content (TFC)

Total flavonoid content was determined by the aluminium calorimetric method (Quettier-Deleu et al., 2000), using rutin as a standard. Briefly, the test samples were individually dissolved in DMSO. Then, the sample solution (150 μl) was mixed with 150 μl of 2% $AlCl_3$. After 10 min of incubation at ambient temperature, the absorbance of the supernatant was measured at 435 nm by using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). Three replicates were made for each test sample. The total flavonoid content was expressed as rutin equivalents in microgram per gram extract (μg RE/g extract).

2.6. DPPH scavenging activity

DPPH scavenging activity of cantaloupe extracts was determined according to the method described by Singh, Murthy, and Jayaprakasha (2002) with slight modifications. In brief, 0.1 ml cantaloupe extract at various concentrations were respectively added to 0.49 ml of methanol and 0.39 ml of DPPH methanolic solution (4 mg/100 ml). Then, the mixtures were vortexed vigorously and allowed to stand in the dark for 60 min. Finally, the absorbance of these mixtures was measured by using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan) at 515 nm. The sample concentration providing 50% of radical scavenging activity (IC_{50}) was obtained through interpolation of linear regression analysis. The lower IC_{50} indicates higher radical scavenging activity and vice versa. Ascorbic acid and α-tocopherol were used as standards.

2.7. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of cantaloupe methanolic extracts was determined by using electron spin resonance spectrometer (JEOL-JES-FA100, Tokyo, Japan). Hydroxyl radical was generated through Fenton reaction, with 5,5-dimethyl *N*-oxide pyroline (DMPO) as the trapping agent. The reaction mixture contained 40 μl of DMPO (400 mM), 37.5 μl of $FeSO_4$ (0.4 mM), 112.5 μl of EDTA (0.1 mM), 60 μl of sample or blank and 150 μl of H_2O_2 (2.0 mM). The electron spin resonance (ESR) measurement was conducted 1 min after preparing each reaction mixture at room temperature. The condition of ESR measurement was as follows: sweeping field, 336.45 ± 5 mT; microwave power, 8 mw; mod width, 0.1 mT; sweep time, 2 min; time constant, 0.1 s and amplitude, 160. Dimethy sulphoxide (DMSO) was used as standard in this experiment and hydroxyl radical scavenging activity of cantaloupe extracts were expressed in gram DMSO equivalent per gram of sample (g DMSOE/g sample).

2.8. β-carotene bleaching activity

Beta-carotene bleaching activity of cantaloupe extracts was determined according to the method of Wettasinghe & Shahidi, 1997. In brief, 3 ml of β-carotene solution (5 mg β-carotene/50 ml chloroform) were added to 40 mg of linoleic acid and 400 mg of Tween 20. Then, the mixture was mixed well and dried under a stream of nitrogen. Immediately, 100 ml of distilled water were added to the dried mixture to form a β-carotene-linoleic acid emulsion. In order to determine the β-carotene bleaching activity of the extract, 1.5 ml of emulsion were respectively added to 20 μl of cantaloupe extracts. After that, these mixtures were incubated in a water bath at 50 °C for 60 min. Finally, the absorbances of the reaction mixtures were read at 470 nm by using a UV–Visible spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). Alpha-tocopherol was used as a standard in this experiment. Antioxidant activity (AA%) of cantaloupe extracts were calculated by using the following equation:

$$AA(\%) = 100(DR_C - DR_S)/DR_C$$

whereby, AA = antioxidant activity; DR_C = degradation rate of the control: $[(a/b)/60]$; DR_S = degradation rate of the sample: $[(a/b)/60]$; a = initial absorbance of the sample; b = absorbance after 60 min of incubation.

2.9. Total antioxidant activity assay

2.9.1. Ferric thiocyanate (FTC) test

FTC test on cantaloupe extracts was conducted according to the method described by Kikuzaki and Nakatani (1993). In this study, 4 mg of cantaloupe extracts were individually dissolved in 4 ml of methanol. Then, the extract solutions were respectively mixed with linoleic acid (4.1 ml), 0.05 M phosphate buffer pH 7.0 (8 ml) and distilled water (3.9 ml). These mixtures were then kept in screw-cap containers at 40 °C in the dark. In order to determine the FTC values, 0.1 ml of these mixtures was respectively added into 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of ferrous chloride solution (in 3.5% HCl) to the reaction mixture, the absorbance of the samples was read at 500 nm by using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). This procedure was repeated every 24 h until the control sample reached its maximum absorbance value. Ascorbic acid and α-tocopherol were used as standard antioxidants in this test.

2.9.2. Thiobarbituric acid (TBA) test

TBA test (Mackeen et al., 2000) was conducted instantly after the control sample from FTC test reached its maximum absorbance value. In brief, 1.0 ml of 20% aqueous trichloroacetic acid and 2.0 ml of 0.67% aqueous thiobarbituric acid were added to 2 ml of sample solutions acquired from FTC test. The mixtures were then placed in boiling water bath for 10 min. After cooling under the running tap water, the mixtures were centrifuged at 3000*g* for 30 min. Finally, the absorbance of supernatants at 532 nm was measured by using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan).

2.10. Statistical analyses

Data was reported as mean ± standard deviation from triplicate determination. Analysis of variance (ANOVA) accompanied with LSD and Tukey tests (SPSS for Windows, Version 15) were conducted to identify the significant difference between samples ($p < 0.05$).

3. Results and discussion

3.1. Extraction yield, total phenolic content and total flavonoid content

Table 1 presents the yield, total phenolic content and total flavonoid content of cantaloupe methanolic extracts. The yield of the extracts varied from 13.7 to 89.6%. Among all tested extracts, the highest and the lowest yields were respectively obtained from the flesh and seed of cantaloupe ($p < 0.05$). The yield of methanolic extracts from different parts of cantaloupe is presented in the following order: flesh > skin > stem > leaf > seed ($p < 0.05$). Low extraction yield of cantaloupe seed is probably due to the low solubility of major components of the seed (for instance, fat, starch and protein that are usually present in large amount in the seed) in methanol. This is supported by the finding of Thitilertdecha, Teerawutgulrag, and Rakariyatham (2008) who reported that the yield of *Nephelium lappaceum* seed obtained through ether extraction was higher than methanol extraction, probably due to the high fat content of the seed (22.2%).

Table 1
Extraction yield, total phenolic content and total flavonoid content of cantaloupe extracts ($n = 3$).

Part of cantaloupe	Extraction yield (% w/w)	Total phenolic content (mg GAE/g extract)	Total flavonoid content (μg RE/g extract)
Seed	13.66 ± 0.52[a]	2.85 ± 0.21[d]	1.62 ± 0.74[d]
Flesh	89.62 ± 0.29[b]	1.68 ± 0.14[e]	2.03 ± 0.16[d]
Leaf	16.29 ± 2.15[c]	26.40 ± 0.34[a]	69.70 ± 3.37[a]
Skin	50.33 ± 4.35[d]	4.70 ± 0.23[c]	5.13 ± 1.32[c]
Stem	23.78 ± 2.09[e]	10.25 ± 0.40[b]	9.68 ± 0.74[b]

Values with different superscript letters within the same column are statistically different ($p < 0.05$).

The content of extractable phenolic compounds in the cantaloupe extracts was determined through a linear gallic acid standard curve ($y = 8.2313x + 0.078$; $r^2 = 0.9971$). The total phenolic content of cantaloupe extracts varied from 1.68 to 26.40 mg GAE/g extract. The highest content of total phenolic compounds was detected in the cantaloupe leaf extract (26.40 mg GAE/g extract) whereas the lowest content was measured in the flesh extract (1.68 mg GAE/g extract) ($p < 0.05$). Total phenolic content of cantaloupe extracts is arranged in the following descending order: leaf > stem > skin > seed > flesh ($p < 0.05$). This finding is in agreement with some previous studies which reported that total phenolic content of leaf extract was higher than other parts of the plant for *Beta vulgaris, Petroselinum crispum* and *Coriandrum sativum* (Pyo, Lee, Logendra, & Rosen, 2004; Wong & Kitts, 2006). This suggests that leaf might be the part that is rich in phenolic compounds in many plants. Several studies have revealed that the phenolic content in the plants are associated with their antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Chang et al., 2001).

Flavonoids are the most common and widely distributed group of plant phenolic compounds, which usually are very effective antioxidants (Yanishlieva-Maslarova, 2001). In this study, the total flavonoid content of methanolic extracts from different part of cantaloupe was evaluated by aluminium colorimetric assay. Rutin was used as a standard ($y = 0.0097x + 0.0127$, $r^2 = 0.9995$) and the total flavonoid content of cantaloupe extract was expressed in microgram of rutin equivalents per gram of extract (μg RE/g extract). The total flavonoid content of cantaloupe extracts was varied considerably from 1.62 to 69.70 μg RE/g extract. The data presented in Table 1 indicates that the highest flavonoid content of 69.70 μg RE/g extract was observed in the extract of leaf and the lowest content was observed in the extract of the seed (1.62 μg RE/g extract) ($p < 0.05$). Total flavonoid content of cantaloupe extracts is arranged in the following sequence: leaf > stem > skin > flesh > seed ($p < 0.05$). The total flavonoid content of cantaloupe extracts is in well correspondence to the total phenolic content ($r = 0.9836$). This indicates that the flavonoids are the major phenolic compounds present in cantaloupe plant.

3.2. Antiradical activity

Table 2 shows DPPH and hydroxyl radicals scavenging activity of cantaloupe methanolic extracts. In general, IC_{50} values of all tested samples through DPPH scavenging activity test were ranging from 0.02 to 25.44 mg/ml and the DPPH scavenging activity is arranged in the following descending order: ascorbic acid ($IC_{50} = 0.02$ mg/ml) > α-tocopherol ($IC_{50} = 0.06$ mg/ml) > leaf > stem > skin > flesh > seed ($p < 0.05$). In this study, DPPH scavenging activity of cantaloupe extracts shows similar trend with the result of total phenolic content ($r = 0.9228$) and total flavonoid content ($r = 0.8478$), indicating that DPPH radical scavenging activity of cantaloupe extracts is highly related to the amount of phenolic compounds especially flavonoids that present in the extracts.

Meanwhile, hydroxyl radical scavenging activities of cantaloupe extracts were ranging from 37.37 to 147.96 g DMSOE/g extract (Table 2). Among all tested extracts, leaf extract exhibited the strongest hydroxyl radical scavenging activity (147.96 g DMSOE/g extract), whilst seed extract showed the least antiradical activity

Table 2
DPPH and hydroxyl radicals scavenging activity of cantaloupe methanolic extracts ($n = 3$).

Cantaloupe Extracts	DPPH radical scavenging activity (IC_{50} (mg/ml))	Hydroxyl radical scavenging activity (g DMSOE/g extract)
Flesh	11.9 ± 1.00[a]	67.19 ± 8.90[a]
Leaf	1.52 ± 0.01[b]	147.96 ± 22.04[b]
Stem	2.16 ± 0.22[c]	55.59 ± 0.35[c]
Seed	25.44 ± 2.83[d]	37.37 ± 2.42[c]
Skin	9.58 ± 0.37[e]	39.11 ± 2.91[d]

Values with different superscript letters within the same column are statistically different ($p < 0.05$).

(37.37 g DMSOE/g extract) ($p < 0.05$). Hydroxyl scavenging activity of cantaloupe extracts is presented in the following descending order: leaf > flesh > stem > skin > seed ($p < 0.05$). Correlation test shows that hydroxyl radical scavenging activity of cantaloupe extracts is well correlated with its total phenolic ($r = 0.9171$) and total flavonoid ($r = 0.9512$) contents, supporting the former statement on the contribution of phenolic compounds (particularly flavonoids) in antiradical activity of cantaloupe extracts.

3.3. β-carotene bleaching (BCB) activity

Antioxidant activity of cantaloupe extracts as measured by bleaching of β-carotene was determined through the interpolation of a linear α-tocopherol standard curve ($y = 16.784x + 3.1533$; $r^2 = 0.9708$) and expressed in mg alpha-tocopherol equivalent (Teq)/g extract. In the BCB assay, linoleic acid produces hydroperoxides during incubation at 50 °C. The presence of hydroperoxides cause rapid discoloration of β-carotene (Wettasinghe & Shahidi, 1999). However, hydroperoxides formed in this system can be neutralised by the antioxidants from the extracts.

A variation in antioxidant activity of cantaloupe extracts ranging from 0.41 to 4.43 mg Teq/g extract was observed. Fig. 1 shows that the cantaloupe leaf extract again exhibited the highest antioxidant activity (4.43 mg Teq/g extract) through BCB assay, whilst seed extract showed the least antioxidant activity (0.41 mg Teq/g extract) towards the bleaching of β-carotene. Antioxidant activity of cantaloupe extracts through BCB assay is arranged in the following order: leaf > stem > skin > flesh > seed ($p < 0.05$). This result is in agreement with the previous study by Elzaawely, Xuan, & Tawata, 2007 who reported that the leaf extract of *Alpinia zerumbet* exhibited higher inhibitory activity towards β-carotene oxidation than other parts of the plant. On the other hand, stem extract of cantaloupe also showed good antioxidant activity in reducing the oxidation of β-carotene in this study ($p < 0.05$). As discussed previously, high antioxidant activity of cantaloupe leaf and stem extracts might due to its high phenolic content, likewise flavonoids in particular.

3.4. Total antioxidant activity (FTC & TBA tests)

Fig. 2 shows the hydroperoxides inhibitory activity of cantaloupe extracts through FTC test. As shown in the Fig. 2, almost all cantaloupe extracts (except for flesh) significantly retarded the formation of hydroperoxides in the linoleic acid emulsion system throughout the incubation period as compared to the control sample ($p < 0.05$). From the third day onwards, the absorbance value of the control was higher ($p < 0.05$) than other samples (except for flesh) and reached its maximum absorbance on the ninth day of incubation. The overall inhibitory activity of cantaloupe extracts against hydroperoxides formation can be established in the following descending order: ascorbic acid > leaf > stem > skin > α-tocopherol > seed > control > flesh. The flesh extract showed greater absorbance value ($p < 0.05$) than the control sample at the end point of the incubation period (ninth day), indicating that cantaloupe flesh extract might possess prooxidative properties that enhances the autoxidation of linoleic acid in the emulsion system and increase the generation of reactive substances.

The cantaloupe leaf extract exhibited the highest hydroperoxides inhibitory activity that is superior to ascorbic acid, α-tocopherol as well as other tested cantaloupe extracts ($p < 0.05$). This result suggests that the leaf extract might contain primary antioxidant compounds, which are able to react aggressively with free radicals, particularly hydroxyl radicals, thereby terminating the radical-chained reaction and retard the formation of hydroperoxides (Frankel, 1991; Shahidi & Wanasundara, 1992). In line with that, previous studies also suggested that leaf extract possesses excellent antioxidant activity for wild blueberry leaves and *B. vulgaris* (Naczk, Amarowicz, Zadernowski, Pegg, & Shahidi, 2003; Pyo et al., 2004).

After the control sample reached its maximum absorbance value in FTC test, TBA test was conducted on the samples subsequently. This test measures the thiobarbituric acid reactive substances content at a later stage of lipid oxidation, involving the quantification of the secondary products formed from lipid oxi-

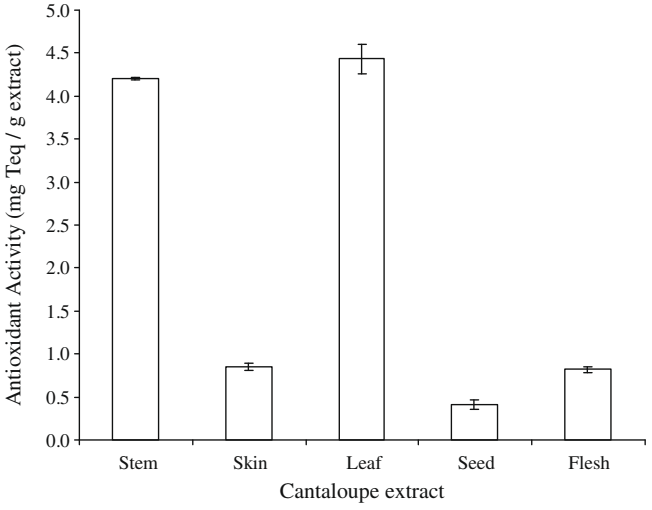

Fig. 1. Antioxidant activity of cantaloupe extracts through BCB assay.

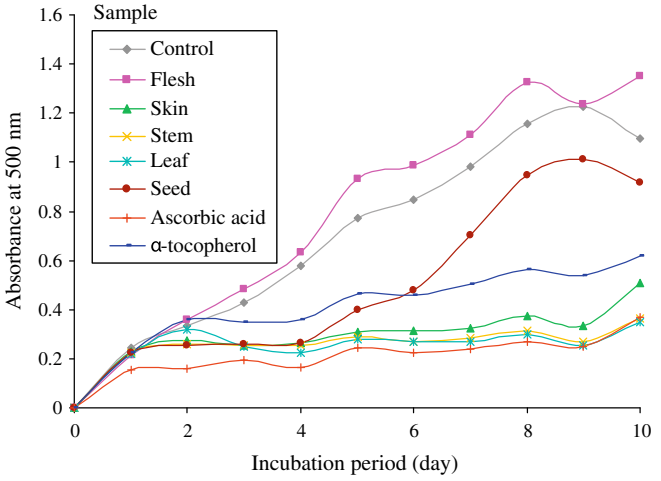

Fig. 2. Hydroperoxides inhibitory activity of cantaloupe extracts through ferric thiocyanate test.

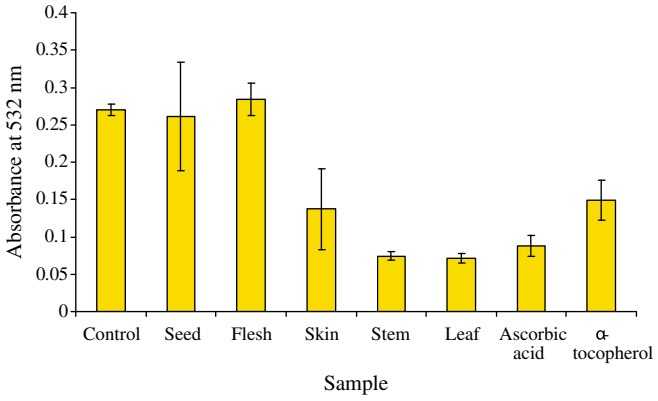

Fig. 3. Thiobarbituric acid reactive substances inhibitory activity of cantaloupe extracts measured by thiobarbituric acid test.

dation. In this test, low absorbance value indicates higher thiobarbituric acid reactive substances inhibitory activity. Fig. 3 shows that the flesh extract exhibited the weakest thiobarbituric acid reactive substances inhibitory activity, whilst the leaf extract possessed the strongest activity ($p < 0.05$). The strength of thiobarbituric acid reactive substances inhibitory activity of leaf and stem extracts were similar to ascorbic acid ($p > 0.05$) but superior to α-tocopherol ($p < 0.05$).

In summary, thiobarbituric acid reactive substances inhibitory activity of all tested samples is presented in the following descending order: leaf > stem > ascorbic acid > skin > α-tocopherol > seed > control > flesh ($p < 0.05$). The trend of thiobarbituric acid reactive substances inhibitory activity of cantaloupe extracts is rather similar to the trend of FTC test in this study. This suggests that reduction of thiobarbituric acid reactive substances content in leaf and stem samples might due to the lower hydroperoxides accumulation in the respective samples, previously. Besides, secondary antioxidant compounds that might present in these extracts may also contribute to the inhibition of hydroperoxides decomposition in these samples.

4. Conclusions

High antioxidant activity is observed in the leaf and stem extracts of cantaloupe as compared to other tested extracts. Thus, these extracts can be considered as new sources of natural antioxidants for food and nutraceutical products, potentially.

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