



Antioxidant Activities and Total Phenolic Content of Multi-colored Fruits and Vegetables in Thailand

Sirikhwan Tinrat*

Department of Biotechnology, Faculty of Applied science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand 10800

*Correspondent author: Sirikhwan.t@kmutnb.ac.th, sktinrat@gmail.com

Abstract

Fruits and vegetables are valuable sources of natural phenolic antioxidants which are known to have beneficial health promoting properties. The main objectives of this study were to compare the antioxidant activities and total phenolic and flavonoid contents of ten ethanolic edible plant extracts (Five species of fruits and vegetables) with varieties of colors. The result showed that purple color of plants, including ethanolic extracts of *Brassica oleracea* Linn. ($40.7 \pm 0.56\%$) and *Vigna sesquipedalis* ($4.52 \pm 0.01\%$) significantly showed the highest TPC and TFC compared with the same species and other species with different colors ($p < 0.01$). Measurement of antioxidant capacity, yellow *Capsicum annum* Linn. ($58.72 \pm 0.52\%$) and purple *Solanum melongena* Linn. extract ($22.44 \pm 1.20\%$) significantly showed the maximum antioxidant activities by DPPH radical scavenging activity assay and ferric ion reducing antioxidant power (FRAP) assay, respectively ($p < 0.01$). Base on shades of color pigment extracts, green and red pigment had more potential antioxidant activities than other colors. The purple and yellow pigments showed the maximum total phenolic contents by Folin-Ciocalteu reagent. These results concluded that purple color of plants; *Brassica oleracea* Linn. and *Vigna sesquipedalis* in this study had the potential to be a suitable source of cheap phenolics and flavonoids as antioxidant compounds.

Keywords : *Edible plant extracts, Antioxidant activity, Total phenolic and flavonoid contents, Fruits and vegetables*

1. Introduction

Antioxidant compounds such as phenolic acids, polyphenols and flavonoids can scavenge free radicals and result in inhibition of oxidative mechanisms which are responsible for many disorders and diseases in humans (1) such as infections,

diabetes, arthritis, cardiovascular diseases, cancer and Alzheimer's diseases (2, 3). Potential sources of antioxidant compounds have been searched in several types of plant materials (4, 5) such as vegetables, fruits, leaves, roots and crude plant extracts (6, 7, 8). Fruits and vegetables are edible plants

which are known as source of beneficial nutrients such as minerals, polyphenols (9) and vitamins which provide health benefits in addition to their nutritional value. They have long been recognized as a rich source of natural antioxidant compounds or phytochemical substances. Fruits and vegetables that are blue, purple, red, orange, yellow and green often contain higher nutrients and antioxidants (10, 11, 12). Different colors indicated different nutrient profiles and potential antioxidant activities (13). On the color wheel, the purple-blue-red-orange spectrum is home to the most antioxidant-rich fruits (14).

Plant polyphenols are known to have multifunctional properties such as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers. Flavonoids and their derivatives are the largest and most important group of polyphenols. Among the variety of phenolic compounds, phenolic acids have attracted considerable interest in the past few years due to their many potential health benefits. As phenolic

compound, phenolic acids are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anti-carcinogenic, anti-inflammatory and vasodilatory actions (15, 16). Though many other plant species have been investigated in the search for novel antioxidants but generally there is still demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore, ten most prominently utilized fruits and vegetables in Thailand were selected for investigation of antioxidant activity.

2. Materials and Method

2.1 Plant materials

A total of 10 edible plant materials (five species of fruits and vegetables) in different shades of color were selected from local market and Big C Supermarket, Bangkok province, in the central region of Thailand. All of them (Table 1) are commonly consumed in Thailand.

Table 1. Description of the 10 studied plant material samples

Scientific name	Family	Common name	Color	Part used
Vegetables				
<i>Capsicum annum</i> Linn.	Solanaceae	Bell pepper	Yellow, Red and Green	Fruits
<i>Solanum melongena</i> Linn.	Solanaceae	Egg plant	Green and Purple	Fruits
<i>Brassica oleracea</i> Linn.	Brassicaceae	Cabbage	Green and Purple	Leaves
<i>Lycopersicon esculentum</i>	Solanaceae	Cherry tomato	Red and Yellow	Fruits
<i>Vigna sesquipedalis</i>	Fabaceae	Yardlong bean	Green and Purple	Seeds
Fruits				
<i>Malus domestica</i>	Rosaceae	Apple	Red and Green	Fruits
<i>Citrullus lanatus</i> (Thunb.)	Cucurbitaceae	Water melon	Red and Yellow	Fruits
<i>Hylocereus polyrhizus</i>	Cactaceae	Dragon Fruit	Red and white	Fruits
<i>Vitis vinifera</i> Linn.	Vitaceae	Grape	Red and Green	Fruits
<i>Citrus maxima</i>	Rutaceae	Pomelo	Yellow and Pink	Fruits

2.2 Sample preparation and extraction

All edible plant materials were thoroughly cleaned and soaked with 70% ethanol for 15 min. The different samples were prepared and soaked in 95% ethanol in a ratio of 1:2 for 1-2 day separately. Then, the liquid extract was filtered and centrifuged at 2000 rpm for 20 min at room temperature to obtain a clear supernatant and was used for analyzing the total phenolic and flavonoid content, reducing power and their free radical scavenging capacity. The ethanolic extracts were stored at -20°C under dark condition until further analysis. The final weight of the crude

extracts were weighted and calculated for the percentage yield.

2.3 Estimation of total phenolics content

The amount of total phenolic content (TPC) of crude ethanolic extracts was determined according to Folin-Ciocalteu procedure (17, 18). Briefly, 100 µl of the tested samples were introduced into test tubes; 750 µl of fresh Folin -Ciocalteu reagent (diluted in distilled water (1:10)) and 750 µl of 6% (w/v) sodium carbonate (Na₂CO₃) was added and allowed to completely react for 90 min at room temperature in the dark condition. Absorbance at 725 nm was measured on

a spectrophotometer. The total phenolic content was expressed as gallic acid equivalents in milligram per gram of fresh weight (mgGAE/100gFW) using the linear equation based on standard calibration curve of the gallic acid (0.02 - 0.1 mg/ml).

2.4 Estimation of total flavonoids content

Total flavonoids content (TFC) in plant extracts were estimated by aluminium chloride colorimetric assay (19). Two hundred microliters of sample extracts were then added in 2.3 ml of 30% of methanol. The mixture was added with 100 μ l of 0.5 M NaNO_2 and 100 μ l 0.3 M AlCl_3 , respectively. Next, the sample solution was thoroughly mixed with vortex and kept in the dark for 5 min. And then, the absorbance at 506 nm was measured using by spectrophotometer. A yellow color indicated the presence of flavonoids. The total flavonoid content was expressed as rutin equivalents in milligram per gram of fresh weight (mg RE/g FW) using the linear equation based on calibration curve of rutin (0.01-0.05 mg/ml)

2.5 DPPH free radical scavenging assay

The antioxidant activities of plant extracts were evaluated through free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. This assay was determined by the method of Akowuah *et al.* (2005) (20). DPPH solution was prepared freshly at a concentration of 0.1 mM in methanol. An aliquot of 100 μ l of each extract solution was mixed with 900 μ l of methanolic DPPH solution. The mixture was thoroughly mixed and left to stand for 15 min in the dark and absorbance was recorded at 517 nm using spectropho-

tometer. Ascorbic acid (0.01-0.05 mg/ml) was used as standard and was prepared using the similar procedure.

2.6 Determination of ferric reducing/antioxidant power assay (FRAP)

The total reducing capacity was determined by using FRAP assay which was performed according to the method of Benzie and Strain (1996) (21) with some modification. The FRAP reagent was initially prepared, consisting of 300mM acetate buffer (pH 5.6), 10mM 2,4,6-Tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution in a ratio of 10:1:1 (v/v/v). An amount of 300 μ l plant extracts was mixed with 2.7 ml of the FRAP reagent in test tubes by vortex. the absorbance was measured at 596 nm by using spectrophotometer after incubation for 30 min. The results were expressed as ascorbic acid equivalents in milligram per gram fresh weight (mg AAE/g FW) based on calibration curve of ascorbic acid (0.01-0.05 mg/ml).

2.7 Statistical Analysis

All data were expressed as means \pm standard deviation of (n=6) measurements. Statistical analyses (ANOVA) were performed with the statistical program MS Excel (Microsoft Office 2010 Professional). P-values less than 0.05 were considered significant (P<0.05).

3. Results and Discussion

3.1 Total phenolics content (TPC)

Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. Phenolic acids are a major class of phenolic

compounds, widely occurring in the plant kingdom especially in fruits and vegetables (9). Natural antioxidants are responsible for inhibiting or preventing the deleterious consequences of oxidative stress (18). The amount of total phenolics compounds of different plant samples was measured by Folin-Ciocalteu method, varied widely in edible plant materials and ranged from 0.84 ± 0.05 (green *Brassica oleracea* Linn.) to 40.70 ± 0.56 (purple *Brassica oleracea* Linn.) mg GAE/100g FW (Table 2).

The maximum content of total phenolics was found in purple *Brassica oleracea* Linn. followed by purple *Solanum melongena* Linn., yellow *Lycopersicon esculentum*, green *Solanum melongena* Linn. and red *Lycopersicon esculentum*, respectively. Among the ethanolic vegetable extracts, the highest level of phenolics was recorded in purple *Brassica oleracea* Linn., whereas the lowest was in green *Brassica oleracea* Linn., which was consistent with the data reported by Bunga et al., (2014) (22).

Among the ethanolic fruit extracts, the highest level of phenolics was found in yellow *Citrus maxima* while the lowest was in red *Citrullus lanatus* (Thunb.) ($P > 0.05$). When considering shades of color, the amount of total phenolics in these plant materials are in descending order of purple > yellow > green > red > white. The total phenolic contents of the vegetable ethanolic extracts had significantly higher than these of the fruit ethanolic extracts ($P < 0.05$).

3.2 Total flavonoids content (TFC)

Flavonoids is one of the most diverse and widespread group of natural compounds which are probably the most important natural phenols (23). Many researchers attempt to find the novel natural source of bioactive compounds such as antioxidant substances (24). Total flavonoid contents (TFC) in various colors of plant materials are given in Table 2. TFC were determined by using spectrophotometric method with aluminum chloride and expressed in terms of rutin equivalent (RE). The total flavonoids content in this study was presented between 0.12 ± 0.05 (yellow *Citrus maxima*) and 4.52 ± 0.01 (purple *Vigna esquipedalis*) mg RE/100gFW. But the content of flavonoids in ethanolic *Citrullus lanatus* (Thunb.) extracts was not found. The total flavonoids content was the highest in purple *Vigna esquipedalis* followed by purple *Brassica oleracea* Linn., red *Lycopersicon esculentum*, green *Malus domestica* and yellow *Lycopersicon esculentum*, respectively. Among the ethanolic vegetable extracts, the highest level of TFC was found in purple *Brassica oleracea* Linn. while the lowest was in green *Capsicum annum* Linn. Among the ethanolic fruit extracts, the highest level of phenolics was found in purple *Vigna esquipedalis* while the lowest was in *Citrullus lanatus* (Thunb.). When considering shades of color, the amount of total phenolics in these plant materials are in descending order of purple > red > green > yellow > white.

Table 2. The yield, total phenolic and flavonoid content and antioxidant activities of edible plants

Plants	Color	%Extract yield (w/w)	TPC (mg GAE/100g FW)	TFC (mg RE/100g FW)	Antioxidant activities	
					DPPH assay (mg AAE/100g FW)	FRAP assay (mg AAE/100g FW)
<i>Capsicum annuum</i> Linn.	Red	6.65±0.12 ^G	13.37±0.67 ^F	0.42±0.05 ^J	54.72±8.06 ^b	17.36±1.67 ^c
<i>Lycopersicon esculentum</i>		6.79±0.13 ^G	18.42±0.14 ^D	1.92±0.04 ^C	21.68±0.41 ^c	15.12±0.89 ^f
<i>Malus domestica</i>		15.88±0.02 ^C	3.58±0.29 ^K	1.26±0.06 ^G	2.70±0.55 ^h	1.50±0.1 ^k
<i>Citrullus lanatus</i> (Thunb.)		9.19±0.22 ^E	0.94±0.05 ^O	ND	0.35±0.06 ^m	0.06±0.02 ^o
<i>Hylocereus polyrhizus</i>		17.75±0.08 ^B	1.63±0.25 ^M	0.39±0.05 ^J	0.94±0.18 ^k	0.86±0.01 ^m
<i>Vitis vinifera</i> Linn.		17.75±0.08 ^B	1.63±0.25 ^M	0.39±0.05 ^J	0.94±0.18 ^k	0.86±0.01 ^m
<i>Citrus maxima</i>		9.7±0.20 ^{DE}	4.33±0.17 ^U	0.13±0.01 ^N	2.66±0.36 ^h	1.54±0.02 ^{jk}
<i>Capsicum annuum</i> Linn.	Green	6.66±0.12 ^G	9.29±0.32 ^H	0.27±0.11 ^L	58.72±4.52 ^a	9.34±0.51 ^h
<i>Solanum melongena</i> Linn.		5.68±0.05 ^{GH}	18.78±0.91 ^D	0.95±0.03 ^H	8.61±0.76 ^e	20.8±1.36 ^c
<i>Brassica oleracea</i> Linn.		4.9±0.06 ^I	0.84±0.05	0.30±0.02 ^K	2.02±0.34 ⁱ	1.74±0.07 ^j
<i>Vigna sesquipedalis</i>		9.02±0.02 ^E	4.90±0.18 ^I	1.77±0.11 ^E	3.84±0.62 ^g	12.66±1.74 ^g
<i>Malus domestica</i>		20.18±0.02 ^A	4.02±0.01 ^J	1.87±1.23 ^D	1.78±0.24 ⁱ	1.25±0.08 ^l
<i>Vitis vinifera</i> Linn.		14.57±0.19 ^C	1.94±0.33 ^L	0.50±0.07 ^I	1.18±0.24 ^j	1.02±0.07 ^l
<i>Capsicum annuum</i> Linn.	Yellow	5.64±0.18 ^H	16.59±0.43 ^E	0.31±0.08 ^K	17.2±2.19 ^d	20.14±2.95 ^c
<i>Lycopersicon esculentum</i>		6.51±0.05 ^G	22.92±0.25 ^C	1.59±0.28 ^F	22.32±0.21 ^c	19.72±0.34 ^d
<i>Citrullus lanatus</i> (Thunb.)		10.43±0.16 ^D	1.10±0.08 ^N	ND	0.37±0.03 ^{lm}	0.05±0.01 ^o
<i>Citrus maxima</i>		8.75±0.23 ^E	4.95±0.59 ^I	0.12±0.05 ^N	2.26±0.29 ^j	2.12±0.04 ⁱ
<i>Solanum melongena</i> Linn.	Purple	5.93±0.12 ^G	31.28±1.85 ^B	1.24±0.12 ^G	8.77±0.73 ^d	22.44±1.20 ^a
<i>Brassica oleracea</i> Linn.		7.76±0.04 ^F	40.70±0.56 ^A	2.67±0.12 ^B	6.50±0.47 ^f	21.32±1.10 ^b
<i>Vigna esquipedalis</i>		7.29±0.13 ^F	11.20±0.66 ^G	4.52±0.01 ^A	6.26±0.73 ^f	19.84±1.87 ^d
<i>Hylocereus polyrhizus</i>	White	17.87±0.87 ^B	0.99±0.08 ^N	0.16±0.02 ^M	0.40±0.01 ^l	0.30±0.06 ⁿ

GAE = Gallic Acid Equivalent; RE = Rutin Equivalent; AAE = Ascorbic acid Equivalent; ND = Not Detected

ABCDEFGHIJKLMN means in the same column with different superscript were significantly different (P < 0.05) by one way ANOVA and paired T-test

abcdefghijklnmo, means in the same column with different superscript were significantly different (P < 0.01) by one way ANOVA and paired T-test

3.3 Antioxidant activities

The antioxidant activities of the ethanolic edible plant extracts were showed significantly different between varieties. It was evidence that the varieties with colored flesh significantly exceed white-flesh varieties (Table 2). DPPH is a stable and free radical. It is commonly used as a substrate to evaluate in vitro antioxidant activity of extracts of fruits, vegetables and medicinal plants compared with other methods. Antioxidants can scavenge the radical by hydrogen donation, which causes a decrease of DPPH absorbance at 517 nm. This method is widely used to evaluate antioxidant activities within a relatively short time when compared with other methods. The result clearly showed that all edible plant extracts had DPPH radical scavenging activity ranging from 0.35 ± 0.06 (red *Citrullus lanatus* (Thunb.)) to 58.72 ± 0.52 (green *Capsicum annum* Linn) mg AAE/100g FW. The ethanolic extracts of green *Capsicum annum* Linn. showed the highest of DPPH value and the red *Citrullus lanatus* (Thunb.) showed its lowest. The DPPH radical scavenging activity of the vegetable ethanolic extracts had significantly higher than that of the fruit ethanolic extracts ($P < 0.05$). When considering shades of color, the total phenolic content in these plant materials decreased in the following order: green > red > yellow > purple > white.

3.4 FRAP

The antioxidant potential of the plant species was ascertained from FRAP assay based on their ability to reduce TPTZ- Fe^{3+} complex to TPTZ- Fe^{2+} . TPTZ- Fe^{2+} is an intensive blue color and can be monitored at 596 nm. Reducing

power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (20, 25). In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of each compound. Maximum reducing capacity was presented in the purple *Solanum melongena* Linn. (22.44 ± 1.20 AAE/100g FW) (Table 2) whereas low reducing power was found in the yellow *Citrullus lanatus* (Thunb.) (0.05 ± 0.01 AAE/100g FW). The reducing capacity of the vegetable extracts had significantly higher than that of the fruit extracts ($P < 0.05$). Moreover, the total phenolic content in these plant materials decreased in the following order: purple > green > yellow > red > white.

Antioxidant compounds (phenolics and flavonoids) and their antioxidant activity in five different colors (purple, green, yellow, red, and white) of the edible plants were investigated. Among these plant extracts, the purple plants had higher level of the total phenolic and flavonoid contents than the other colors, while the white plants had lower level of the total phenolic and flavonoid contents than the other colors. The varieties with colored flesh significantly exceed traditional white (Table 2). This fact is confirmed by data in Figure 1-2, which compare the groups of varieties with different flesh color. In this study, the colors in the ethanolic extracts of samples were pigments that found in general plants including the expected carotenoids (red and orange), beta-carotene (yellow and orange), anthocyan lycopene (red), anthocyanins (purple), flavonoids (yellow), betalains, and chlorophylls (green). All of them act as

antioxidant compounds. The pigments found in plants play important roles in plant metabolism and visual attraction in nature.

They are also important for humans, attracting our attention and providing us with nutrients.

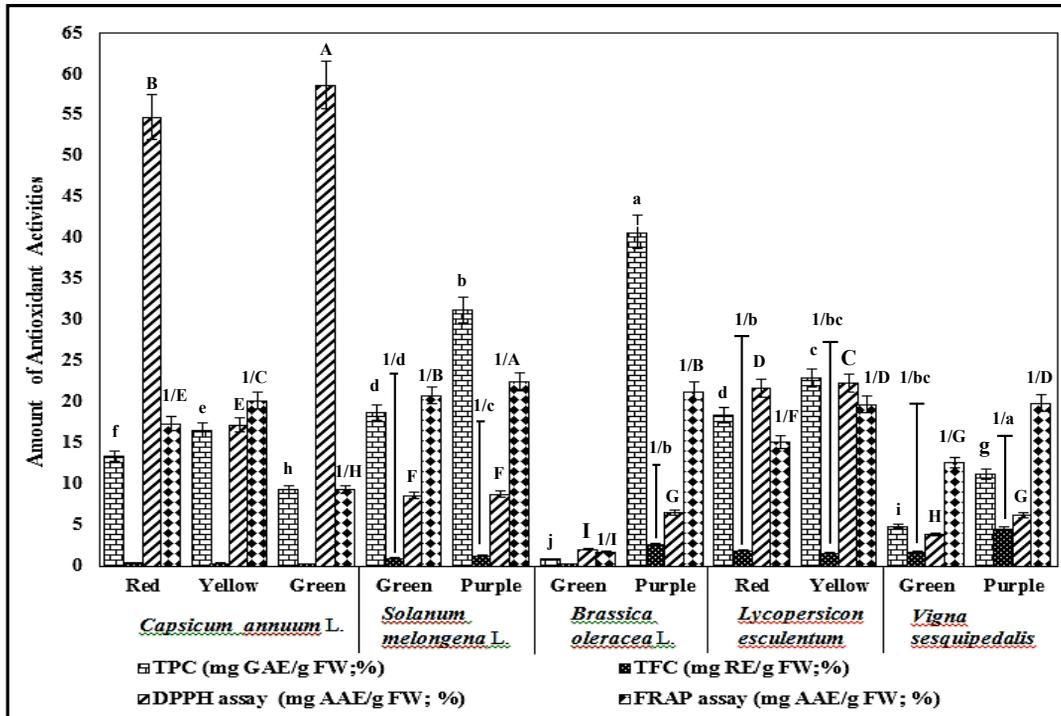


Figure 1. Impact of varieties with different flesh color on the total phenolics and flavonoids content and antioxidant activity of vegetable materials (a-j, 1/a-d: $p < 0.05$; one way ANOVA and paired T-test and A-I and 1/A-G: $p < 0.01$; one way ANOVA and paired T-test)

4. Conclusion

The antioxidant activities of edible fruits and vegetables were determined. Based on these studies, it can be concluded that the total phenolics and flavonoids contents were dependent on pigments in plant extracts. Purple color of the plants (*Brassica oleracea* Linn. and *Vigna sesquipedalis*) showed the highest TPC and TFC. Moreover, the antioxidant activity of the ethanolic extract varies with its ability to react with the harmful free radicals.

Among all these fruits, green *Capsicum annuum* Linn. and purple *Solanum melongena* Linn. showed the highest antioxidant capacity. Therefore, these edible fruits and vegetables could be exploited as antioxidant additives or as nutritional supplements. The providing data can enrich the existing comprehensive data of antioxidant activity of wild fruits and its biological value. In this study, the differences in the total phenolics and flavonoids contents were statistically significant

between the different varieties of species. The frequent consumption of vegetables

and fruits in various colorful will be the positive impact on the health.

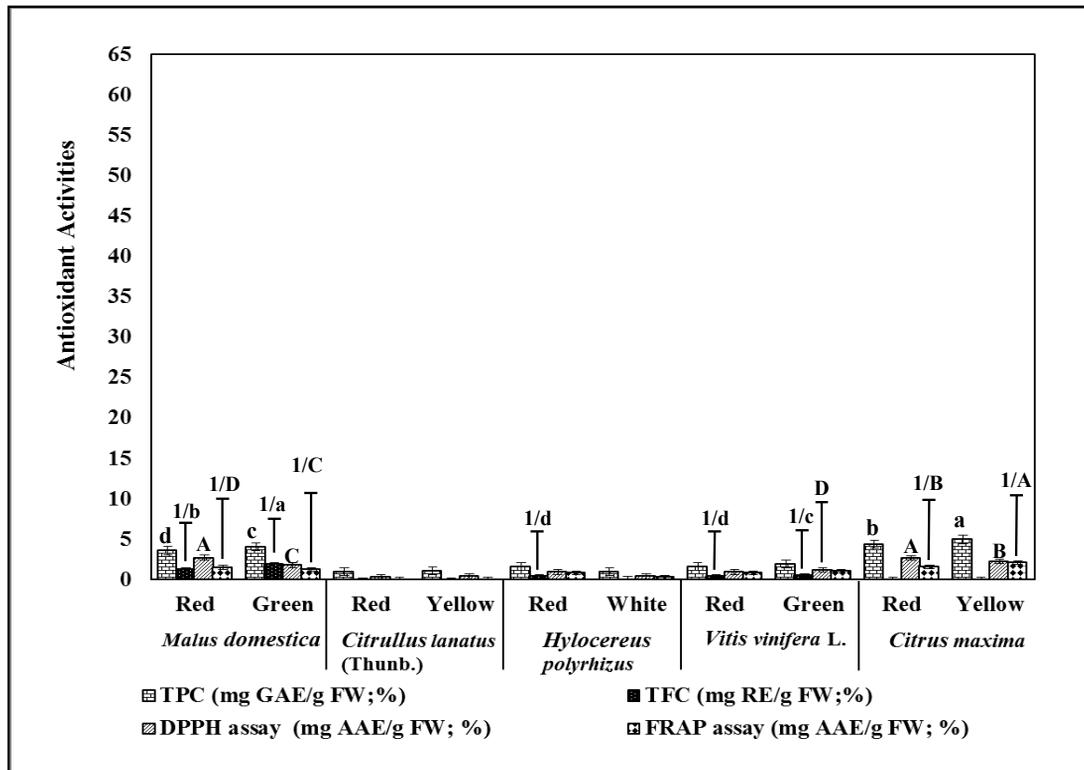


Figure 2. Impact of varieties with different flesh color on the total phenolics and flavonoids content and antioxidant activity of fruit materials (a-d, 1/a-d: $p < 0.05$; one way ANOVA and paired T-test and A-D and 1/A-D: $p < 0.01$; one way ANOVA and paired T-test)

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