

PHENOLIC, CAROTENOID, ASCORBIC ACID CONTENTS AND THEIR ANTIOXIDANT ACTIVITIES IN BELL PEPPER

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ABSTRACT

Essential nutritional components are quantified in the study in addition to major antioxidants, carotenoids, phenols and flavonoids. Their antioxidant activities were also analyzed using DPPH, ABTS and hydrogen peroxide radical scavenging activities and by determining their iron reducing power. Results indicated that the highest quantity of nutritional and antioxidant components was obtained in red variety (4.63 mg/g ascorbic acid, 10.32 mg/g total carotenoid content, 61.50 mg/g total flavonoid content, 310.27 mg/g total phenolic content) followed by orange, yellow and green varieties. Similar trend was observed while analyzing their antioxidant activities by different methods. In studying correlation between components and antioxidant activity, a strong correlation was obtained for ABTS radical scavenging activity with total phenolic content ($R^2 = 0.722$) and total carotenoid contents ($R^2 = 0.709$), while moderate correlation was observed for ABTS radical scavenging activity with total flavonoid contents ($R^2 = 0.517$) and ascorbic acid ($R^2 = 0.673$).

Key words: nutraceutical, carotenoids, radical scavenging, polyphenols

INTRODUCTION

Today's lifestyle makes people prone to hazardous chemicals and unhealthy foods that may lead to the formation of undesirable free radicals in human body. These free radicals (reactive oxygen species/reactive nitrogen species) are produced in the body during normal metabolic processes or under stress. Free radicals generated in this way may damage essential cell components such as RNA and DNA, thus damaging the whole metabolic machinery resulting in cancer, cardiovascular diseases, brain damage, etc. Their control is necessary in order to prevent life from threatening diseases through the use of antioxidants that scavenge free radicals, thus inhibiting the

damage caused by them. Great awareness and concern of people for their health issues make them to switch to natural remedies that are safe alternatives for disease prevention. People are moving towards nutraceutical foods that are very beneficial in disease prevention by providing protons to free radicals thus scavenging them or preventing their formation.

Fruits and vegetables are most renowned nutraceutical foods that are preferred over artificial antioxidants, as these are abundant in polyphenols, minerals and vitamins. Among these, bell peppers are widely considered to explore for their positive potential, because these are being used in daily life, either

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in fresh or processed form. Bell peppers are available in wide range of colors showing their different varieties such as green, yellow, orange, red, purple, brown, white, etc. This wide range of colors is due to the presence of carotenoid pigments providing additional antioxidant benefits of bell peppers, thus increasing their importance among nutraceutical foods [Britton et al. 2009]. Some carotenoids in bell peppers also have property to be converted into vitamin A, so increasing their nutritional value. In addition to carotenoids, bell peppers are also abundant source of other phytochemicals including phenols, flavonoids, vitamins, etc. that have already been studied for prevention of cancer, iron chelation, etc. [Maoka et al. 2001, Oboh et al. 2007].

Presence of important nutritional components in large number in bell pepper and its property to be used as nutraceutical food product makes it highly demanding in industry. This high demand as nutraceutical food product and their high production shows a need to study their nutritional components. Therefore, this study was conducted to quantify the important nutritional components, carotenoid and ascorbic acid, in bell peppers and to determine and compare the antioxidant activity, total phenolics and flavonoids in four bell pepper varieties having different colors. In addition, their antioxidant activity was compared by different methods and its correlation with antioxidant compounds was determined.

MATERIAL AND METHODS

Sample collection and preparation. Four bell pepper varieties of green, yellow, orange and red color were collected from Hydroponic Research Institute PMAS-Arid Agriculture University Rawalpindi in 2017. These were cut and chopped into small pieces. Chopped bell pepper fruits were lyophilized, converted into powder through grinding and stored at $5 \pm 1^\circ\text{C}$ in air tight containers until analyzed.

Determination of total carotenoid content. Total carotenoid content was quantified according to the method outlined by Campos et al. [2013]. About 1 g sample was homogenized in 20 mL acetone and the solution was filtered. The process was repeated until attaining complete removal of colored pigments. Acetone was evaporated and dry sample extract dissolved in 60 mL petroleum ether, filtered, transferred

to 100 mL volumetric flask and volume completed with petroleum ether. Then, 2 mL of this solution was placed in a test tube with 8 mL petroleum ether. Absorbance was read at 475 nm using UV-Vis spectrophotometer (UV-9200, Biotech Engineering Management Co., UK) and concentration was calculated with a standard β -carotene curve.

Ascorbic acid determination. Ascorbic acid content was determined applying AOAC official method 967.21 [1990]. Powdered sample (5 g) was homogenized in 100 mL metaphosphoric acid-acetic acid solution in ratio of 1.0 : 0.5 and filtered. The resulting solution was titrated using phenol-2,6-dichloroindophenol dye (prepared using 50 mg 2,6-dichloroindophenol and 42 mg NaHCO_3 in approximately 50 mL water and dilute to 200 mL with water) until light pink color was obtained persisting for at least 5 seconds. Ascorbic acid solution was used to standardize the dye.

Preparation of extracts. Soluble antioxidant components in bell peppers were extracted by homogenizing the dry samples in ethanol for several times and the solutions were filtered. Solutions were combined and dried to obtain total extracts [Toh et al. 2016].

Determination of total phenolic content. Total phenolic content of sample extracts was determined by Folin-Ciocalteu method [Tundis et al. 2013]. Absorbance was measured at 765 nm using UV-Vis spectrophotometer. Gallic acid was used as a standard to determine total phenolic content of bell pepper samples.

Determination of total flavonoid content. Flavonoid contents were determined using a method based on flavonoid-aluminum complex formation [Loizzo et al. 2013]. Absorbance measured at 510 nm using UV-Vis spectrophotometer and quercetin was used as a standard to calculate the total flavonoid content.

DPPH free radical scavenging activity. Free radical scavenging ability of extracts was evaluated against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals as described by Adedayo et al. [2010]. Appropriately diluted extracts were mixed with 1 mL of 0.4 mmol/L methanol solution containing DPPH radicals. The mixture was left in dark for 30 min and absorbance was measured at 516 nm. DPPH free radical scavenging activity was subsequently calculated with respect to the reference (containing all reagents without the test sample).

ABTS radical scavenging activity. The 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) or ABTS radical scavenging activity was determined by the method described by Kim et al. [2010]. ABTS radicals cations (ABTS⁺) were produced by reacting 7 mM ABTS solution with 2.5 mM potassium persulfate in dark at room temperature for 12 hours until the oxidation of ABTS reached the maximum level and became stable. This solution was diluted with ethanol to absorbance of 0.70 ± 0.15 at 735 nm. Appropriately diluted sample extracts were added to 0.5 mL of this solution. Absorbance was measured at 735 nm using UV-Vis spectrophotometer after 6 minutes incubation in darkness.

Hydrogen peroxide radical inhibition assay. The method described by Shotorbani et al. [2013] was used to determine the hydrogen peroxide scavenging ability of extracts. Ten μL of extract was dissolved in 3.4 mL of 0.1 M phosphate buffer (pH 7.4) solution and mixed with 600 μL of 43 mM solution of hydrogen peroxide (prepared in the same buffer). Absorbance of these solutions was measured at 230 nm against the corresponding blank solutions. Hydrogen peroxide scavenging capacity of extracts was calculated using the following equation:

$$\begin{aligned} \text{Scavenging percentage} &= \\ &= (\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{blank}} \times 100 \end{aligned}$$

where, $\text{Abs}_{\text{sample}}$ = absorbance of reaction mixture and $\text{Abs}_{\text{blank}}$ = absorbance of blank mixture (distilled water instead of extract).

Determination of reducing property. Reducing property of extracts was determined by assessing the ability of extracts to reduce FeCl_3 solution as described by Oboh et al. [2007]. Diluted extracts (2.5 mL) were mixed with 2.5 mL sodium phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min and then 2.5 mL trichloroacetic acid (10%) was added. The mixture was centrifuged for 10 min and 5 mL supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm and higher absorbance indicates greater reducing power.

Statistical analysis. Experimental data were analyzed using Statistix 8.1 software. Correlations were found using SPSS software and values above $R^2 = 0.7$ were accepted as a strong correlation.

RESULTS AND DISCUSSION

Total carotenoid content. The carotenoid pigments attribute different colors to bell peppers, which is evident from the results of studied varieties showing high significance ($p < 0.05$). Carotenoid contents in these varieties ranged from 6.21–10.32 mg/g DM with the highest content obtained in red variety (10.32 ± 0.41 mg/g DM) followed by orange variety contributing 9.42 ± 0.613 mg/g DM, yellow variety contributing 8.35 ± 0.46 mg/g DM and the least content obtained by green variety (6.21 ± 0.413 mg/g DM) (Fig. 1). The figure shows that the variation in total carotenoid contents of orange variety is highest (8.4–10.2 mg/g DM) and all varieties showed even distribution of carotenoid contents in samples. Some important carotenoids contributing the color to these products may include capsorubin, capsanthin, β -carotene, β -cryptoxanthin, etc. that often gradually decrease as the color shade is reduced in the varieties. Yellow-orange bell pepper varieties tend to have α - and β -carotene as major carotenoids pigments along with fair amounts of zeaxanthin, lutein and β -cryptoxanthin coloring pigments [Ademoyegun et al. 2011]. However, the color produced in a bell pepper is due to a complex mixture of carotenoid as well as chlorophyll pigments [Ha et al. 2007]. This fact is evident in green bell peppers that are abundant in chlorophyll compounds contributing green color to the variety, thus masking the carotenoid contribution to color in these varieties. Some carotenoids have antioxidant activity that can be used to scavenge free radicals, thus making them suitable for nutraceutical formulations.

Ascorbic acid content. Ascorbic acid in different bell peppers varieties indicated significant variation ($p < 0.05$). Interestingly, it was observed that bell peppers are such high in ascorbic acid content that they may fulfill its daily recommended dietary allowance (60–75 mg) by the intake of as much as 18 grams per day. Higher ascorbic acid in red bell peppers makes it perfect for fortification purposes as it may be required in least amount due to the presence of higher amounts of ascorbic acid content (4.63 ± 0.23 mg) among tested varieties. This was followed by orange and yellow bell pepper, while green bell pepper is required in highest amount, because these are the least in ascorbic acid content (3.55 ± 0.23 mg) (Fig. 2). Ascorbic acid content in orange variety re-

vealed the highest variation with higher number of samples containing less ascorbic acid. Green variety samples were also found to have greater saturation at lower interquartile range in terms of ascorbic acid content, while the other two varieties showed even distribution of samples in their ascorbic acid content. Difference in ascorbic acid content observed in tested varieties are attributed to genetic variation and varieties with higher ascorbic acid content may have more potential to be used as nutraceutical products [Alós et al. 2013].

Total phenolic content. Great diversity of total phenolic contents exists in bell pepper with significant differences ($p \leq 0.05$) in four varieties with corresponding effects on health, because these are responsible for maintaining a proper health and preventing the occurrence of lethal diseases due to their nutraceutical property [Hervert-Hernández et al. 2011]. It was observed that health promoting total

phenolic contents are the highest in red variety followed by orange, yellow and green variety (Fig. 3). Red variety samples showed close values for total phenolic contents having narrow interquartile range (290–327 mg), which was followed by orange and yellow varieties that also showed even distribution of samples referring to total phenolic contents. The interquartile range for green variety was largest with more sample saturation at lower quartile range, showing that more samples had total phenolic content between 145–170 mg. The reason for differences in phenolic content among the four varieties may be the environmental and weather variations during their production [Aguilar-Meléndez et al. 2009]. However, a difference in phenolic content of these varieties from values found in literature observed by other scientists may occur due to cultivar differences and their variable extraction procedures [Zhang and Hamazu 2003].

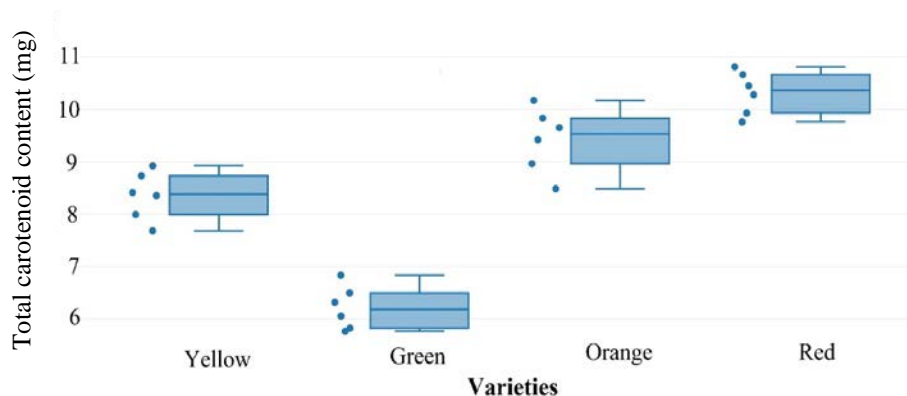


Fig. 1. Total carotenoid content of four bell peppers

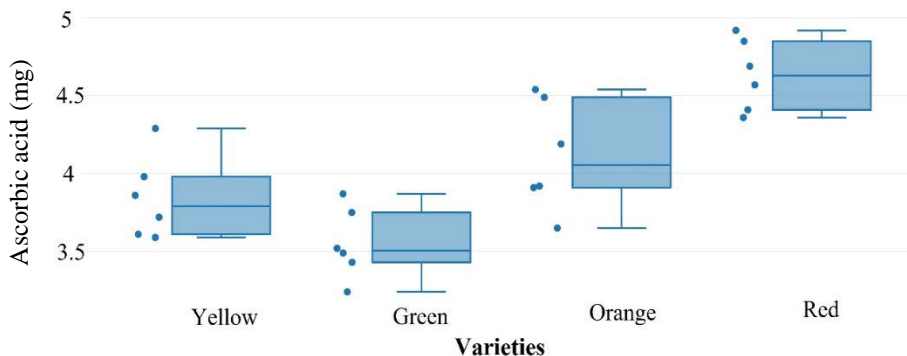


Fig. 2. Ascorbic acid content of four bell peppers

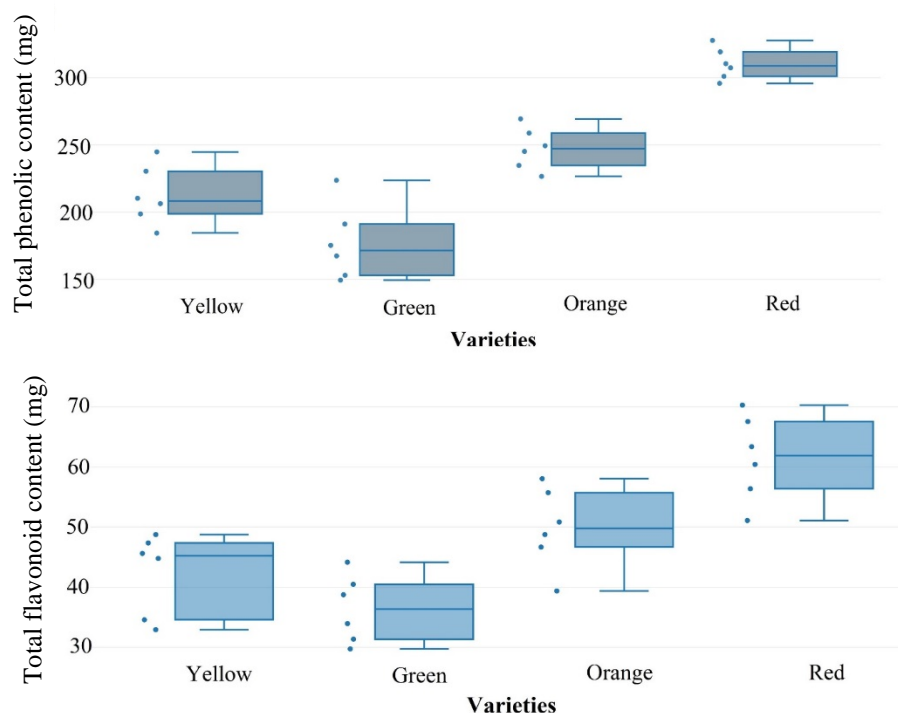


Fig. 3. Total phenolic content (A) and total flavonoid content (B) of four bell peppers

Total flavonoid content. Flavonoids are polyphenolic compounds that have been found to be associated with cancer risk reduction as a nutraceutical product due to their antioxidant activity [Lee et al. 1995]. This benefit can be achieved by high intake of bell peppers that are abundant source of flavonoids. Among studied varieties, red variety had the highest flavonoid content (61.51 ± 7.11 mg), therefore it would be highly efficient in cancer risk reduction. The other varieties are beneficial in cancer risk reduction in the following order corresponding to their flavonoid content: orange > yellow > green. Thus, red variety consumption should be preferred where the food is consumed solely for nutraceutical purpose, especially to prevent the cancer risk. The highest spread in total flavonoid content was found for yellow variety that also showed some overlapping with the results of green variety (Fig. 3). However, most of the values exist in upper interquartile range for yellow variety. Green and red varieties show almost uniform distribution of samples in terms of total flavonoid contents. While in orange variety, majority of samples lie in the quartile range below the median line showing total flavonoid content in the range of 47–51.5 mg. These high flavonoid-containing bell peppers can

be beneficially employed in food industry to produce nutraceutical products.

Radical scavenging antioxidant activity. Purplish colored DPPH radicals are scavenged by proton donation of phenolic contents, which results in discoloration of solution. The highest DPPH radical scavenging ($72.95 \pm 4.75\%$) was observed in red variety extracts, followed by orange ($62.03 \pm 4.30\%$) and yellow variety ($56.13 \pm 3.71\%$) (Fig. 4). This radical scavenging activity may occur due to the presence of huge number of variable antioxidant compounds in bell peppers. Owing to least antioxidant compounds such as phenols, flavonoids, carotenoids and a nutritional component – ascorbic acid in green bell pepper, these have the least DPPH radical scavenging activity [Bae et al. 2012].

ABTS and hydrogen peroxide radicals are similarly scavenged by proton donation of antioxidant compounds. Similar trend in ABTS and hydrogen peroxide radical scavenging activity was observed among the four studied varieties having the highest (88.78% ABTS and 64.50% hydrogen peroxide) antioxidant activity in red, while the lowest (66.53% ABTS and 48.38% hydrogen peroxide radical scavenging) activity in green variety.

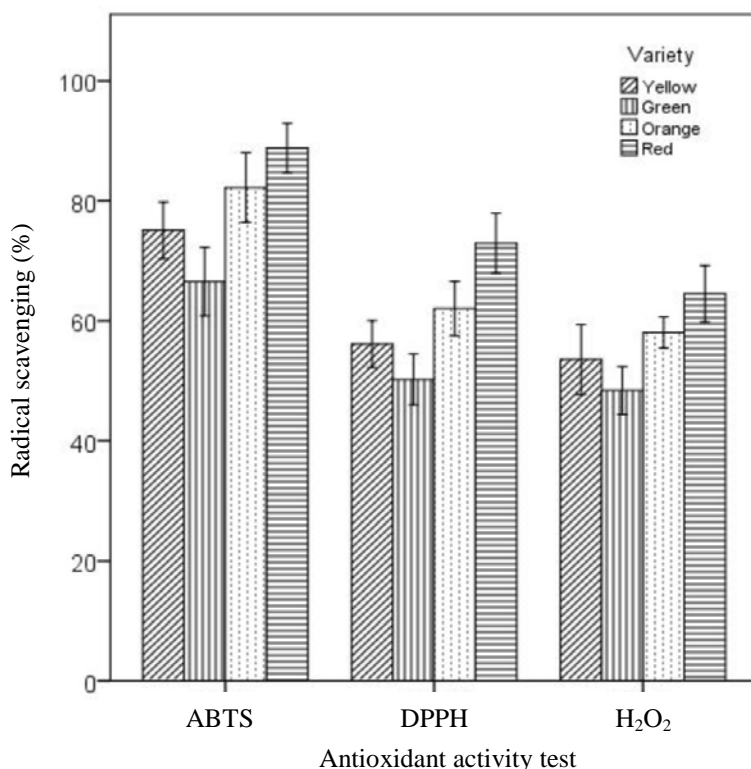


Fig. 4. Antioxidant activity determined by ABTS, DPPH and hydrogen peroxide radical scavenging activity of bell pepper extracts

It can be clearly concluded from the results of DPPH, ABTS and hydrogen peroxide radical scavenging activity of four bell pepper varieties that antioxidant activity is directly related to the content of antioxidant compounds present in the food product. In all the radical scavenging assays, the highest radical scavenging activity was observed in red variety, followed by orange, yellow and green varieties. This difference in antioxidant activity exists due to differences in ripening stages and agricultural practices during their production in addition to their cultivar differences [Lee et al. 2005].

Reducing power. Absorbance at 700 nm by the reaction mixture is an indicator of reducing power of the extract. The more the radicals are scavenged by antioxidant compounds of sample extracts, the higher is the absorbance of mixture. Highly significant results ($p < 0.05$) were obtained for reducing power of four studied varieties. The highest reduc-

ing power was obtained for red variety (0.96 ± 0.023), which may be due to the highest levels of reducing agents in red variety. Orange variety had reducing power of 0.83 ± 0.016 and stands second according to antioxidants presence. Yellow bell peppers have phenols, flavonoids and ascorbic acid contents less than orange but more than green bell peppers. Therefore, similar trend was observed in reducing power and yellow bell peppers showed 0.733 ± 0.024 reducing power. Least reducing power was observed in green bell peppers (0.62 ± 0.021) due to the lowest antioxidant compounds contents. This iron reducing power of bell peppers shows their effect in chelation and deactivation of transition metals, thereby preventing such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal catalyzed reactions in the body, which are responsible for occurrence of various diseases [Sun et al. 2007].

Correlation. Correlation of ABTS radical scavenging activity with total phenols, flavonoids, carotenoids and ascorbic acid showed that there is a strong correlation between ABTS radical scavenging with phenolic ($R^2 = 0.722$) and carotenoid ($R^2 = 0.709$), while ABTS radical scavenging activity correlates moderately with flavonoid ($R^2 = 0.517$) and ascorbic acid contents ($R^2 = 0.673$) in bell pep-

pers (Fig. 5). These results suggest that the ABTS radical scavenging activity of bell peppers is more interrelated to total phenolic and carotenoid contents, but there may also be some other components responsible for this activity. While moderate correlation with flavonoids and ascorbic acid contents suggests more involvement of other factors and constituents in antioxidant activity.

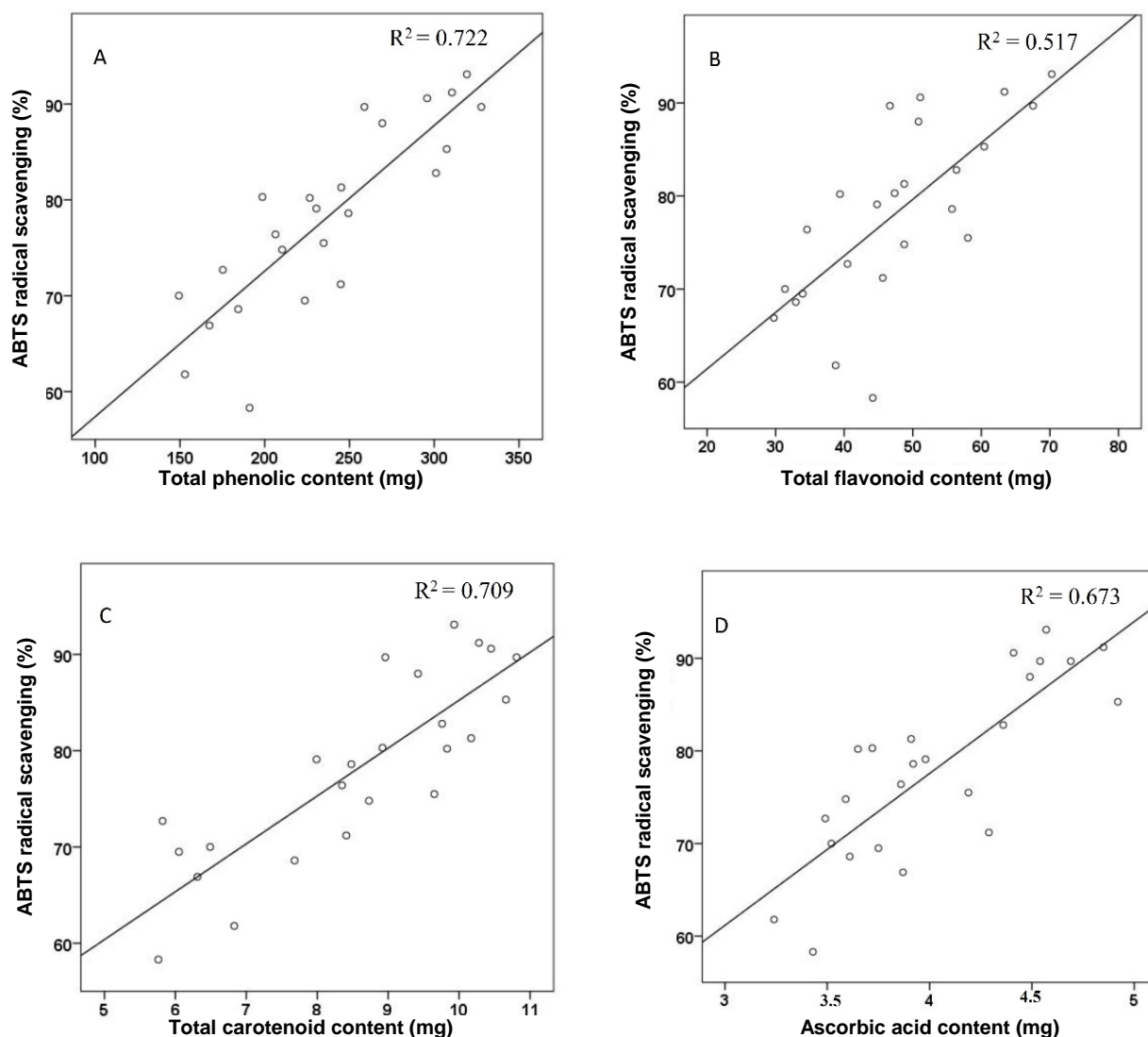


Fig. 5. Correlation between (%) ABTS radical scavenging activity with (A) total phenolic content, (B) total flavonoid content, (C) total carotenoid content, (D) ascorbic acid content

CONCLUSIONS

Four colored varieties (red, orange, yellow and green) were studied for their phenolic, flavonoid, carotenoid and ascorbic acid contents. From results of the study, it is concluded that the highest contents of antioxidant compounds (phenols, flavonoids, carotenoids, ascorbic acid) are present in red variety. Due to this higher concentration in red variety, its antioxidant activity observed through DPPH, ABTS, and hydrogen peroxide radical scavenging activity and through determining the reducing power is also the highest. Orange variety stands second and yellow variety stands third in this comparison. While green variety has the least antioxidant contents and the activity. These results suggest that red variety being highest in nutritional and antioxidants has the highest nutraceutical benefits and it can prevent from development of various diseases and it may also successfully replace the artificial antioxidants.

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