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EVALUATION OF ANTIOXIDANT-RELATED PROPERTIES OF SIX COLORED VARIETIES OF CARROTS (*Daucus carota* L.)

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ABSTRACT

Carrots (*Daucus carota* L.) are a prevalent vegetable in human diet nutrition and are an excellent source of vitamins, alpha- and beta-carotene, and antioxidant compounds. Carrots are categorized primarily according to the pigmentation of their tuberous roots, which can range from white, yellow, orange, purple, and black. This study aims to determine the antioxidant-related properties of six carrot varieties collected in Vietnam, including the total phenolic content (TPC), the total flavonoid content (TFC), DPPH scavenging activity, and the antioxidant enzyme activities. The results showed that the black carrot extract had the lowest IC_{50} value whereas the highest belonged to the orange carrot. Furthermore, the findings revealed that black carrots exhibited the highest TPC, which was 1.24 ± 0.01 (g GAE/100 g sample) whereas the red-orange carrots had the lowest TPC, which was 0.3 ± 0.05 (g GAE/100 g sample). Additionally, the black carrots also had the highest TFC, which was 161.32 ± 1.68, and the red-orange carrots had the lowest TFC value which was 27.6 ± 0.80 (g QE/100 g DW). The antioxidant enzyme activities resulted in the highest peroxidase activity of black carrots (149.48 ± 2.25 U/mg protein) and the highest catalase activity of purple carrots (93.19 ± 3.28 U/mg protein). Phytochemical analysis indicates that black and purple carrots exhibit higher antioxidant potential, suggesting their applicability in treatments and as agents for food preservation.

Keywords: Antioxidant activities, *Daucus carota*, DPPH, total flavonoid content, total phenolic content.

INTRODUCTION

Carrots, a member of the Apiaceae family, are the most popular vegetable after potatoes. Therefore, the bioactive compounds found in carrot taproot, which is the main storage organ, are widely investigated. These include cyanidin 3- (2-xylosylgalactoside), cyanidin 3-xylosylglucosylgalactoside, and cyanidin 3-ferulylxyloglucosyl galactoside (anthocyanins), chlorogenic acid, dicaffeoylquinic acids (phenolic acids), and flavonoids (Bystricka *et al.*, 2015). Moreover, carrots are also rich in various vitamins and minerals, including *β*-carotene, ascorbic acid, tocopherol, potassium, calcium, magnesium, and phosphorus (Tang, 2010). Carrots have been shown to exhibit potent antioxidant and radical-scavenging properties, attributed to their bioactive

compounds and robust enzymatic antioxidant activities (Zhang and Hamauzu, 2004). The non-enzymatic antioxidant system includes low-molecular-weight molecules such as phenolic acids, carotenoids, and ascorbic acid (Kasote *et al*., 2015), while the enzymatic antioxidant system encompasses enzymes like catalase, peroxidase, and superoxide dismutase. Many studies have demonstrated a reduction in the risk of developing serious diseases associated with carrot consumption (Akhtar *et al.*, 2017; Singh *et al.*, 2021).

Carrot pigment influences the concentration of bioactive substances in a substantial way. Orange carrot is a rich source of the antioxidants α-carotene and β-carotene, which are critical for the maintenance of healthy vision and the immune system (Sharma *et al*., 2011). The lycopene compound present in red carrots has been linked to a decreased risk of death from cancer and cardiovascular disease. Yellow carrot contains lutein and xanthophyll, which have been found to substantially decrease the likelihood of developing atherosclerosis, macular degeneration, and cancer (Molldrem *et al*., 2004). Carrots that possess anthocyanin, which gives them a deep purple and black, are acknowledged for their antibacterial, antifungal, and anti-inflammatory properties. In addition, white carrots, because of lacking pigment, have the least health effect (Zhang and Hamauzu, 2004).

Given the significant potential applications of carrots in human health and the wide range of their color variations, this study evaluated the antioxidant properties of methanol extracts from carrots of various colors, including orange, white, yellow, purple, red-orange, and black. The assessment involved the analysis of antioxidant enzyme activities (peroxidase and catalase), DPPH radical scavenging activity, total flavonoid content, and total phenolic content, to explore the pharmacological effects associated with these diverse carrot varieties.

MATERIALS AND METHODS

Materials

Plant materials

The carrot taproots (*Daucus carota* L.), including black, purple, red-orange, orange, yellow, and white varieties (Figure 1), which were cultivated in a farm in Dalat, Vietnam, were used as plant materials in this study.

Figure 1. Six different varieties of carrots (*Daucus carota* L.) were used in the study.

Methods

Methanol extraction and extraction yield

The fresh carrot taproots were thoroughly rinsed under tap water and cut into small slices. After that, they were dried in the oven for 5 days at 60°C to reach the constant weight. Once dried, the roots were ground into a fine powder. Next, the samples were incubated with absolute methanol (Sigma-Aldrich) and sonicated at 38°C for 30 minutes for a better extraction (Nguyen and Scarlett, 2016). After sonication, the falcon tubes were centrifuged for 6 minutes at 10000 rpm. The supernatant, containing the crude extract, was then evaporated in an oven set at 60°C to remove the methanol and concentrate the bioactive compounds.

The percentage of extraction yields was calculated as follows:

$$\text{Yields (\%)} = \frac{W1 \times 100\%}{W2}$$

Where:

W1: dry weight of the obtained dry extract after solvent evaporation (mg)

W2: dry weight of carrot sample (mg)

Finally, a suitable volume of methanol was added to the extracts to have a concentration of 200 mg/mL.

DPPH scavenging assay

DPPH radical scavenging activity was determined by using the spectrophotometer method to analyze the antioxidant capacity (Khan *et al.*, 2019). Briefly, in a 96-well plate, each well contained a mixture of 10 µL carrot extract and 190 µL DPPH 0.25 mM (Sigma-Aldrich) which was incubated at 37°C for 20 minutes in the dark. The absorbance was measured at 517 nm using a spectrophotometer (SpectraMax® iD5). The protocol to build the standard curve was the same as with carrot extract except the extract was replaced by ascorbic acid. The percentage of radical inhibition was calculated as follows:

$$\text{Inhibition (\%)} = 100\% - \left(\frac{ODs}{ODc} \times 100\%\right)$$

Where:

ODs: optical density of the carrot sample.

ODc: optical density of the control.

IC_{50} values were determined as the concentration of the extract at which 50% of the radical was inhibited.

Total phenolic content (TPC)

The TPC was measured using the Folin-Ciocalteu assay by Baba and Malik, (2015). The gallic acid was used to build the standard curve with six concentrations from 0.125 to 2 mg/mL. In each well, 40 µL of each sample was mixed with 480 µL Folin-Ciocalteu reagent (diluted 10 times with water) (Sigma-Aldrich) in 1 minute. Then, 480 µL Na_2CO_3 6% was added to the mixture. After that, the final solutions were incubated at 40°C in 15 minutes. The absorbance of the blue complex obtained after incubation was read at 765 nm. The TPC value was expressed as grams of gallic acid equivalents per 100 grams of the extract (g GAE/100 g DW).

Total flavonoid compound (TFC)

The TFC was determined as described by Chang *et al.*, (2002). Quercetin was used to build the standard curve with six concentrations from 0.025 to 0.8 mg/mL. In each well, 40 µL of extract sample and 10

µL $NaNO_2$ 5% (Sigma-Aldrich) were incubated for 6 minutes at room temperature. Then, 10 µL $AlCl_3$ 10% (Sigma-Aldrich) was added and incubated for 6 minutes more followed by adding 80 µL NaOH 1M (Sigma-Aldrich) and 60 µL ethanol 30% (Vietnam) and incubated for another 15 minutes at room temperature. The absorbance was measured at 510 nm. The TFC was calculated based on the standard curve of gallic acid and the results were displayed as g of gallic acid equivalent per 100 g dried weight (g GAE/100 g extract).

Antioxidant enzyme activities

The fresh carrots were frozen in liquid nitrogen and ground in a 100 mM phosphate buffer solution at a pH of 7.8. The sample then was centrifuged at 10.000 rpm for 20 minutes at 4^{o}C. The upper phase was collected for determining catalase (CAT) and peroxidase (POD) activities. The estimation of CAT and POD activities was conducted according to the method of Chen and Zhang (2016). Briefly, the CAT solution mixture consisted of enzyme extract, phosphate buffer (100 mM, pH 7.0) (Sigma-Aldrich), and H_2O_2 30% (Vietnam). Similarly, the POD solution mixture consisted of enzyme extract, phosphate buffer (100 mM, pH 7.0), H_2O_2 30%, and guaiacol 0.2% (Sigma-Aldrich). The CAT and POD reaction mixtures were measured at wavelengths of 240 nm and 470 nm, respectively, using an Analytik Jena ScanDrop 250 spectrophotometer after a 15-second interval.

Correlation and principal component analysis

The correlation between parameters was investigated using GraphPad Prism version 8.0. The principal component analysis (PCA) was analyzed by using RStudio version 4.2.1 by the FVIZ_PCA function and visualized by GGPLOT2 R package (Le *et al*., 2022).

Statistical test

The statistical differences were analyzed using one-way ANOVA and the Turkey post-hoc test with a significance level of $p < 0.05$ using GraphPad Prism. The data represented the average value obtained from three replications per treatment ± standard deviations.

RESULTS

Extraction yield of six carrots

Firstly, the extraction yields of six carrot varieties in methanol solvent combined with ultrasonic amplification were investigated (Table 1). The results showed that the purple carrot had the greatest extraction yield, which reached approximately 33.8%. Following closely behind with a substantial yield of 27.8% was the white carrot. The orange and yellow carrots had the lowest extraction yield of 7.82% and 8.6%, respectively.

Table 1. Extraction yield of six carrot samples in methanol solvent in combination with ultrasonic.

Samples	Sample weight	The weight of the extract obtained	Extraction yield
Orange carrot	5000 mg	391 ± 5.5 mg^{f}	7.82 ±0.001 %^{d}
Yellow carrot	5000 mg	430 ± 4.45 mg^{e}	8.6 ± 0.001 %^{d}
Red-orange carrot	5000 mg	620 ± 7.86 mg^{d}	12.4 ± 0.002 %^{c}
Black carrot	5000 mg	1320 ± 10.35 mg^{c}	26.4 ± 0.002 %^{b}
White carrot	5000 mg	1390 ± 11.26 mg^{b}	27.8 ± 0.002 %^{b}
Purple carrot	5000 mg	1690 ± 13.80 mg^{a}	33.8 ± 0.003 %^{a}

Different letters indicate the statistically significant differences between groups within the same criterion ($p < 0.05$).

Six carrot varieties showed a diversity of DPPH scavenging activities

Table 2 indicates the IC_{50} values of six carrot color varieties: black carrot was 3.53 ± 0.26, white carrot was 34.72 ± 1.52 mg/mL, purple carrot is 22.74 ± 0.13 mg/mL, yellow carrot was 43.53 ± 0.41 mg/mL, red-orange carrot was 70.8 ± 1.45 mg/mL, and orange carrot was 431.86 ± 420.58 mg/mL. The IC_{50} value of the black carrot was significantly lower than that of the orange carrot ($p < 0.001$), indicating that the black carrot exhibited the highest DPPH scavenging activity among the six carrot color varieties studied. Conversely, the orange carrot had the highest IC_{50} value, reflecting the lowest DPPH scavenging activity.

A lower IC_{50} value indicates greater anti-oxidative activity. Accordingly, the black carrot exhibited the highest antioxidant activity, followed in descending order by the purple carrot, white carrot, yellow carrot, red-orange carrot, and orange carrot.

Table 2. IC_{50} values of six carrot extracts using DPPH scavenging assay.

Color	Black carrot	Purple carrot	White carrot	Yellow carrot	Red-orange carrot	Orange carrot
IC_{50} of extracts (mg/mL)	3.53 ± 0.26^{f}	22.74 ± 0.13^{e}	34.72 ± 1.52^{d}	43.53 ± 0.41^{c}	70.8 ± 1.45^{b}	431.86 ± 420.58^{a}

Different letters indicate the significant difference between groups ($p < 0.05$).

TPC of six investigated carrot varieties showed a diverse

Figure 2 illustrates the varying concentrations of TPC in the six carrot cultivars, with values ranging from 0.3 to 1.2 g GAE/100 g extract. The black carrot continued to show the highest TPC at 1.24 ± 0.01 g GAE/100 g DW, indicating high potential benefits associated with antioxidants. In contrast, the TPC in the orange carrot was relatively low, measuring 0.3 ± 0.05 g GAE/100 g DW. This value was significantly lower compared to the TPC observed in other analyzed carrot varieties ($p < 0.05$). The middle TPC group includes red-orange, black, white, yellow, and purple carrots. The findings from this present study highlight the distinct phenolic profiles of various carrot varieties and are alert to the potential variations in the health benefits they contain.

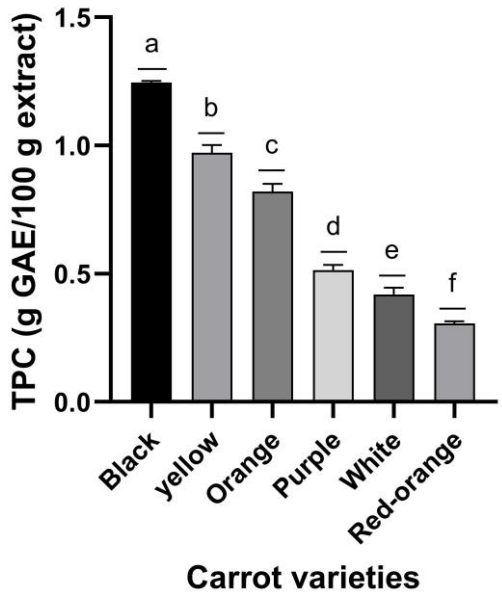

Figure 2. TPC values of six carrot extracts. Different letters indicate the significant difference between groups ($p < 0.05$).

TFC of six investigated carrot varieties showed a diverse

Figure 3 indicates the TFC values of six carrot color varieties where black carrot had 161.32 ± 1.68 g QE/100 g extract, yellow carrot had 111.45 ± 2.48 g QE/100 g extract, orange carrot had 107.128 ± 1.1 g QE/100 g extract, purple carrot had 79.6 ± 1.15 g QE/100 g extract, white carrot had 50.44 ± 0.6 g QE/100 g extract, and red-orange carrot had 27.6 ± 0.8 g QE/100 g extract. The results consistently indicated that black and yellow carrots exhibited the highest TFC values, suggesting superior antioxidant activity compared to the other carrot varieties analyzed in this study.

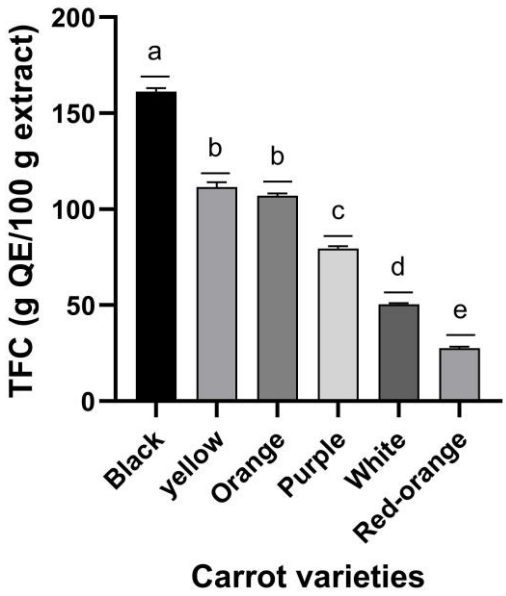

Figure 3. TFC values of six carrot extracts. Different letters indicate the significant difference between groups ($p < 0.05$).

Antioxidant enzyme activities

The antioxidant enzyme activities, POD, and CAT, exhibited a diversity in six studied carrot varieties (Table 3). More importantly, the black and purple carrots steadily showed notable greatest of POD and CAT at approximately 149.474 and 93.19 U/mg protein, respectively. This indicates that the antioxidant enzyme activities are higher in black and purple carrots, which will decompose hydrogen peroxide more efficiently than other carrot varieties in stress conditions. In contrast, the white and yellow carrot varieties demonstrated the lowest POD and CAT enzymatic activities, with measured values of approximately 3.18 U/mg protein and 0.67 U/mg protein, respectively. The results suggest that purple and black carrots, in particular, may offer significant antioxidant benefits because of their robust POD and CAT activities.

Table 3. Catalase and peroxidase activities of six colored carrot varieties.

	Black carrot	**Purple carrot**	**Orange carrot**	**Yellow carrot**	**Red orange carrot**	**White carrot**
POD activity	149.48 ± 2.25^{a}	18.49 ± 0.45^{b}	14.02 ± 0.34^{c}	8.41 ± 0.26^{d}	7.95 ± 0.46^{d}	3.18 ± 0.12^{e}
CAT activity	4.6 ± 0.18^{c}	93.19 ± 3.28^{a}	6.54 ± 0.24^{b}	0.67 ± 0.023^{e}	1.06 ± 0.01^{d}	4.61 ± 0.35^{c}

Different letters indicate the significant difference between groups in each parameter ($p < 0.05$).

Correlation between five investigated parameters

The results from Figure 4 showed that we obtained an extremely high correlation between TFC and TFC values where R reached 0.98. In fact, in black carrots, TPC and TFC were the highest among the six investigated varieties. Relatively high correlations were also obtained between POD and TFC, POD and TPC, and POD and DPPH, where all these three R-values were higher than 0.6. A negative correlation was observed between CAT and TPC, TFC, and POD.

	TPC	*TFC*	*POD*	*CAT*	*DPPH*
TPC	1				
TFC	0.978746	1			
POD	0.714777	0.748025	1		
CAT	-0.25643	-0.08057	-0.1134	1	
DPPH	0.201247	0.27876	0.653349	0.367117	1

Figure 4. Correlation analysis between five investigated parameters.

Principal component analysis to visualize the discrimination in six studied colored carrots

Five investigated parameters were used to visualize the discrimination in six carrot varieties. The first principal component could explain up to 57.34% of variability while it was 28.09% in the second principal component; which made up 85% in total. Thus, those two principal components could simply analyze the complicated data with lesser dimension. Figure 5 shows the clear separation of six carrot varieties in the PCA plot. Black and purple carrots were predominantly positioned on the lower left or right sides, indicating that these two varieties exhibited greater distinction compared to the other four-color variations. In contrast, the proximity of orange and yellow varieties, as well as red-orange and white varieties, within the plot indicates a relatively close relationship based on the five tested parameters.

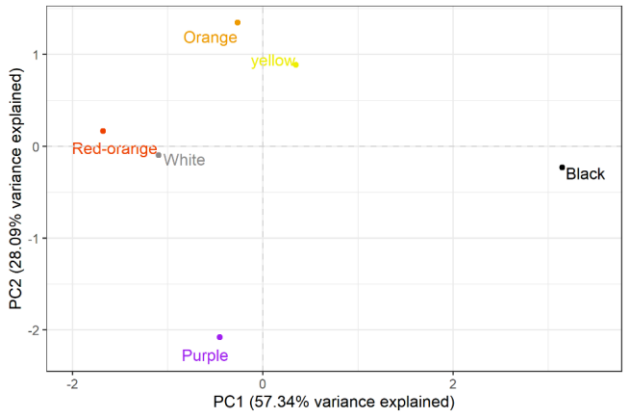

Figure 5. PCA score plot of five investigated parameters in six studied colored carrot varieties.

DISCUSSION

This is the first study in Vietnam to provide systemic information about the antioxidant properties of six different cultivated Vietnamese-colored carrot varieties, including orange, red-orange, purple, black, yellow, and white carrots. Numerous studies have demonstrated the antioxidant properties of carrots of various colors (Sun *et al*., 2009; Haq and Prasad, 2017; Scarano *et al*., 2018). Our study provides further support for those findings. However, amounts of phytochemicals that determine the antioxidant activity found in carrots may vary depending on harvesting, transportation, and processing (Czepa and Hofmann, 2003). The findings of our study revealed that black carrot extract demonstrated the highest antioxidant capacity, as evidenced by its superior DPPH scavenging activity. Additionally, it exhibited the highest concentrations of phenolic and flavonoid compounds. Furthermore, black carrots displayed the greatest POD activity among the six colored carrot varieties analyzed. Our results also indicated a strong correlation between TPC and TFC content with an R-value equal to 0.98. Higher antioxidant capacity was thought to relate to the higher TPC and TFC (Grassmann *et al.*, 2009). Analogous results were reported by Sun *et al.* (2009), as they observed a direct correlation between antioxidant activity and TPC. We also obtained a relatively high correlation between antioxidant enzyme activity POD and TPC and TFC with an R-value higher than 0.6, which was similar to the results of Singh *et al.* (2018) performed in tropical carrots.

Further consideration should be given to purple carrot because of its elevated phytochemical activities. The purple carrot variety had the highest CAT activity and was the second highest in DPPH scavenging activity. Similar outcomes were documented in a prior investigation that assessed the antioxidant capacity, anthocyanin, phenolic, and carotenoids in seven distinct carrot varieties (including purple carrots), analyzed by Sun *et al.* (2009). The study revealed a strong correlation between antioxidant capacity and anthocyanin and phenolic content, with correlation coefficient values ranging from 0.77 to 0.99. Leja *et al.*, (2013) strongly indicated that among the different investigated colored carrots, purple carrots exhibited the highest antioxidant capacity due to their higher phenolic compound concentration. The TPC and antioxidant activity of the purple carrot were approximately 8 times higher than those of the orange or yellow carrots (Cefola *et al.*, 2012). Moreover, the TPC was estimated to be 2.7 ± 1.7 g/100 g gallic acid equivalents, as determined with the Folin–Ciocalteu reagent, which is relatively similar to our results, where we obtained the highest TPC of 1.24 ± 0.01 g GAE/100 g sample (Oviasogie *et al.,* 2009).

More importantly, metabolites from the black carrot variety are effective against many serious diseases, including breast cancer cell lines (Longnecker *et al*., 1997), diabetes (Akhtar *et al*., 2017), and cardiovascular diseases (Wright *et al*., 2013). Additionally, red carrot pigment was applied to sunflower oil as a natural antioxidant to prevent sunflower oil from going rancid. Therefore, these results suggest a high potential for carrots with high antioxidant activities can be used in therapeutic applications or food preservation. Furthermore, this information is also useful for horticulturists to develop carrot varieties with higher antioxidant capacity.

Lastly, by PCA analysis, using the results of five investigated parameters, we experienced a clear separation of black and purple carrot varieties from four other color varieties, which is in agreement with Singh *et al.* (2018). These two-color varieties possess an exceptionally high content of TPC and TFC and thereby lead to the highest antioxidant ability in terms of POD and CAT. The selection based on TPC, TFC, or antioxidant enzyme activities could be pivotal in future breeding to harness the genetic wealth of carrots efficiently.

CONCLUSION

This study investigates the antioxidant properties of six colored carrot varieties cultivated in Vietnam. The results indicate that black and purple carrots demonstrate the highest levels of DPPH radical scavenging activity, TPC, TFC, and antioxidant enzyme activities. These attributes identify them as the most promising candidates for further exploration of their potential therapeutic applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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