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DETERMINATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY IN VEGETABLES CONSUMED IN MEKELLE TIGRAY ETHIOPIA

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ABSTRACT

Vegetables are widely consumed in Mekelle and imported from different regions and zones of the countries. This study was performed to evaluate the phenolic content and antioxidant activity of vegetables commonly consumed in Mekelle. Kale, carrot, cabbage and barcoli were used in the study. This research studies in detail the contents of phenolic compounds determined by the Folin–Ciocalteu reagent and the antioxidant activities determined by DPPH (using diphenyl-*p*-picrylhydrazyl radical). Among all vegetables kale showed highest phenolic content followed by barcoli, cabbage and carrot and the highest antioxidant activity were barcoli followed by kale, cabbage and carrot which was expressed in % of DPPH consumption after 30 minutes of reaction. The data shows the importance of antioxidant activity of vegetables in the diet.

Keywords: Organic Food, Bioactive Compounds, Vegetables, Antioxidant, DPPH.

INTRODUCTION

Fruits and vegetables play a number of important roles in human health. They provide antioxidants such as vitamin A, C and E that are important in neutralizing free radicals (oxidants) known to cause cancer, cataracts, heart disease, hypertension, stroke and diabetes (Sreeramulu and Raghunath, 2010; Szeto et al., 2004).

Diets high in fruits and vegetables are widely recommended for their health-promoting properties. Fruits and vegetables have historically held a place in dietary guidance because of their concentrations of vitamins, especially vitamins C and A; minerals, especially electrolytes; and more recently phytochemicals, especially antioxidants. Additionally, fruits and vegetables are recommended as a source of dietary fiber. Vegetables and fruits are an important part of a healthy diet, and variety is as important as quantity and no single fruit or vegetable provides all of the nutrients you need to be healthy. A diet rich in vegetables and fruits can lower blood pressure, reduce risk of heart disease and stroke, prevent some types of cancer, lower risk of eye and digestive problems, and have a positive effect upon blood sugar which can help keep appetite in check (Halliwell and Gutteridge, 1989; Nakatani, 1996).

According to the previous study, in Ethiopia the production of horticultural crops is much less developed than the production of food grains. These endeavors obviously require sound and up-to-date information on the extent of market availabilities; own Production/cultivation and consumption of fruits and vegetables. Fruits and vegetables are home for many antioxidants. These health benefiting phyto-chemical compounds firstly; help protect the human body from oxidant stress, diseases, and cancers, and secondly; help the body develop the capacity to fight against these by boosting immunity (Papas, 1999).

Their contribution as source food antioxidants can further be substantiated if more studies are done on their potential. Thus, it is the purpose of this study to determine the total antioxidant activity and phenolic content of fresh vegetables of organic carrot (*Daucuscaro-ta L*), broccoli (*Brassica oleraceavar. Italica*), kale (*Brassicaoleraceavar. Acephala*) and cabbage (*Brassica oleraceavar.Capitata*) available in the market of mekelle city in the state of tigray Ethiopia.

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MATERIALS AND METHODS

Sample extraction from the vegetables was performed according to the method described by Kähkönen *et al*., 1999) with some modifications. Samples were first frozen at 18°C and then lyophilized. After that, the material was ground and 1 g of each vegetable powder was placed in Falcon tubes, mixed with 20 mL of 80% ethanol (v/v), and shaken thoroughly. Subsequently, the tubes were solicited for 5 min, centrifuged for 15 min at 5000× g, and the supernatant collected for analysis. All samples were extracted in triplicate.

Antioxidant Activity through the DPPH Radical Scavenging Process: The DPPH and free radical scavenging activities of solutions of the vegetable extracts and synthetic antioxidant substances used in the study prepared in methanol at concentrations of were determined in accordance with the Shimada (1992) method, which is based on the principle of scavenging the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. DPPH was added to the solutions prepared with plant extracts and standard antioxidant substances and stirred. Each mixture was kept in the dark for 15, 30 and 60 min and the absorbance was measured at 517 nm against a blank (Shimada *et al*., 1992).

By the action of an antioxidant or a radical species, the DPPH is reduced creating 2,2-diphenyl-1-picrylhydrazyl, of a yellow color, and consequent absorption disappearance, so it can be monitored by the decrease of absorbance. Based on the results obtained it is possible to obtain the free radical antioxidant or scavenging activity percentage and/or the DPPH remaining percentage in the reaction environment (Assimopoulou *et al*., 2005; Nilsson *et al*., 2005; Zhou and Yu, 2006; Roy *et al*., 2007; Llorach *et al*., 2008; Chu *et al*., 2002).

The percent DPPH scavenging effect was calculated by using following equation:

*%DPPH consumed = 100- (Abs sample- Abs white)*100/Abs control*

Total Phenolic Compound Analysis: The amount of total phenolics in plant foods extracts were determined with the Folin-Ciocalteu reagent using the method of Spanos and Wrolstad (1990) as modified by Lister and Wilson (2001). About 50 mL of each sample (3 replicates), 2.5 mL 1/10 dilution of Folin-Ciocalteau's reagent and 2 mL of Na_2CO_3 (7.5%, w/v) were added and incubated at 45°C for 15 min. The absorbance of all samples was measured at 765 nm using a SPECTRAmax-PLUS384 UV-vis spectrophotometer. Results were expressed as milligrammes of gallic acid equivalent per gramme of dry weight (mg GAE g^{-1} dw).

Statistical analysis: All data were reported as mean ± standard deviation of three replicates. The data were analyzed using one-way variance analysis (One Way ANOVA) and Tukey's Post-test. A statistical analysis was performed using the SPSS 16.0 software package.

RESULTS AND DISCUSSION

The antioxidant activity of the vegetables consumed in mekelle were analyzed and presented in table 1, showed that there was no significant difference ($p < 0.05$) in the consumption percentage of DPPH after 15, 30 and 60 minutes of reaction for all the extracts of the vegetables analyzed.

Among all vegetables the highest antioxidant activity were barcoli followed by kale, cabbage and carrot which expressed in % of DPPH consumption after 30 minutes of reaction but no significance difference observed between broccoli and kale at different reaction time ($p<0.05$).

According to differentiated antioxidant capacity among these vegetables, researchers showed that the methanol extracts of kale, tomato, potatoes, cauliflower, green cabbage, spinach and leaf lettuce, showed inhibition percentages higher than 70% and which is more effective in scavenging of free radicals. The methanol extracts of butter lettuce, white onion and string bean showed moderate action (60% - 70% inhibition), while the red onion, chayote, cucumber, cabbage and carrot showed the weakest capacity in scavenging the DPPH radical.

Table 1. Total phenolic compounds and antioxidant activity (DPPH % consumed) in organic vegetables extracts.

Vegetables	Phenolic content in mg GAE/ml	DPPH% Consumed		
		15 min	30 min	60 min
Broccoli	0.611± 0.011	64.69 ± 4.49^{a}	68.35 ± 5.29^{a}	71.11 ± 8.26^{a}
Cabbage	0.314± 0.002	32.68 ± 1.51^{c}	36.72 ± 3.39^{c}	39.20 ± 5.64^{c}
Kale	0.706± 0.01	62.31 ± 3.25^{ab}	65.21 ± 3.85^{ab}	68.53 ± 4.49^{ab}
Carrot	o.214± 0.031	20.46 ± 0.62^{d}	23.39 ± 1.13^{d}	26.92 ± 1.29^{d}

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According (Faller and Fialho, 2009) the antioxidant activity reacting with DPPH after 60 minutes, was 61.0 for broccoli stalk, 73.8 for broccoli flowers, 79.2 for leafs of organic broccoli, 61.9 for organic carrot peel and 19.0 for organic carrot pulp. These amounts were very similar to the ones found in this study for the organic carrot (20.92% ± 1.29%) and organic broccoli (72.11% ± 8.26%), after 60 minutes reacting with the DPPH.

Among other factors the antioxidant action of the bioactive compounds depends on its chemical structure and concentration (Melo *et al*., 2006). The results indicate a possible presence of high antioxidant compounds in the vegetables. The bioactive compounds with a recognized antioxidant activity present in the carrot are, according to (Singh *et al*., 2012), vitamin C, β-carotene, α-carotene, lycopene and lutein, which may vary, among others, according to the cultivation and the nutrient supplementation during the cultivation (Singh *et al*., 2012). Nevertheless, the result of the carrot's antioxidant activity was lower than the expected.

Among all vegetables kale showed highest phenolic content followed by barcoli, cabbage and carrot.

Several studies have reported on the relationships between phenolic content and antioxidant activity. Some authors found a correlation between the phenolic content and the antioxidant activity, while others found no such relationship. Velioglu *et al.* (1998) reported strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products but in this study, the findings do not show any relation-ship between antioxidant activity and total phenolic contents.

CONCLUSION

The present study allowed to estimating the levels of phenolic compounds and antioxidant activities among vegetables widely available in the market of mekelle. The major vegetables which displayed high antioxidant activities were broccoli kale, cabbage and carrot. Therefore, these vegetables may be considered as good sources of bioactive compounds. Their consumption as sources of nutraceutics could be recommended particularly in groups of people where the incidence of oxidative stress-induced diseases is quite important. For an optimal use of these significant sources of natural antioxidants, more research may be envisaged to characterize the antioxidant compounds and to determine the effects of post-harvest factors and culinary process on their final antioxidant activities.

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