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POLYPHENOL CONTENTS OF TEN MOST WIDELY CULTIVATED AND CONSUMED VEGETABLES IN ETHIOPIA

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ABSTRACT. Vegetables are the fresh and edible portions of herbaceous plants. Vegetables have been used as a source of food due to their abundant bioactive chemical contents. This study was aimed to determine phenolics contents of ten most widely consumed vegetables (beetroot, cabbage, cauliflower, carrot, chili, eggplant, lettuce, potato, Swiss chard, and tomato) by UV-Visible spectrophotometry. The highest level of bound phenolics was found in cabbage (401 mg GAE/100 g) whereas the lowest level was found in tomato (92 mg GAE/100 g). Higher total phenolic contents were found in the extracts of cabbage (3171 mg GAE/100 g), chili (2006 mg GAE/100 g), Swiss chard (1313 mg GAE/100 g), lettuce (1160 mg GAE/100 g), eggplant (1143.2 mg GAE/100 g) and cauliflower (1086 mg of GAE/100 g). While potato had the lowest total polyphenol contents (217.5 mg GAE/100 g) among the studied vegetables. Presence of substantial amounts of total phenolics in vegetables is responsible for their effective antioxidant potency. Therefore, there is a high potential for the use of vegetables as a health promoting and disease preventing source.

KEY WORDS: Vegetables, Polyphenols, Gallic acid, Ethiopia

INTRODUCTION

Vegetables are the fresh and edible portions of herbaceous plants. They are eaten with the main course of a meal and commonly salted and boiled or used for desert and salads. They are important food and highly beneficial for the maintenance of health and prevention of diseases. Vegetables have been used as a source of food and medicine due to their abundant chemical properties since time immemorial [1]. Crude extracts of vegetables and other plant materials rich in phenolics and flavonoids are of boosting interest in food industry and medical field. Vegetables not only provides calorie but also ensure an adequate intake of most vitamins and nutrients, dietary fibres, and phytochemicals. These components of vegetables can bring a much needed measure of balance back to diets. They also contribute to solve many of nutrition problems in both a quantity and a quality issues and certain hormone precursors in addition to protein and energy source [2].

Cultivated and wildly grown leafy vegetables are rich sources of vitamins, proteins, minerals and a variety of bioactive compounds such as phenolic compounds, which provide health benefits beyond basic nutrition [3-6]. Consumption of green leafy vegetables plays important role in the prevention of human diseases, such as cancer, blood pressure, cardiovascular diseases and aging, in which free radicals are involved [4].

Polyphenolic compounds are commonly found in both edible and non-edible plants, and they have been reported to have multiple biological effects, including antioxidant activity [7, 8]. Polyphenols are the most common phytochemicals in human diet and comprise a variety of compounds with a great diversity of structures, ranging from simple molecules to polymers with high molecular weight [9]. Polyphenols are plant secondary metabolites present in all plant tissues, and their primary role is to protect plants from insects, ultraviolet radiation, and microbial infections and to attract pollinators [10]. Fruits, vegetables, whole grains, chocolate, and drinks

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like tea and wine are good sources of polyphenols, but due to diverse chemical structures, it is difficult to estimate the total polyphenol content in foods [10-13].

High consumption of fruits and vegetables has been considered to reduce the risk of a number of major diseases [14, 15]. These effects are mainly associated with biologically active components that are naturally present in the fruits and vegetables, the most important of which being the phenolic compounds, carotenoids, vitamins, minerals, etc. [2, 16, 17]. The term "antioxidant" is defined as: "a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiologic functions in humans" [18]. Phenolics, as natural antioxidants, have come to the attention of nutritionists since the mid-1990s [19].

Polyphenolic antioxidants from dietary sources are current topics of interest due to widespread scientific agreement that they may help to lower the incidence of certain cancers, cardiovascular and neurodegenerative diseases, and DNA damage and even may have anti-aging properties [20, 21]. A large number of plant-based foods around the world contain health-promoting phenolic compounds, which contribute to the prevention of various chronic diseases [8, 22-28]. The probability of having coronary heart disease and stroke is inversely proportional to dietary intake of polyphenols such as flavanols, especially quercetin [29].

Phenolic compounds possess ideal structure for free radical scavenging activities because they have: (1) phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical; (2) extended conjugated aromatic system to delocalize an unpaired electron.

It is well known that phenolic compounds exist in both free and bound forms in plant cells, and that the free phenolic compounds are solvent extractable [30]. In contrast, the bound phenolic compounds, which are covalently bound to the plant matrix, cannot be extracted into water or aqueous/organic solvents mixtures [31].

Simple phenolic acids and flavonoids are the most common phenolic compounds and they generally occur as soluble conjugated (glycosides) and insoluble forms [32]. In nature, phenolic acids occur mostly in the insoluble or bound forms whereas flavonoids present as glycosides with a single or multiple sugar moieties linked through an OH group (O-glycosides) or through carbon–carbon bonds (C-glycosides) [33].

Phenolics in the insoluble forms are covalently bound to cell wall structural components such as cellulose, hemicellulose (e.g. arabinoxylans), lignin, pectin and rod-shaped structural proteins [34]. These phytochemicals play important functions in the cell wall as providing both physical and chemical barriers, protection against pathogen invasion and astringency that deters attack by insects and animals, antibacterial, antifungal and antioxidant functions [35]. Phenolic acids, such as hydroxycinnamic and hydroxybenzoic acids, form ether linkages with lignin through their hydroxyl groups in the aromatic ring and ester linkages with structural carbohydrates and proteins through their carboxylic group [36, 37].

Ethiopia, with its diverse agro-ecological zones, cultivates a variety of vegetables that are integral to the diet of its population. However, there is limited data on the polyphenol content of locally grown vegetables. Understanding the level of polyphenols in widely consumed Ethiopian vegetables can provide insights into their nutritional value and potential health benefits, promoting better dietary choices among the population. Therefore it is worthwhile to determine the total polyphenol contents of the locally grown and consumed vegetables. Hence, the objective of this study was to determine the total polyphenol content of a methanolic extracts of ten most widely cultivated and consumed vegetables in Ethiopia by UV-Visible spectrophotometry.

The vegetables selected for the present study are beetroot, cabbage, carrot, cauliflower, chili, eggplant, lettuce, potato, and Swiss chard, and tomato. These vegetables were selected for the present study because they are most widely cultivated and consumed in Ethiopia. Beetroot, cabbage, potato and Swiss chard are most commonly consumed as cooked vegetable by lower and middle income people. They are common components of fasting food called beiyanet. Carrot is commonly consumed by middle and higher income people as a component of salad and also as a

cooked vegetable. Cauliflower is also commonly consumed by middle and higher income people as a component of pizza and also as a cooked vegetable. It is one of the most common vegetable served in the hotels. Chili is the most commonly used spice and mixed in all types of cooked vegetable. Chili is also a common component of salad. One piece of chili is always served in the fasting food (beiyanet). Chili sauce is commonly served with pizza. Eggplant is commonly consumed by middle and higher income people as a cooked vegetable. It is also consumed after roasting and mixing with chili, tomato, onion and other spices. Lettuce is a common components of salad and consumed by middle and higher income people. It is commonly served in the hotels. Tomato is another most commonly used vegetable in several forms. It is a common component of salad. It is also cooked and mixed with other vegetables. It is also used in the vegetarian pizza and vegetarian sand witch. Tomato is also commonly used as sauce. Evaluating the polyphenol contents of these vegetables will provide baseline information for the future study on these vegetables.

EXPERIMENTAL

Apparatus and instrument

Grinder (mortar and pestle), electronic balance (Model: ARA520, China), water deionizer system (Model: Molatom510d, Mole water System Co., Ltd), ultrasonic cleaner (Model: K240HTD, China), centrifuge (Model: 80-2, China), and pH meter (Eutech Instruments, pH 700) were used for sample preparation. UV–VIS–NIR spectrometer (Model: Lambda 950, Perkin Elmer, UK) were used for the recording the spectra and quantitative determination of total polyphenols of the vegetable samples.

Chemicals

Folin-Ciocalteu's reagent (BDH Chemicals Ltd, Poole, England), gallic acid (Carlo Erba reagents, France), sodium hydroxide (ScharlauChemie S.A., European Union), and sodium carbonate (Research Lab Fine Chem Industries, Mumbai, India) were used. Deionized water was used throughout the experiment.

Sample collection area

The fresh vegetable samples were collected from the shops in Addis Ababa. In this study, ten vegetables namely beetroot, cabbage, carrot, cauliflower, chili, eggplant, lettuce, potato, Swiss chard, and tomato were used. Table 1 summarizes the scientific names and parts of vegetable samples analyzed in this study.

Table 1. The scientific names and parts of vegetable samples analyzed in this study.

No.	English name	Scientific name	Amharic name	Analyzed parts
1	Beetroot	*Beta vulgaris* L. var. rapacea Koch	Kaiser	Root
2	Cabbage	*Brassica oleracea* var. capitata	Tikil goman	Leaves
3	Carrot	*Daucus carota ssp. sativus*	Carrot	Root
4	Cauliflower	*Brassica oleracea* L.	Ababa goman	Flower
5	Chili	*Capsicum annuum* L.	Karya	Fruit
6	Eggplant	*Solanum melongena* L.	Dabarjan	Fruit
7	Lettuce	*Lactuca sativa*	Salata	Leaves
8	Potato	*Solanum tuberosum*	Dinnich	Root
9	Swiss chard	*Beta vulgaris* L.var. cicla Pers	Kosta	Leaves
10	Tomato	*Solanum iycopersicum* L.	Timatim	Fruit

Sample preparation

The vegetable samples collected from the local market were washed with tap water to remove all dust and soil. The non-edible parts were manually removed with the help of a sharp knife. One kilogram of each vegetables namely, beetroot, cabbage, carrot, cauliflower, chili, eggplant, lettuce, potato, and Swiss chard, and tomato were chopped in to almost equal small pieces, and then dried in the shade at room temperature. Once dried, the samples were ground with mortar and pestle, sieved and stored in clean plastic containers until analysis.

Preparation of reagents

Preparation of 7.5% Na_2CO_3 solution

A 7.5 g of Na_2CO_3 was taken in a 100 mL volumetric flask and small amounts of deionized water was added in it and shaken to dissolve Na_2CO_3 and the volume was made up to the mark by adding deionized water.

Preparation of standard gallic acid

A 0.5 g of gallic acid was dissolved in to 500 mL of distilled water to prepare stock solution of 1 mg/mL. Then serial dilution was performed in order to prepare different concentration of solution for the preparation of calibration curve.

Sample extraction

Ultrasound-assisted extraction of free phenolic fractions. 0.5 g of each vegetable samples were mixed with 15 mL of aqueous methanol (90% v/v) and subjected to sonication in an ultrasound bath at 40 ℃ for a period of 35 min followed by centrifugation at 3,500 rpm for 20 min. Samples were filtered using Whatman No. 93 filter paper and stored in vials in dark area to prevent the oxidation of polyphenols. Once again the residue was mixed with 15 mL of aqueous methanol (90% v/v) and subjected to sonication followed by centrifugation at 3,500 rpm for 20 min and filtration. The clear supernatant was collected and stored in vials in dark area for analysis [38].

Ultrasound assisted extraction of bound phenolic fractions. After the free phenolics extraction, obtained residues were rewashed twice with 15 mL of deionized water to extract bound phenolic compounds. Water was then removed and samples were blended with 15 mL of 0.5 M NaOH for 35 min to liberate ester or ether linked phenolics in an ultrasonic bath. The mixture was centrifuged at 3500 rpm for 20 min. This procedure was repeated once more. The supernatants from the two extracts were combined and adjusted to pH 4.0–5.0 by using 0.6 M HCl and was used as a bound phenolics fraction [38]. The clear supernatant was collected and stored in vials for analysis.

Determination of total polyphenolics content (TPC)

Folin-Ciocalteu method was used for determination of total phenolic contents as described by Pérez *et al.* [39] with some modifications. F-C method is based on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybedic phosphotungstic acid complexes to form blue colored complexes that are determined spectrophotometrically at 760 nm. 50 mL volumetric flask was taken and, 0.5 mL of the extract was poured into the flask then mixed with 1 mL of Folin–Ciocalteu reagent and 12 mL deionized water. After 3 min, 6 mL of 7.5% Na_2CO_3 solution was added and incubated in dark for 1.5 h at room temperature before the

absorbance was taken at 760 nm. Similarly, a calibration curve was constructed using gallic acid standard over a concentration range of 1, 60, 100, 220, 300, 420, and 500 mg/L and the result was reported as milligrams of gallic acid equivalent (mg GAE/100 g on dry basis) of the samples. All the determinations were carried out in triplicate. The total phenolic contents in all the samples were calculated by the using the formula:

$$\text{TPC} = c\frac{V}{m}, \quad (1)$$

where TPC = total phenolic content mg GAE/100 g, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in mL, and m = mass of extract in gram.

RESULTS AND DISCUSSION

Total polyphenols

In this study, total polyphenol content was measured and expressed as GAE. The UV-Vis spectra of the standard gallic acid solutions at different concentrations are shown in Figure 1. The UV-Vis spectra of the polyphenol extracted from the vegetables are shown in Figure 2. As can be seen from Figures 1 and 2, the spectra of the polyphenols extracted from the studied vegetables matched with the spectrum of standard gallic acid, which clearly indicate the presence of polyphenols in the vegetables. After confirming the presence of polyphenols in the vegetables, the calibration curve was constructed with standard gallic acid in the concentration range of 1-500 mg/L. The calibration curve is shown in Figure 3. Then the levels of total polyphenols in the samples were determined with the regression equation and the results of total polyphenols of the vegetables have been summarized in Table 2.

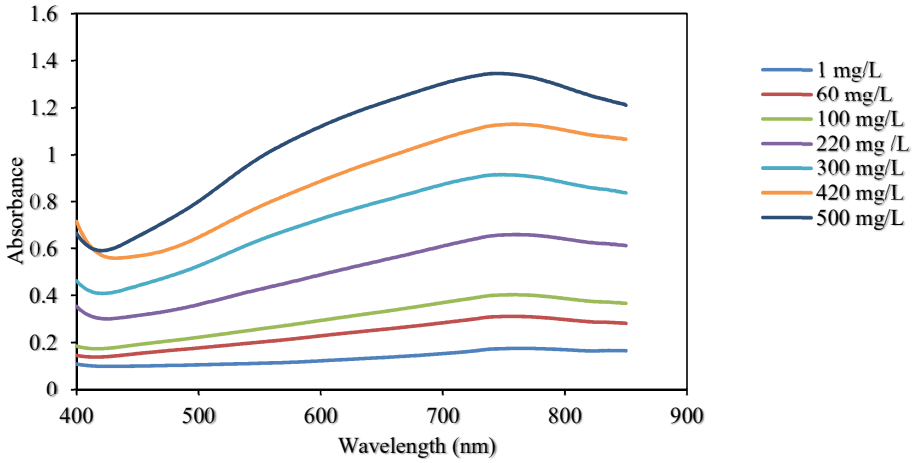

Figure 1. The UV-Vis spectra of the standard gallic acid solutions at different concentrations.

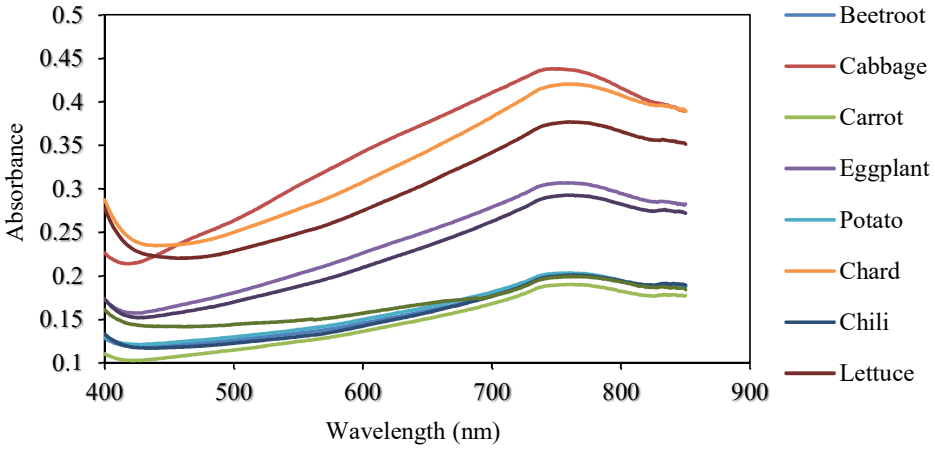

Figure 2. UV-VIS spectra of the polyphenols in ten selected vegetables.

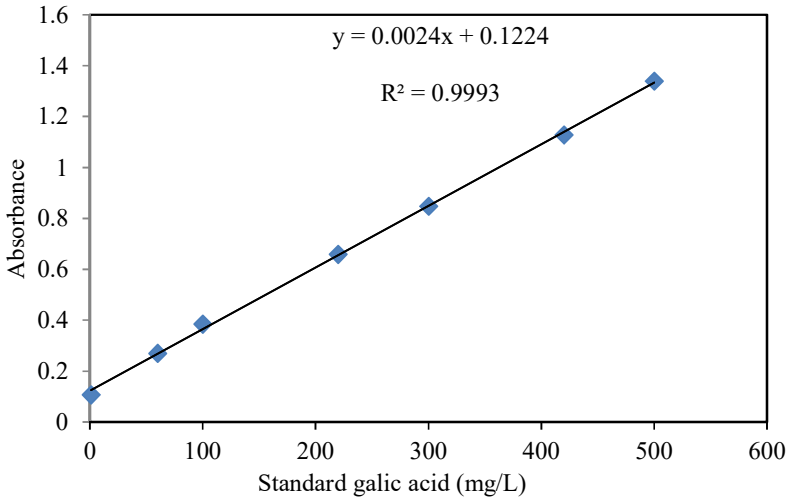

Figure 3. Calibration curve of gallic acid for total polyphenol determination.

The levels of total polyphenol (free and bound polyphenol) were expressed in terms of gallic acid equivalent (GAE), determined by the method of Folin-Ciocalteu [40] and quantified from the equation of regression line: $y = 0.0024x + 0.1224$ with $R^2 = 0.9993$ where y is mean absorbance, x is concentration in mg/L. The graphical presentation of the comparison of total polyphenols in the vegetables are shown in Figure 4. While the comparison of free and bound polyphenol contents of the vegetables are shown in Figure 5. The correlation between the free polyphenols and the bound polyphenols is shown in Figure 6.

Table 2. Polyphenol content (mg of GAE/100 g) on dry basis of the vegetable samples (n = 3, triplicates analysis).

No.	Vegetable samples	FPC (mg/100 g)	BPC (mg/100 g)	TPC (mg/100 g)
1	Beetroot	594 ± 0.10	101 ± 0.09	695 ± 0.19
2	Cabbage	2770 ± 0.13	401 ± 0.08	3171 ± 0.21
3	Carrot	864 ± 0.08	85 ± 0.08	949 ± 0.15
4	Cauliflower	882 ± 0.15	204 ± 0.78	1086 ± 0.93
5	Chili	1613 ± 0.13	393 ± 0.08	2006 ± 0.21
6	Eggplant	916 ± 0.07	228 ± 0.07	1143 ± 0.21
7	Lettuce	867 ± 0.05	293 ± 0.16	1160 ± 0.21
8	Potato	115 ± 0.07	103 ± 0.04	218 ± 0.12
9	Swisschard	1202 ± 0.07	111 ± 0.17	1313 ± 0.24
10	Tomato	532 ± 0.12	92 ± 0.08	624 ± 0.20

FPC = free polyphenols, BPC = bound polyphenols, TPC = total polyphenols.

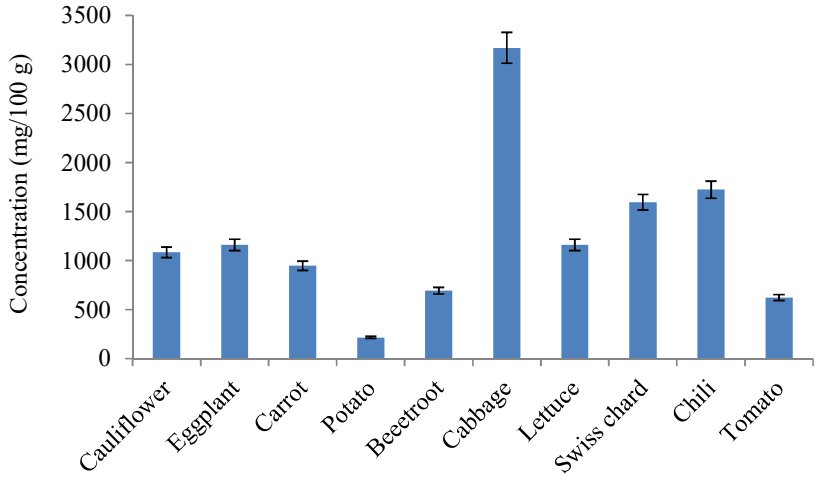

Figure 4. The content of total polyphenol (mg GAE/100 g db) (the data was expressed as mean ± SD).

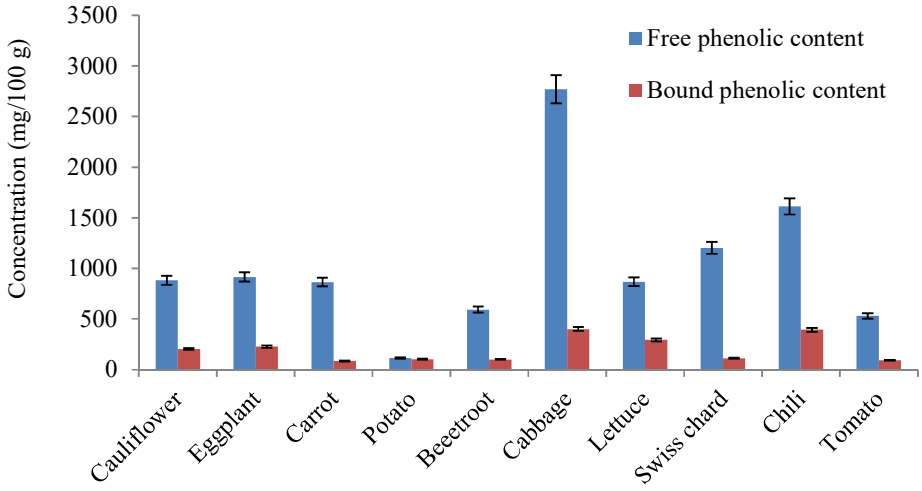

Figure 5. Comparison of free and bound polyphenol contents of the ten selected vegetables in (mg GAE/ 100 g db).

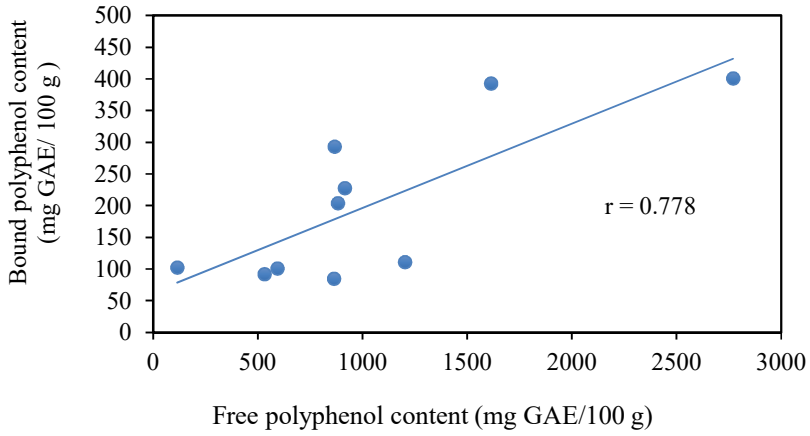

Figure 6. Correlation between free polyphenol and bound polyphenol content of the vegetables.

The total phenolic content (TPC) (sum of free and bound phenolic extracts) in the vegetables by shed drying methods are given in Table 2. The free phenolic content of each kind of vegetable are significantly higher than that of the bound phenolic content. In this research, the bound phenolic contents comprise an average of 16.25% of the total phenolic contents and the free phenolic contents were 83.75% present in the studied vegetables. These results generally indicate that the phenolic compounds in vegetables existed primarily in free form rather than in bound form. Free phenolic contents in vegetables were increased in order: potato < tomato < beetroot < carrot < lettuce < cauliflower < eggplant < Swiss chard < chili < cabbage, in which cabbage

contained the highest amount of free phenolic compound (2770 ± 0.13 mg GAE/100g) and potato had the lowest (115 ± 0.073 mg GAE/100g). The highest level of bound phenolics was found in cabbage (401 ± 0.08 mg GAE/100 g), whereas the lowest bound phenolics was in tomato (92 ± 0.081 mg GAE/100 g). Generally, the higher total phenolic content (TPC) values were found in cabbage, chili, Swiss chard, lettuce, eggplant and cauliflower, these were respectively 3171 ± 0.21 mg GAE/100 g, 2006 ± 0.21 mg GAE/100 g, 1313 ± 0.24 mg GAE/100 g, 1160 ± 0.21 mg GAE/100 g, 1143.2 ± 0.21 mg GAE/100 g and 1086 ± 0.93 mg of GAE/100 g. While potato has the lowest total polyphenol contents in the studied vegetable which has 217.5 ± 0.12 mg GAE/100g. The TPC of selected vegetables in this study was ranges from 217.5 to 3171 mg GAE/100 g of dry basis. Alam *et al.* [41] reported that the TPC of selected unconventional vegetables grown in Bangladesh was in the range of 82.410 - 2711.054 mg GAE/100 g DW of vegetable extract. Hung and Duy [42] reported that free phenolic contents of the selected vegetables ranged from 36.5 to 454.6 µg GAE/g sample. In this study, the bound phenolic contents of the selected vegetables ranged from 92 to 401 mg GAE/100 g sample. In other study, Hung and Duy [42] reported that bound phenolic contents of the selected vegetables ranged from 3.3 to 19.2 µg GAE/g sample. Compared to other studies, the selected vegetables under this study had slightly higher TPC than some frequently consumed leafy and non-leafy vegetables. Several factors could be added to be responsible for differences in total phenolic content of vegetables. These include variation in vegetable cultivars, climatic condition, soil characteristics, extraction methods specially extraction solvents, harvest and post-harvest handling and storage conditions, processing techniques during analytical determinations. The results indicate that extracts of cabbage, chili, Swiss chard, lettuce, eggplant and cauliflower are good source of phenolic compounds and have more benefits to human health.

Statistical analysis

Statistical analysis using one-way ANOVA ($\alpha = 0.05$) was performed to test whether there are statistically significant differences between the mean concentration of the free polyphenol and bound polyphenol content across different vegetable samples. Since the p-value (0.0022) is much less than the common significance level of 0.05, means there is a statistically significance difference between the free and bound polyphenol contents in the vegetable samples.

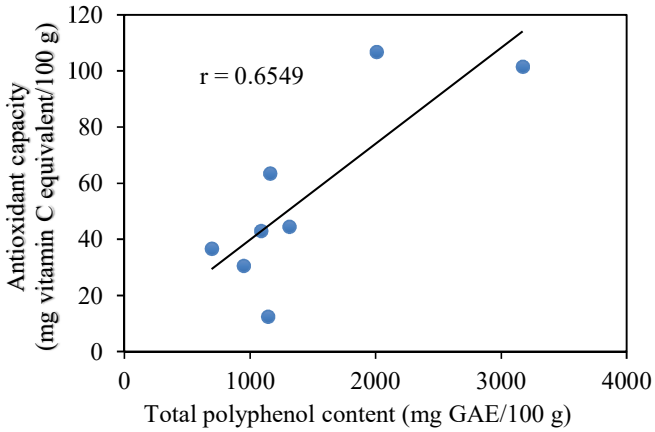

Figure 7. Correlation between total polyphenol content and antioxidant capacity of the vegetables.

The Pearson correlation coefficient r = 0.778 (Figure 6), indicates a strong positive correlation between FPC and BPC across the vegetable samples. Implies that vegetables with higher free polyphenol content also tend to have higher bound polyphenol content. This relationship suggests that these two forms of polyphenols might be influenced by similar factors in the vegetables, such as their metabolic pathways, environmental conditions, or genetic factors.

The correlation between total polyphenols content and antioxidant capacity [43] of the studied vegetables has been shown in Figure 7. The correlation coefficient (r = 0.6549) clearly indicates that there is strong positive correlation between the total polyphenols content and antioxidant capacity of studied vegetables. Therefore, consuming the vegetables is good for the health. Similar conclusion has also been drawn in the recently published literature on vegetables [43].

CONCLUSION

The current study evaluates the total polyphenol of ten vegetables. The presence of polyphenols was confirmed using chemical analytical methods followed by UV-Vis spectroscopy techniques. Among the vegetables, the extracts of cabbage, chili, Swiss chard, lettuce, eggplant and cauliflower exhibited the highest total polyphenol contents.

In this research, the contents of bound polyphenol comprise an average of 16.25% of the total polyphenol while the contents of free polyphenol were 83.75%. The total polyphenol content (TPC) in the free fraction were much higher than in bound fraction, indicating that free components were main contributors to the total antioxidant capacities.

The presence of substantial amounts of total phenolics in vegetables may be responsible for their effective antioxidant activity, which is a key fighting against various diseases related to oxidative stress. Therefore, there is a high potential using for vegetables as a health promoting and disease preventing source.

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Disclosure statement

The authors declare that there are no conflicts of interest.

Data availability statement

All the data are included in the manuscript. There are no additional data with the authors.

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