Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense*)

Lat. Am. J. Pharm. **43** (1): (2024)

Nutraceutical Potential and Health Benefits of Different Polyphenol-Rich Vegetables Stalk Extracts by Exploring Antioxidant Activities

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Abstract:
Edible stalk portion of vegetable is essential constituents of well-balanced diet enriched with minerals, dietary fiber, vitamins and phytochemicals. The main aim of the present research work was to determine the antioxidant of different polyphenol-rich vegetables stalk extracts (Cauliflower Stalk (CS), Broccoli Stalk (BC), Garlic Stalk (GC), Onion Stalk (OS), Parsley Stalk (ps), Young Bamboo Shoot (YBSS), Sarson Stalk (SS) and Sugar Cane SCS). The methanol crude extracts of all the stalks were prepared by orbital shaker. Quantification of phenolic acids and flavonoids was evaluated by RP-HPLC. In-vitro antioxidant activity was carried out by measuring the total phenolic contents, total flavonoid contents, DPPH radical scavenging activity, reducing potential and inhibition of linoleic acid peroxidation. HPLC analysis showed the presence of gallic acid, p-coumaric acid, chlorogenic acid and benzoic acid was found as major phenolic acids, while catechin was found as a major flavonoid in many stalk extracts. TPC was ranged from (0.41 to 1.26 and 0.05 to 0.18 mg/g of dry weight of stalk). Highest TPC and TFC were found in garlic stalk (1.26 g/100 g of dry stalk measured as gallic acid) and broccoli stalk (0.19 g/100 g of dry weight of stalk measured as catechin equivalent). Garlic stalk (17.23 mg/mL) showed the excellent radical scavenging activity. Among these vegetables' stalks, Garlic stalk showed the promising DPPH radical scavenging activity, reducing potential and inhibition of linoleic acid peroxidation. HPLC analysis showed four phenolic acids were detected: gallic acid, p-hydroxyl benzoic acid, caffeic acid and ferulic acid. Gallic acid and p-hydroxy benzoic acid were the major phenolic acids present in abundance in extracts of all the stalks. Two flavonoids—catechin and quercetin—are being reported as the major flavonoids in edible stalk extract, analyzed.

Keywords: Polyphenol-rich vegetables stalk; Antioxidant activity; Gallic acid; p-hydroxyl benzoic acid; Caffeic acid; Ferulic acid

Introduction

A man is distinguished from all other more complex life forms and homo culinarians in his survival strategy by eating natural organic substances like "food" (fruits and vegetables) [1]. The lowest consumption of edible and non-edible portion like stalk and peels of vegetables results in significant food waste [2]. The loss of edible food parts can also be due to the reduction in the edible food mass during the processing, washing, slicing or peeling. Almost 1.3 billion tons of wastage of food occurs from the production till the end of the use of consumers per annum [3]. In many vegetables, the curd is being consumed and the stalk is thrown away which is also account for the remarkable nutritional values[4].The loss of stalk and other edible parts of vegetables is a problem to industrial development as well as environmental protection. So, there is a need for the research on the utilization of vegetables stalk. The development of value-added stalk portion of the vegetables is the main focus of attention due to the presence of phytochemicals and metabolites which can also help to promote the wellbeing of mankind and improvement of human health [1].

Stalk is also considered to be the useful source of the nutrients as well as the potential functional components to get the added value products to overcome the hidden hunger [5]. The stalk with long or short length of different vegetables comprises a remarkable amount of biological active components. They are enriched with phenolic

compounds with low molecular weight secondary metabolites consisting of polyphenols, alkaloids, saponins, proteins, polysaccharides and carotenoids [6]. Polyphenols possess chemo-preventive, antioxidant and various kinds of different pharmacological activities. From the last two decades, the dietary polyphenols as antioxidants exert a potential health benefits. 100 g of average fresh vegetables comprises of almost 300 mg of polyphenols [7].

Cauliflower stalk (*Brassica oleracea var. botrytis*), broccoli stalk (Brassica *oleracea* var. *italic*), sugar cane (*Saccharum officinarum*), garlic stalk (Allium *sativum*), onion stalk (Allium *cepa*), parsley stalk (Petroselinum *crispum*), young bamboo shoot (*Bambusa vulgaris*), sarson stalk (Brassica *campestris* var. sarson) are rich source of micro and macro nutrients like proteins, polysaccharides, polyphenols, low fats and high fiber contents as well as starch with low glycemic index. These stalks are good source of antioxidants and antimicrobial, anticancer and anti-inflammatory activities by lowering the oxidative stress. The chopped and intact stalks of these vegetables can be used in salad, soups, pastas and stir fries [8]. Various economics and nutritional benefits linked with the vegetables stalks and is important to carried out the systematic investigation of antioxidants and pharmacological status of stalks as there is a plenty of literature on the antioxidant activity of vegetables curd is present but not much literature focusing on the importance of stalk. So, the present study aims to investigate the antioxidant potential of different vegetables stalks. The polyphenolic profile was done by RP-HPLC.

Material and Methods

4.1. Reagents, Reference compounds and chemicals

The standards and chemicals used in this study were analytical grade and were purchased from the Sigma Chemical Co. (St Louis, MO, USA) and the Solvents were purchased from Merck (Darmstadt, Germany).

4.2. Pretreatment of plant material

Stalks of all plant materials like cauliflower stalk, broccoli stalk, garlic stalk, onion stalk, parsley stalk, young bamboo shoot, sarson stalk and sugar cane were collected from the Ayub Agriculture Research Institute, Faisalabad, Pakistan (AARI). The plant materials were further identified and authenticated by taxonomist in Department of Botany, Government College University Faisalabad. The stalks of all samples were washed, dried at room temperature and ground to fine powder (80-mesh) using commercial electrical grinder. The ground samples were stored in air tight polythene bags for further use.

4.3. Samples preparation

The ground stalk of all the plant materials (20 g) were extracted using orbital shaker (Utech products Inc. Albany New York 12203 USA) with absolute methanol (200 mL) in a conical flask for 8 hours [9]. The extracts of all sample stalk were filtered and then concentrated using rotary evaporator (Rotavapor R-300, BÜCHI, Labortechnik AG, Flawil, Switzerland). The concentrated extracts were weighed and yields were calculated.
Yield (%) = Weight of extract / Weight of dry material × 100

4.4. Evaluation of antioxidant activity

4.4.1. Total phenolic content: The total phenolic contents were determined by Folin Ciocalteu method described [10]. Briefly, 0.5 mg of each extracts was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL deionized water. The mixture was allowed to stand at room temperature for 10 minutes, and then 1.5 mL of 20% sodium carbonate (w/v) was added and then the mixture was heated in a water bath at 4 °C for 25 minutes and then cooled in an ice bath. The absorbance was measured at 755 nm by UV-Vis double beam spectrophotometer (Lambda 25, Perkin-Elmer, Shelton, CT, USA). TPC were calculated by using gallic acid calibration curve. The total phenolic contents were expressed as mg/g of DW of plant material; measured as Gallic Acid Equivalent (GAE).

4.4.2. Total flavonoid content: Total flavonoids contents of all extracts were determined by method reported by Zafar *et al*. (2022) [11]. A volume of 1 mL of each extract was mixed with 5mL distilled water in a 10 mL volumetric flask followed by the addition of 0.3 mL of 5 % $NaNO_2$ (w/v). After 5 min, 6 mL of 10% $AICl_3$ was added to this mixture. After another 5 min, 2mL of 1M NaOH was also added and then make the volume up-to 10 ml with distilled water. The absorbance was recorded by UV-Vis double beam spectrophotometer (Lambda 25, Perkin-Elmer, Shelton, CT, USA). at 515 nm). TFC was reported in mg/g of DW of all plant materials and measured as Catechin Equivalent (CE).

4.5. Reducing power

Reducing power of all the extracts were determined to investigate the antioxidant potential by the method reported [11]. 5 mL of sodium phosphate buffer (0.2 M, pH= 6.6) was mixed with 1 mL of each stalk extracts followed by the addition of 5 mL of 1% potassium hexacyanoferrate solution and then incubated at 50^0C for 20 min in a water bath. Then 5 mL of 10% Trichloroacetic acid was added in the mixture and centrifuged at 3000 rpm for 10 minutes at 5^oC in a refrigerated centrifuge (CHM-17; Kokusan Deriki, Tokyo, Japan). The upper layer (5 mL) of supernatant was separated and diluted with 5 mL distilled water followed by the addition of 1 mL of 0.1 % $FeCl_3$. Absorbance was recorded at 700 nm by using a double beam spectrophotometer (Lambda 25, Perkin-Elmer, Shelton, CT, USA). at 515 nm).

4.6. Inhibition of Linoleic acid peroxidation

Antioxidant activity of stalk extracts were determined by measuring the inhibition of linoleic acid peroxidation by the method reported [12]. The stock was prepared by dissolving the 0.1 mg β-carotene, 100 mg tween40, 20 mg linoleic acid in 1 ml chloroform. Chloroform was removed by the rotary evaporator under vacuum at 50^oC. Then, 50 ml distilled water saturated with oxygen (30 min, 100 ml/min) was added and the mixture was stirred carefully. Then, 5 ml of reaction mixture was poured into a test tube consisting of 200 µL of extracts and the absorbance was measured at 490 nm against the blank containing emulsion without β-carotene. Emulsion was incubated at room temperature for 50 hours and the absorbance was measured at different time intervals within 180 hours. Percent inhibition was calculated by the following formula:

Inhibition (%) = 100 – [((Absorbance increase in sample) / (Absorbance increase in negative control)) × 100]

4.7. Determination of DPPH

The antioxidant activity of each stalk extracts was determined by DPPH radical scavenging activity according to the method reported [11]. 0.5 ml of each stalk extracts was mixed with 3 mL DPPH solution (90 µM) and then this mixture was incubated at room temperature for 30 minutes. The absorbance was measured at 517 nm. DPPH solution was used as blank and the Butylated Hydroxytoluenen (BHT) was used as the positive control. Percentage radical scavenging activity was measured by the following formula:

DPPH radical scavenging (%) = (Absorbance of DPPH solution – Absorbance of sample solution) / (Absorbance of DPPH solution) × 100

4.8. Quantification of polyphenols by RP-HPLC

Quantification of phenolic acids and flavonoids were carried out by RP-HPLC as described by the method reported [11]. Fresh sample was prepared by re-dissolving the 10 mg dried extract of each stalk sample into 1 ml methanol and then filtered. HPLC profile of phenolic acids and flavonoids was done by Chromera HPLC (Perkin Elmer, 520 South Main St., Suite 2423, Akron, OH, USA), where a binary solvent gradient system equipped with C-18 column (250 × 4.6 mm internal diameter, 5 µm particle size), a non-linear gradient system consisting two solvent systems such as solvent A (0.5 % glacial acetic acid and distilled water) and solvent B (methanol: acetonitrile; 30:70) and UV-Vis LC detector. UV-Vis spectra were measured at 275 nm. The quantification was carried out by standard addition method by matching the retention times and spikes of known standard concentration.

4.9. Chromatographic conditions

The Agilent 1260 infinity HPLC system (Agilent, Santa Clara, CA, USA) with a C-18 column (150 × 4.6 mm internal diameter, 2.7 micrometer (µm) particle size) equipped with a gradient binary pump system (G7112B), 1260 autosampler (G7129A) and multiwavelength detector (G7165A) was used. The non-linear gradient with methanol was acetonitrile (30:70 as solvent A) and 0.1% acetic acid in distilled water as solvent B. The following gradient program was developed: 10% A from 0 to 5 min; 10–30% A from 5 to 25 min; 30–40% A from 25 to 40 min; 40–90% A from 40 to 60 min and kept at 90% A from 60–65 min. The MWD detector settings were 250, 270, 290, 310, 330, 350, 370 nm 1.2 nm resolution and 10 points/s sampling rate. Qualitative analysis was performed using the matching of retention times with the authentic standards and spiking of standards in the samples, while for quantitative analysis, the standard addition method was applied.

4.10. Statistical analysis

All the analysis was repeated in triplicated manner and the results were reported as mean value ± standard deviation. One Way analysis of Variance (ANOVA) using STATISTICA 5.5 (Stat Soft Inc., Tulsa, OK, USA) software and the probability value of $p \leq 0.05$ exhibited a statistical significance difference.

Results and Discussion

5.1. Yield

The yield of crude methanol extracts of all the vegetables stalks (cauliflower stalk, broccoli stalk, garlic stalk, onion stalk, parsley stalk, young bamboo shoot, sarson stalk and sugar cane) was ranged from 3.5 to 32.5 g/100g of dry stalk material represented in Table 1. Broccoli stalk showed the highest yield (32.5 g/100g) followed by sugar cane (4.8 g/100g), parsley stalk (5.1 g/100g), young bamboo shoot (6.3 g/100g), cauliflower stalk (9.7 g/100g), onion stalk (13.6 g/100g) and sarson stalk (26.2 g/100g), respectively. The significant difference in yield of different stalk extracts was found to be significant ($p \leq 0.05$). A study [13] reported the 67-70 % yield of fresh broccoli stalk. The extraction yield of cauliflower stalk was also expressed by another report [14] reported the extraction yield of cauliflower stalk of about 53.07 %. Another study [15] explained the extraction yield of parsley stalk ranging from 25.81-49.68 %. Another report published [16] which reported the yield (57.72 µg/g) of sugar cane. The choice of solvent and the extraction method plays a vital role in the variation of extraction of bioactive compounds from specific plant material and have a remarkable effect on the quality and the yield of extracted bioactive compounds [17].

Table 1: Yield, spectrophotometric quantification of total phenolic acids and total flavonoid contents, radical scavenging activity and Inhibition of linoleic acid peroxidation of vegetables stalks extracts.

Sr. No.	Extract	(g/100 g of dry weight)			DPPH (IC_{50}, µg/mL)	% Inhibition of linoleic acid peroxidation
		Yield	TPC (GAE)	TFC (CE)		
1	Allium *sativum*	3.5±0.2^{a}	1.26±0.06^{d}	0.18±0.01^{c}	17.23±0.86^{b}	70±4^{c}
2	Allium *cepa*	13.7±0.7^{f}	0.85±0.04^{b}	0.07±0.01^{a}	19.74±0.98^{c}	80±4^{e}
3	Petroselinum *crispum*	5.1±0.3^{c}	0.81±0.04^{b}	0.08±0.01^{a}	23.71±1.18^{e}	60±3^{a}
4	*Brassica oleracea var. botrytis*	9.7±0.5^{e}	0.82±0.04^{b}	0.14±0.01	20.21±1.01^{d}	78±4^{d}
5	Brassica *oleracea* var. *italic*	32.6±2^{h}	1.01±0.05^{c}	0.19±0.01^{c}	20.12±1.01^{d}	84±4^{ef}
6	*Brassica campestris* var. sarson	26.2±1^{g}	0.41±0.02^{a}	0.09±0.01^{ab}	19.82±0.99^{c}	80±4^{e}
7	*Bambusa vulgaris*	6.3±0.3^{d}	0.42±0.02^{a}	0.12±0.01^{b}	23.25±1.16^{e}	60±3^{a}
8	*Saccharum officinarum*	4.9±0.24^{b}	0.48±0.03^{a}	0.05±0.01^{a}	24.71±1.24^{ef}	61±3^{b}
9	BHT	---	---	---	5.96±0.29^{a}	91±1^{g}

Values are the mean ± standard deviation of experiments in triplicate manner and different letters in superscript exhibits significant difference among extracts of different vegetables stalks.

5.2. Antioxidant activities

5.2.1 Total phenolic contents and total flavonoid contents: Total phenolic contents and total flavonoid contents of all the vegetables stalks (cauliflower stalk, broccoli stalk, garlic stalk, onion stalk, parsley stalk, young bamboo shoot, sarson stalk and sugar cane) represented in Table 1 and Figures 1 and 2.

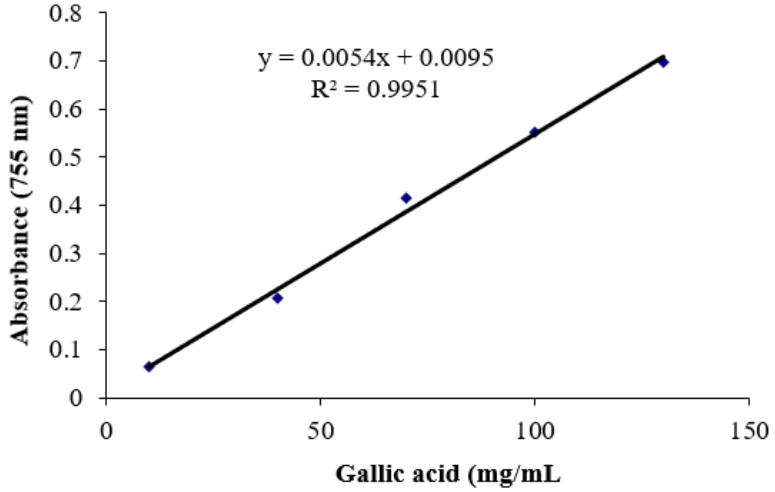

Figure 1: Calibration curve of gallic acid.

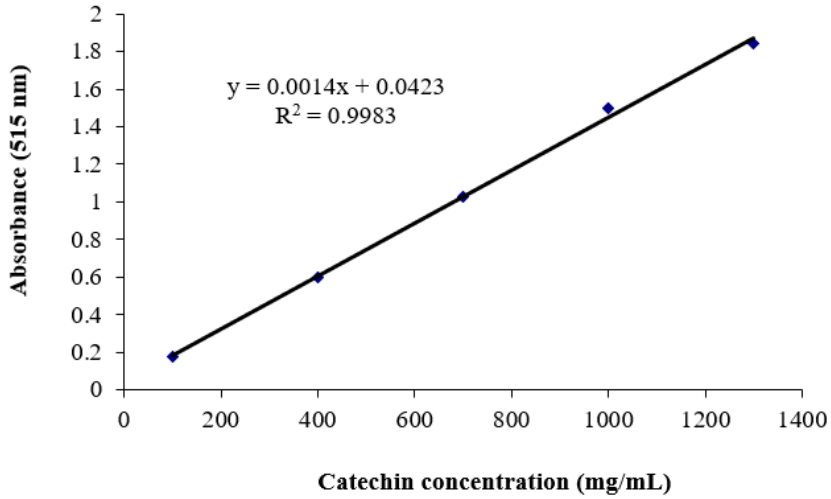

Figure 2: Catechin standard curve.

Total phenolic contents were reported in mg/g of dry weight of stalk measured as Gallic Acid Equivalent (GAE). Total phenolic contents of different vegetable stalks were ranged from (0.41 to 1.26 g/100 g of dry weight of stalk). Highest TPC were found in garlic stalk (1.26 g/100 g of dry stalk measured as gallic acid) while the lowest TPC were found in sarson stalk (0.41 g/100 g of dry stalk measured as gallic acid). Total flavonoid contents were reported in mg/g of dry weight of stalk measured as Catechin Equivalent (CE). TFC were ranged from (0.05 to 0.18 g/100g of dry weight of stalk) and the highest TFC were exhibited by broccoli stalk (0.19 g/100 g of dry weight of stalk measured as catechin equivalent) while the lowest TFC were exhibited by sarson stalk (0.19 g/100 g of dry weight of stalk measured as catechin equivalent) as compared with other vegetables stalks. Statistical analysis showed that the different stalk extracts have a potential effect ($p \leq 0.05$) on TPC and TFC. Our results are different to the previously published results [18] explained the TPC (14.62-27.83 mg gallic acid/g) and TFC (0.92-2.49 mg QE/g) of young bamboo shoots, respectively. Another report published by Rungjang *et al*. (2022) who reported the TPC of different forms of sugar cane where the TPC of stalk of sugar cane was (1.75 g GAE/kg). Another report [19] explained the TPC and TFC of onion (2.86 mg GAE/g and 3.76 mg CE/g of dry matter) and garlic (0.72 mg GAE/g and 0.26 mg CE/g of dry matter), respectively. The TPC and TFC are considered to be the best parameters for the assessment of antioxidant potential. It is confirmed by several reports that the amount and composition of phenolics and the flavonoid contents are different among different species in cellular and sub-cellular level. Variation in the TPC and TFC in current and previously reported results might be due to the variation in the geographical, seasonal and agro-climatic conditions and handling techniques [11]. Many reports exhibited that there is a good relationship between the UV radiations and higher or lower production polyphenols. So, the substantial level of polyphenols among different vegetables and the surrounding environment. Because variation in the atmospheric condition can cause the variation in the growth and development of plants as a result the variation in the synthesis of secondary metabolites and bioactive components [20, 21].

5.2.2. DPPH radical scavenging activity: Free radical scavenging activity of all the extracts of vegetables stalks (cauliflower, broccoli, garlic, onion, parsley, young bamboo shoot, sarson and sugar cane) increased in the concentration dependent manner where the concentration exhibited the 50% scavenging (IC_{50}) is presented in Table 1. The radial scavenging activity (IC_{50}) of stalk extracts (cauliflower, broccoli, garlic, onion, parsley, young bamboo shoot, sarson and sugar cane) was ranged from 17.23 to 24.71 µg/mL. The lowest IC_{50} was exhibited by garlic stalk (17.23 µg/mL) which showed the excellent radical scavenging activity while the highest IC_{50} was exhibited by sugar cane stalk (24.71 µg/mL) which showed the lowest radical scavenging activity. The synthetic antioxidant BHT exhibited the best radical scavenging activity in terms of lowest IC_{50} (24.71 µg/mL). A significant variation ($p \leq 0.05$) was reported among the stalk extracts (cauliflower, broccoli, garlic, onion, parsley, young bamboo shoot, sarson and sugar cane). Here garlic stalk extract exhibited the maximum TPC and TFC and hence showed the highest radical scavenging activity because the antioxidant activity is associated with the large amount of polyphenols found in the specific plant extract. Previous results published [18] reported the DPPH radical scavenging activity of different young bamboo shoots ranging from (347.48-2489.60 µg/mL). In another study [22], reported the inhibition by DPPH method was 45.2 % for sugar

cane. Previously published study [19] reported the antioxidant activity in terms of DPPH radical scavenging activity of onion (12.66 and garlic μM) and garlic (6.81 μM).

5.2.3. Inhibition of Linoleic acid peroxidation: Antioxidant activity of vegetables stalks extracts (cauliflower, broccoli, garlic, onion, parsley, young bamboo shoot, sarson and sugar cane) can also determine in terms of inhibition of linoleic acid peroxidation and is represented in Table 1. Linoleic acid is a polyunsaturated fatty acid, which makes peroxide on oxidation and is also considered as the hydrogen transfer-based assay. Higher absorbance showed the higher magnitude of peroxides formed in the reaction and as a result antioxidant activity will be lower [23]. A significant difference ($p \leq 0.05$) was found between the percentage inhibition of linoleic acid peroxidation of each stalk extract. Broccoli stalk showed the highest percentage inhibition of linoleic acid peroxidation (83.60 %) while young bamboo shoot showed the lowest percent Inhibition of linoleic acid peroxidation (60.03 %). Published data [23] showed the percentage inhibition of linoleic acid (85. 0%) of cauliflower. There is strong correlation between the polyphenolic contents, DPPH radical scavenging activity and percentage linoleic acid. Higher the polyphenol contents and antioxidant activity, higher will be the percentage inhibition of linoleic acid peroxidation [23, 24]. This also support the present results that the higher the antioxidant activity of garlic stalk higher the percentage inhibition of linoleic acid.

5.2.4. Reducing power: Antioxidant activity of all the extracts of vegetables stalks (cauliflower, broccoli, garlic, onion, parsley, young bamboo shoot, sarson and sugar cane) can also carried out by reducing power and the results are expressed in Figure 1. The reducing power based on electron donating ability in concentration dependent manner up-to 0-10 mg/ml where the antioxidant activity increases as the concentration of extract increase. In comparison with the reducing power of all the vegetable stalk extracts, the maximum reducing power was exhibited by garlic while the minimum reducing power was exhibited by the sugar cane. Maximum reducing power of garlic might be associated with the fact that the reducing potential is correlated with antioxidant capacity such as there is a good association between reducing power and polyphenol contents. As the garlic stalk exhibited the highest phenolic contents, so it also showed the maximum reducing [25, 26]. Present results are different from the results reported in previous reports [25] reported the reducing power of cauliflower. Another report [27] who explained the reducing power of garlic between different solvents where distilled water extract of garlic stalk showed the maximum reducing potential.

5.2.5. Quantification of phenolic Acids and flavonoid by RP-HPLC: The phenolic acid and flavonoid compositions of edible stalk extracts were determined by RP-HPLC using a Multi-Wavelength Detector (MWD). Chromatograms showing the separation of phenolic acids and flavonoids from the extract at 250, 270, 290, 350 and 380 nm are presented in Figure 3. Qualitative and quantitative data from all the extracts are represented in Table 2. Four phenolic acids were detected: gallic acid, p-hydroxyl benzoic acid, caffeic acid and ferulic acid. Gallic acid (0.21–0.96 mg/g of extract) and p-hydroxy benzoic acid (0.12-2.59 mg/g of extract) were the major phenolic acids present in abundance in extracts of all the stalks. Two flavonoids—catechin and quercetin—are being reported as the major flavonoids in edible stalk extract, analyzed. Catechin was reported to have the highest concentration (0.14-3.18 mg/g of extract) whereas concentration of quercetin was in the range of 0.36-3.54 mg/g of extract.

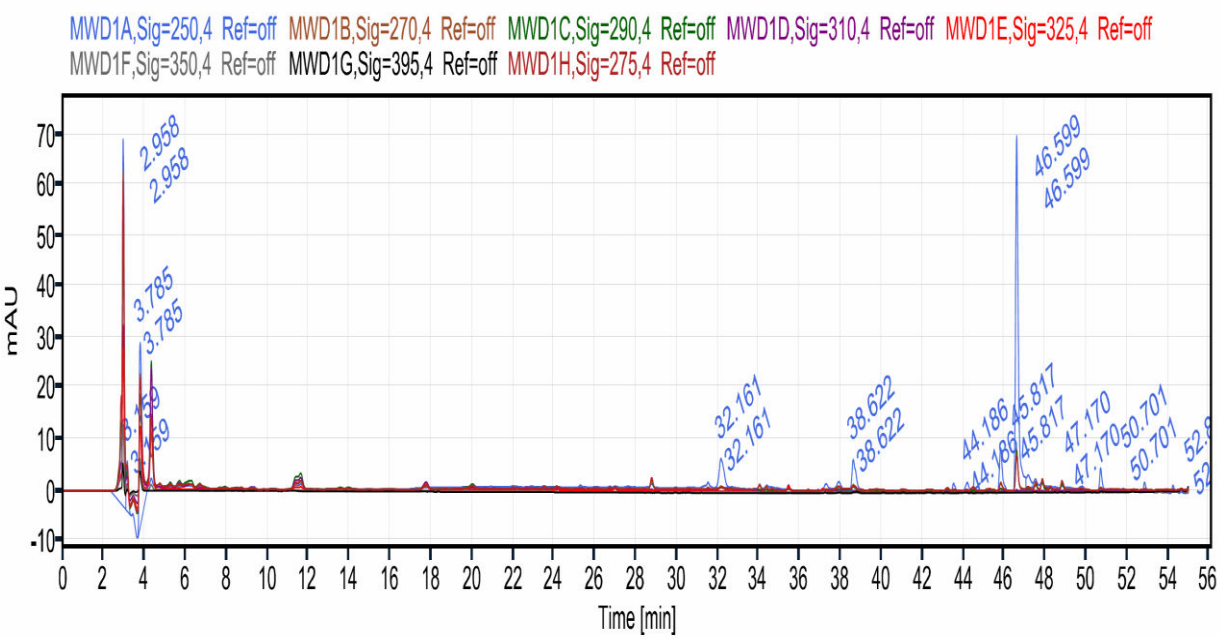

Figure 3: Typical chromatogram showing the separation of polyphenols from *Brassica oleracea.*

Table 2: Composition of phenolic acids and flavonoids from extracts of edible stalks by RP-HPLC.

Extract	Concentration (mg/g of extract)					
	Gallic acid	**PHBA**	**Caffeic acid**	**Ferulic acid**	**Catechin**	**Quercetin**
Allium *sativum*	0.27	1.27	1.56	1.08	0.89	3.26
Allium *cepa*	0.51	0.12	---	---	0.23	1.96
Petroselinum *crispum*	0.25	0.42	---		2.59	0.91
Brassica oleracea var. botrytis	0.56	2.59	1.56	1.33	1.90	3.54
Brassica *oleracea* var. *italic*	0.21	1.08	0.37	0.49	3.18	1.86
Brassica campestris var. sarson	0.96	0.56	---	0.23	1.44	0.36
Bambusa vulgaris	0.58	0.64	---	---	0.14	---
Saccharum officinarum	0.23	0.47	0.38	---	---	1.06

Values are the mean ± standard deviation of experiments in triplicate manner and different letters in superscript exhibits significant difference among extracts of different vegetables stalks.

Conclusion

The edible stalk portion of different vegetables like (cauliflower, broccoli, garlic, onion, parsley, young bamboo shoot, sarson and sugar cane) are rich source of natural phenolic acids and flavonoids. Among these vegetables' stalks, Garlic stalk showed the promising DPPH radical scavenging activity, reducing potential and inhibition of linoleic acid peroxidation. HPLC analysis showed four phenolic acids were detected: gallic acid, *p*-hydroxyl

benzoic acid, caffeic acid and ferulic acid. Gallic acid and *p*-hydroxy benzoic acid were the major phenolic acids present in abundance in extracts of all the stalks. Two flavonoids—catechin and quercetin—are being reported as the major flavonoids in edible stalk extract, analyzed.

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