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# A comparative study focusing on antioxidant properties of the selected green leafy vegetables: *Chenopodium album, Brassica juncea* and *Brassica campestris*

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## Abstract

Green leafy vegetables (GLVs) are believed to possess strong antioxidant potential. In this study, antioxidant properties of three GLVs were examined. Methanolic and ethanolic extracts of *Chenopodium* album, *Brassica* juncea and *Brassica* campestris were analyzed for total phenolic contents (TPC), total flavonoid contents (TFC), DPPH radical scavenging activity and Ferric reducing antioxidant power (FRAP). The results indicated that methanolic extract of *B. campestris* credited for the highest amount of TPC (226.48 µg GAE/mg) whereas the lowest amount was found in the ethanolic extracts of *C. album* (174.63 µg GAE/mg). The trend of TPC was found as *B. campestris* > *B. juncea* > *C. album* for both methanolic and ethanolic extracts. TFC in the selected GLVs ranged from 14.81 to 31.14 µg QE/mg for both extraction solvents. The order of TFC was *B. juncea* > *B. campestris* > *C. album* in the methanol extract and *B. juncea* > *C. album* > *B. campestris* in the ethanol extract. *B. Juncea* exhibited the highest percentage of DPPH inhibition in methanol extract as 43.36 % and ethanol extract as 42.68 %. Similarly, both methanolic and ethanolic extracts of *B. Juncea* also found maximum reducing power as 135.85 µg AAE / mg and 85.64 µg AAE /mg respectively. The positive correlation of TPC with antioxidant activities suggested their contribution to antioxidant activity.

**Keywords:** Green leafy vegetables (GLVs), Methanolic and Ethanolic Extract, Total phenolic contents, Total flavonoid contents, Antioxidant activity.

## 1. Introduction

Bangladesh is blessed with a large area of fertile arable land which ultimately supports its inhabitants to cultivate a variety of vegetables throughout the year. Vegetables cover a significant part of our daily diet and are important source of nutrients. More specifically daily intake of leafy vegetables keeps us healthy and fit. Green leafy vegetables are rich in bioactive compounds like polyphenols, vitamins, and minerals [1]. Different parts of plants are consumed as food for sound health as they are highly nutritious; moreover, nutrition, and health care are interconnected [2].

Bathua (*Chenopodium album*), Mustard leaf (*Brassica juncea*), and Field Mustard (*Brassica campestris*) are widely consumed green leafy vegetables in the different regions of Bangladesh. *Chenopodium album* which is also known as 'Bathua' cultivated as a leafy vegetable and an important subordinate grain crop for human and animal foodstuff basically for about centuries [3]. *Brassica juncea* is a medicinal plant and its leaves are used as tonics, diuretics, and expectorants [4]. *Brassica campestris* is cultivated mainly as oilseed crop as well as a green leafy vegetable and are grown worldwide for their high erucic acid oil.

Crude extracts of GLVs were used in most of the studies to demonstrate their potentiality of health benefits. They possess a high amount of antioxidants, vitamin C, and consist of flavonoids and carotenoids in varying amounts. The regular consumption of dietary antioxidants is now believed to be effective in the reduction of the risk of various serious diseases [5]. Besides, a high amount of chlorophyll in GLVs has been proven to assist in the production of red blood cells and decline the risk of stroke, heart disease, and certain cancers [6]. Moreover, GLVs can contribute to the detoxifications of unwanted impurities and harmful metals accumulated in our body from highly processed food items [7].

Antioxidants are chemical compounds having the capability of inhibiting the oxidative chain reactions to retard or constrain the oxidation of lipids or other molecules [8]. To detect and determine the antioxidant activity of leafy vegetables, large numbers of scientific research studies have been carried out [9]. Several GLVs are reported by Gupta *et al.* [10] as rich sources of antioxidant vitamins and have been designated as

'nature's anti-aging wonders'. On this account, to ascertain the various nutritional qualities of commonly consumed GLVs in Bangladesh, comprehensive research will be decisive.

Therefore, the main purpose of our investigation was to evaluate the antioxidant activity of these GLVs by in vitro analysis and to find out the correlation with their total phenolic and total flavonoid contents and thus to probe their potency as nutriment for native residents.

## 2. Materials and Methods

### 2.1 Chemicals and Reagents:

Folin-Ciocalteu reagents, 35% sodium carbonate ($Na_2CO_3$), 2% $AlCl_3$ solution, 2,2- Diphenyl -1-picrylhydrazyl (DPPH), phosphate buffer, 1% potassium ferricyanide, 10% trichloroacetic acid, standard gallic acid, standard quercetin, standard ascorbic acid, methanol, ethanol and all other reagents of analytical grade were used. Distilled water was used in the preparation of standard solutions.

### 2.2 Plant Materials:

Three selected freshly harvested GLVs namely *Chenopodium album*, *Brassica juncea*, and *Brassica campestris* were harvested in the winter season and purchased in December 2017 from a local vegetable market located at Madina market region, Sylhet in Bangladesh. The leaves were washed to remove dirt, and any physical damage or insect infected areas were discarded. The leaves were further rinsed with distilled water. Each of the samples was 1kg in amount and kept at normal temperature.

### 2.3 Extract Preparation:

Leaves (1kg) of GLVs were spread on perforated stainless steel trays and placed in an oven dryer ( WTC binder, TUTTLINGEN, Germany) with a temperature set 65±5°C for 12h. Dried leaves were then ground into a fine powder using a blender (Panasonic MJM-176P, Malaysia ) and kept in airtight jars at 4°C, until further analyses. Absolute methanol and ethanol were used to extract the samples. About 10 g of each ground samples were poured into 100 ml of absolute methanol and ethanol individually and placed on a table-top shaker for 48 h. The mixture was filtered to yield the crude extract that was concentrated by removing the solvent at 40°C temperature using a vacuum rotary evaporator (HS-2005S-N Hahnshin, S&T Co. Ltd, Korea). The crude concentrated extracts were then analyzed for Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Antioxidant activity. The prepared extracts were stored at -20°C until further analysis.

### 2.4 Spectrophotometric Determination of Total Phenolic Contents and Total Flavonoid Contents:

The total phenolic contents (TPC) in methanolic and ethanolic extracts were analyzed using the Folin-Ciocalteu's colorimetric assay as reported by Amorim *et al*. [11] with some modifications. Briefly, aliquots (0.2 ml of 1000µg/ml) of diluted sample extract was added with 8.3 ml of distilled water. Then 0.5 ml of Folin-ciocalteu Phenol reagent was added. After keeping at room temperature for 30 minutes, 1 ml of 35% $Na_2CO_3$ solution was added and the mixture was shaken well. Absorbance was then measured at 765 nm using UV Spectrophotometer (Jenway 6405, Germany) after 20 minutes. Water was used to prepare blank instead of the sample. A gallic acid calibration curve (0-100 µg/ml) was plotted to measure TPC content. The results were expressed as microgram of gallic acid equivalent per milligram of dry extract (µg GAE/mg). All measurements were performed in three repetitions. The measurement of Total Flavonoid Contents (TFC) was carried out using a modified aluminum chloride colorimetric method [12]. Approximately 1.5 ml (1000µg/ml) of the extracts were placed in a 10 ml flask and 2% $AlCl_3$ solution was then added. After adding 6 ml of water the solution was vortexed. The absorbance of the solutions was measured after 30 minutes at 415nm against a blank. The total flavonoid compound was determined as microgram of quercetin equivalent per milligram (µg GAE/mg) of dry extract. The measurements of samples were done in triplicates.

### 2.5 Determination of antioxidant activity:

**2.5.1 DPPH radical scavenging activity:** DPPH radical scavenging activity was determined using the slightly modified method reported by Brand-Williams *et al*., 1995 [13]. An aliquot of 1 ml from the extract (1000µg/ml) was mixed with 4 ml DPPH solution in a tube. The tubes were vortexed well and kept in dark at room temperature to react for 30 min before the absorbance was recorded at 517 nm. The control sample was prepared with a DPPH solution without adding the aliquot. The results were expressed in percentage. The DPPH radical scavenging effect was calculated using the following equation, DPPH radical scavenging effect (%) = {1-(Absorbance sample/Absorbance blank)} × 100.

**2.5.2 Ferric reducing antioxidant power (FRAP assay):** FRAP assay was performed following a modified method described by Oyaizu *et al*., 1986 [14]. Briefly, 0.3 ml of sample extracts (1000µg/ml) were added with 0.85 ml of 0.2 M phosphate buffer (PH 6.6) and 0.85 ml of potassium 1% ferricyanide [$K_3Fe(CN)_6$]. The mixtures were then vortexed and incubated for 20 minutes at 500ºC. 0.85 ml of trichloroacetic acid (10%) was added and vortexed. Finally, 2.85 ml of distilled water and 0.57 ml $FeCl_3$ (1%) were added and the mixture was incubated at 25ºC for 30 minutes. At the end of the second incubation, absorbance was measured at 700 nm. Instead of aliquot, distilled water was used in blank preparation. The results were expressed as microgram of ascorbic acid equivalent per milligram of dry extract (µg AAE/mg).

### 2.6 Statistical analysis:

All experiments were repeated three times (n=3). All data were expressed as a mean (M) ± standard error means (SEM) and SPSS V.20 was used to analyze all experimental data and differences were considered significant at $p<0.05$.

## 3. Results and Discussion

### 3.1 Total Phenolic and Flavonoid Contents:

Quantitative estimation of total phenolic contents showed that the amount of TPC ranged from 187.22 to 226.48 µg GAE/mg of dry extract in methanolic extract and 174.63 to 186.65 µg GAE/mg of dry extract in ethanolic extract (Table-01). The maximum amount of TPC was possessed by methanolic extract of *B. campestris* (226.48 ± 1.51 µg GAE/mg of dry extract) as methanol has a higher polarity which ultimately increases its extraction efficiency [15] and the lowest amount of TPC was found in ethanolic extracts of *C. album* (174.63 ± 1.08 µg GAE/mg of dry extract). In *B. juncea* extracts, TPC ranged from 181.68 to 214.82 µg GAE/mg of dry extract depending on extraction solutions which was higher than the total phenol quantity obtained in acetone extract of B. juncea leaves (120.15 ± 15.58 µg GAE/mg) reported by Khandayataray *et.al*. [16]. Variety of vegetables and drying procedures might play an important role in this variation as the study of Capecka *et.al.* [17] claimed that the air-drying of peppermint resulted in significant increases in total phenols. A variation was observed between methanolic and ethanolic extracts and ethanolic extracts were recorded to give better results. In the case of both methanolic and ethanolic extracts, the trend of TPC of the selected GLVs can be expressed in the order as *B. campestris* > *B. juncea* > *C. album.* Statistical analysis (Table-03) showed that the value of the correlation coefficient is 0.464 which implies there is a positive correlation association between TPC and antioxidant activity (FRAP assay and DPPH radical scavenging activity). Selected GLVs were found to contain high TPC which suggests that they have the capability of treating inflammatory diseases and can be implicated in wound healing which can be targeted by pharmacists in the treatment of various diseases [18].

**Table- 01: TPC and TFC in Methanol and Ethanol extract of *B. campestris, B. juncea* and *C. album***

| *Sample* | *Extract Solvent* | TPC (µg GAE/mg of dry extract) | *TFC (µg QE/mg of dry extract)* |
|---|---|---|---|
| *B. campestris* | MeOH | 226.48±1.51 $^{a}$ | 26.67±1.86 $^{d}$ |
| | EtOH | 186.65±2.04 $^{A}$ | 14.81±1.66 $^{D}$ |
| *B. juncea* | MeOH | 214.82±1.66 $^{ab}$ | 31.14±1.45 $^{c}$ |
| | EtOH | 181.68±2.86$^{AB}$ | 29.88±1.64$^{CD}$ |
| *C. album* | MeOH | 187.22±2.40 $^{b}$ | 24.74±0.45 $^{d}$ |
| | EtOH | 174.63±1.08 $^{B}$ | 22.74±1.82 $^{D}$ |

Note: Values followed by different letters in a row are significantly ($p<0.05$) different from each other. The superscript small and capital letters are used for MeOH and EtOH extract solvent respectively.

Flavonoids are part of antioxidant compounds consisting flavonols, flavones, flavanones, flavanols, anthocyanins, and isoflavonoids. In this study, TFC was calculated as microgram of quercetin equivalents (QE) per milligram of dry extract in methanol and ethanol solvents. TFC in the methanolic extract was also found to be higher than ethanolic extracts and it ranged from 14.81 to 31.14 µg QE/mg of dry extract (Table-01). *B. juncea* was found to possess the maximum amount of TFC in both methanolic (31.14 µg QE/mg) and ethanolic (29.88 µg QE/mg) extract whereas lowest amount of TFC was recorded in ethanolic extract of *B. campestris* (14.81 µg QE/mg). The value of the correlation coefficient is -0.044 (Table-04) which implies there is a negative correlation association between TFC and antioxidant activity (DPPH radical scavenging activity and FRAP assay). In our research work, the trend of TFC was *B. juncea* > *B. campestris* > *C. album* in methanol extract and *B. juncea* > *C. album* > *B. campestris* in ethanol extract. Flavonoids are also very essential as they possess the ability of anti-inflammatory and antimicrobial activity.

### 3.2 Antioxidant Activity of *B. campestris, B. juncea* and *C. album:*

Phytochemicals and polyphenols can donate hydrogen atoms and/or electrons and act as antioxidants [19]. Therefore, these compounds can reduce oxidizing agents by being self-oxidized and prevent oxidation. In the living and nonliving systems, free radicals produce during redox reactions and antioxidants have the ability to scavenge them. Various radical scavengers are believed to be useful in protecting cell tissues from free radicals and prevent diseases especially cancer [20]. The antioxidant capacity of plant extracts depends on various factors such as extract composition, analysis system, etc. and it is important to determine antioxidant capacity through more than one measurement mechanism [21].

**Table 02: Antioxidant Activity of *B. campestris, B. juncea* and *C. album***

| *Sample* | *Extract Solvent* | *DPPH % of Scavenging (%I) (1000 µg/ml)* | *FRAP (µg AAE/mg of dry extract)* |
|---|---|---|---|
| *B. campestris* | MeOH | 38.18±1.64$^{b}$ | 76.03±1.24$^{d}$ |
| | EtOH | 24.81±1.06$^{B}$ | 70.47±1.91$^{D}$ |
| *B. juncea* | MeOH | 43.36±1.28 $^{a}$ | 135.85±2.08 $^{c}$ |
| | EtOH | 42.68±0.59$^{A}$ | 85.64±1.15$^{C}$ |
| *C. album* | MeOH | 37.01±1.04 $^{b}$ | 76.45±2.55$^{d}$ |
| | EtOH | 33.79±0.72 $^{B}$ | 67.90±2.85$^{D}$ |

Note: Values followed by different letters in a row are significantly ($p<0.05$) different from each other. The superscript small and capital letters are used for MeOH and EtOH extract solvent respectively.

In DPPH assay, higher scavenging or inhibition percentage of free radicals means the more hydrogen atom-donating capacity which indicates a high potentiality of antioxidant activity [22]. DPPH scavenging activity of methanolic and ethanolic extracts of *B. campestris, B. juncea* and *C. album* was estimated at concentrations of 1000 µg/ml. Methanolic extracts showed a higher scavenging percentage (%I) than the ethanolic extracts of selected GLVs (Table-02). The highest percentage of inhibition was found in *B. juncea* for both methanolic extracts (43.36%) and ethanolic extracts (42.68%). Scavenging percentage of *B. campestris* was 38.18% and 24.81% in MeOH and EtOH respectively. About 37.01% and 33.79% scavenging activity was found in the methanolic and ethanolic extracts of *C. album* whereas Kumar and Kumar, 2009 [23] found that aqueous extract of *C. album* exhibited 38.9% and 86.42% scavenging effect at a concentration of 1000 µg/ml and 2500 µg/ml respectively.

Ferric reducing antioxidant power (FRAP) assay determines the reducing capability by monitoring the reduction of Ferric ($Fe^{3+}$) to ferrous ($Fe^{2+}$) ion [24]. The reducing power was determined as µg AAE/mg of dry extract. Table-02 indicated that the reducing power of selected leafy vegetables ranged from 70.47 to 135.85 µg AAE/mg of dry extract where *B. juncea* was found to possess maximum reducing power in both methanolic (135.85 µg AAE/mg) and ethanolic (85.64 µg AAE/mg) extracts. In this study, ferric reducing antioxidant power of *B. campestris* and *C. album* in methanolic extracts were very similar (76.03 µg AAE/mg and 76.45 µg AAE/mg respectively) whereas in ethanolic extracts the reducing power of *B. campestris* was 70.47 µg AAE/mg and *C. album* was 67.90 µg AAE/mg. The FRAP value of *B. campestris* reported by An-Na Li *et.al.* [25] was 24.26lmol Fe (II)/g.

**Table 03: Correlations among TPC, DPPH and FRAP**

| Control | Variables | | DPPH | FRAP |
|---|---|---|---|---|
| Total phenolic content (TPC) | DPPH | Correlation | 1.000 | .464 |
| | | Significance (2-tailed) | . | .061 |
| | | df | 0 | 15 |
| | FRAP | Correlation | .464 | 1.000 |
| | | Significance (2-tailed) | .061 | . |
| | | df | 15 | 0 |

*Correlation is significant at the 0.01 level (two tailed test)

The correlation coefficient value is 0.464 which implies there is positive correlation association between the variables.

**Table 04: Correlations among TFC, DPPH and FRAP**

| Control | Variables | | DPPH | FRAP |
|---|---|---|---|---|
| Total Flavonoid Content (TFC) | DPPH | Correlation | 1.000 | -.044 |
| | | Significance (2-tailed) | . | .866 |
| | | df | 0 | 15 |
| | FRAP | Correlation | -.044 | 1.000 |
| | | Significance (2-tailed) | .866 | . |
| | | df | 15 | 0 |

*Correlation is significant at the 0.01 level (two tailed test)

The correlation coefficient value is (-) 0.044 which implies there is a negative correlation association between the variables.

## 4. Conclusion

The findings of our study reveal that the selected GLVs were found to acquire potential antioxidant activity as they were effective in free radical scavenging and ferric reducing activities. Among three leafy vegetables, *B. juncea* has been found to possess better antioxidant activity and total flavonoid content while maximum total phenolic content was detected in *B. campestris*. In every measurement methanolic extract showed better results than ethanolic extracts. The observations of this study could increase the interest in the GLVs for nutritional and pharmaceutical applications and consumption of these vegetables might prevent free radicals induced human diseases. However, further investigations are required to explore details about the phytochemicals in these GLVs and their antioxidant properties.

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