Inter-Varietal Variation in Phenolic Profile, Sugar Contents, Antioxidant, Anti-Proliferative and Antibacterial Activities of Selected Brassica Species

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Abstract: The main objective of this research work was to evaluate the variation in nutritional profile, antioxidant, anti-proliferative and antibacterial activities of selected species of Brassica. Five locally grown Brassica species (cauliflower, broccoli, red cabbage, white cabbage and Chinese cabbage) were collected from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. Polyphenolic rich extracts of these Brassicaceae species were prepared by Soxhlet extraction technique using ethanol. Phenolic acids, flavonoids and sugar contents of the investigated species were determined and quantified by RP-HPLC. Antioxidant activity was carried out by measurement of total phenolic contents (TPC), total flavonoid contents (TFC), reducing potential and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity. Anti-proliferative activity of all the extracts was determined by MTT assay on lung cancer cell line A549. Antibacterial activity was tested against the two bacterial strains, i.e., Bacillus cereus (B. cereus) and Escherichia coli (E. coli). HPLC analysis revealed the presence of gallic acid, p-coumaric acid, chlorogenic acid and benzoic acid as the major phenolic acids, whereas catechine was the major flavonoid in most of the extracts. The TPC ranged from 9.7 to 32.8 mg/g of dry plant material, measured GAE and TFC ranged from 7.7 to 23.7 mg/g of dry plant material, measured as CE. Higher TPC and TFC were found in red cabbage extract followed by cauliflower, broccoli, white cabbage and Chinese cabbage. Red cabbage extract also showed higher DPPH radical scavenging activity (IC50 = 2.3 µg/mL) followed by cauliflower, broccoli, white cabbage and Chinese cabbage. Maltodextrose was the major sugar followed by fructose in all species of Brassica. Promising anti-proliferative and antibacterial activities were also recorded by the selected Brassica extracts.

Keywords: antioxidant capacity; Brassicaceae; anti-proliferative; antibacterial activities

1. Introduction

Functional food is a food product rich in natural components with particular physiological and health promoting benefits [1]. Functional foods have potential application against oxidative stress and various degenerative diseases including cancer, heart diseases, diabetes and many other chronic diseases [2,3]. Some foods which have high polyphenolic contents are also considered to be potential functional foods due to antimicrobial, immune modulatory and anti-inflammatory and antioxidant activities [4,5]. The polyphenols are
secondary metabolites with an ideal structural chemistry for the free radical scavenging activity and are proved to be health beneficial antioxidants [6].

The *Brassica* genus is considered to be the most important genera in the Brassicaceae family and consists of a different group of species incorporating vegetables and oilseed crops along with a broad range of agronomic traits [7,8]. Brassicaceae vegetables are considered to be a good dietary source and grown worldwide including several vegetable cultivars called cole crops. These cole crops contain many subspecies, such as cauliflower (*Brassica oleracea* L. var. *botrytis*), broccoli (*Brassica oleracea* L. var. *italica*), red cabbage (*Brassica oleracea* L. var. capitata f. Rubra), kohlrabi (*Brassica oleracea* var. gongylodes), Chinese cabbage (*Brassica rapa*, subspecies *Pekinensis*) and white cabbage (*Brassica oleracea* L. var. *capitata*). These vegetables consist of genus, species and subspecies which show huge differences in their intravital, genetic and morphological changes [9]. Due to varietal, genetic and morphological variations, different species and subspecies may change their internal characteristics and these changes may be noticed in primary and secondary metabolites [10]. Thus, there is a need to characterize each variety and cultivar and report primary and secondary metabolites by applying state of the art techniques. *Brassica* vegetables are also used in traditional medicines as well as in decreasing risk of several human diseases due to cardioprotective, anti-aging, antibacterial, neuroprotective, gastrointestinal disorder, antidiabetic and anti-cancer potentials [11,12]. The chemo-preventive characteristics of *Brassica* vegetables are due to the higher amount of phytochemicals, such as polyphenols, which are potential natural antioxidants [13]. Thus, the consumption of different types of Brassicaceae vegetables exerts a beneficial effect to prevent different types of degenerative diseases [13].

Thus, the present study was aimed to investigate inter-varietal variation in the total phenolic and total flavonoid contents and free radical scavenging capacity, antioxidant, antibacterial and anti-proliferative activities of some selected species of *Brassica* genus, i.e., cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli. Sugar contents were determined by high performance liquid chromatography (HPLC). Moreover, the identification and quantification of phenolic acids and flavonoids of all selected species was carried out in single run by reverse phase high performance liquid chromatography (RP-HPLC).

2. Materials and Methods

2.1. Collection and Pretreatment of Plant Materials

Cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli were obtained from the Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. The plant materials were further identified and authenticated by a taxonomist from the Department of Botany, Government College University, Faisalabad, Pakistan. The edible parts of all of these varieties were washed, cut into equal small pieces and dried under shade at room temperature. The dried samples were ground into fine powder (80-mesh) by a commercial electrical grinder and stored in an air tight polythene bag until further use for extraction. All the chemicals used in this study were of analytical grade and purchased from Sigma Chemical Co. (St Louis, MO, USA) and Merck (Darmstadt, Germany).

2.2. Extract Preparation

Extracts of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli were prepared by using Soxhlet extraction technique [14]. Briefly, 50 g of dry powder of each sample was taken in a thimble and extracted for four extraction cycles in 300 mL ethanol solvent in a 500 mL Soxhlet apparatus. The obtained extracts were filtered by Whatman filter paper (No. 1) and concentrated using rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd., Tokyo, Japan) under reduced pressure. The crude extracts were weighed and yields were calculated. The extracts were stored in a refrigerator at −4 °C until further use.
2.3. Quantification of Phenolic Acids and Flavonoids by HPLC

Quantification of phenolic acids and flavonoids compounds was carried out by RP-HPLC, as reported by Dominic et al. [15] with few modifications. The sample was freshly prepared by redissolving the 10 mg dried extract of each sample into 1 mL methanol and filtered. The HPLC profile for the phenolic acids and flavonoids was carried out by Chromera HPLC (Perkin Elmer, 520 South Main St., Suite 2423, Akron, OH, USA), a binary solvent gradient system equipped with a C-18 column (250 × 4.6 mm internal diameter, 5 µm particle size), a UV/Vis LC detector and a non-linear gradient system containing two solvent system like solvent A (methanol: acetonitrile; 30:70) and solvent B (0.5% glacial acetic acid and distilled water). The UV/Vis spectra were recorded at 275 nm where the maximum absorption was observed. Quantification was done by standard addition method by matching the retention times and spikes of known concentrations of standards (2.5, 5, 10 ppm).

2.4. Quantification of Sugar Profile by HPLC

Quantification of major sugars were done by HPLC using an already reported method [15]. Dry powder (1 g) of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli was homogenized with 10 mL distilled water. All the solutions were centrifuged for 10 min at 3000 rpm and the upper layer was separated and the supernatant was filtered by 0.25 µm Millipore filters and dried at room temperature. The dried extracts for sugar analysis were stored in a refrigerator for further use. An Agilent 1100-series HPLC system equipped with vacuum degasser (G1379a), a quaternary pump (G1311A) and Bio-Rad Aminex HPX-87K 300 × 7.8 mm column (Cat # 1250142) with the BIO-Rad Guard column having ultra-pure water as a mobile phase with flow rate of 0.50 mL/min. Individual sugars were detected by maintaining the refractive index of the detector at 40 °C. For detection of carbohydrates, 20 µL of the sample was injected into the chromatographic column. Maltose, glucose, maltodextrine and fructose were detected and identified by comparison of their retention times and peak areas with the calibration curve of standards [15].

2.5. Evaluation of Total Phenolic Contents

Total phenolic contents from ethanol extract of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli were calculated using Folin–Ciocalteu reagent as reported by Iftikhar et al. [16]. Briefly, 0.5 mL of 10 mg/mL extract concentration was mixed with 0.5 mL Folin–Ciocaltu reagent and 7.5 mL distilled water was also added in a test tube. Then the mixture was kept at room temperature for 10 min. After this 1.5 mL of 20% sodium carbonate solution (w/v) was added into the reaction mixture. The mixture was then heated in a water bath for 20 min at 40 °C and cooled in an ice bath. Absorbance was measured at 755 nm by spectrophotometer (Bio Tek Instrument, Inc., Winooski, VT, USA). Total phenolic contents were calculated by using gallic acid calibration curve (0.1, 0.2, 0.4, 0.8 mg/mL). The total phenolic contents were reported as mg/g of DW of plant material; measured as gallic acid equivalent (GAE).

2.6. Evaluation of Total Flavonoids Contents

Total flavonoids contents from ethanol extracts of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli were estimated by the method reported by Iftikhar et al. [16]. According to this method, 1 mL of extract solution (10 mg/mL extract concentration) was poured into a 10 mL volumetric flask followed by the addition of 5 mL distilled water. Then, 0.3 mL of 5% NaNO₂ (w/v) was added. After 5 min, 6 mL of 10% AlCl₃ (w/v) was also added. After another 5 min, 2 mL of 1 M sodium hydroxide was added and finally the volume was increased to 10 mL with distilled water. The absorbance was recorded at 510 nm by spectrophotometer (Bio Tek Instrument, Inc., Winooski, VT, USA). Total flavonoid contents were calculated by comparing calibration curves of catechene (0.1, 0.2, 0.4, 0.8 mg/mL). Total flavonoids contents were reported in mg/g of DW of plant material and measured as catechene equivalent (CE).
2.7. Radical Scavenging Activity (DPPH)

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was done as reported by Iftikhar et al. [16]. Furthermore, 0.5 mL of ethanol extracts (0.1–1000 µg/mL) of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli were mixed with 3 mL of DPPH solution (90 µM). The reaction solution was incubated for 30 min at room temperature and the absorbance was recorded at 517 nm. The DPPH solution was used as a blank and butylated hydroxytoluene (BHT) was used as a positive control. The percent inhibition was measured by the following given formula:

\[
\text{Radical scavenging (\%)} = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100
\]

where, \( A_{\text{blank}} \) is the absorbance of DPPH solution, while \( A_{\text{sample}} \) is the absorbance of sample solution.

2.8. Reducing Potential

Reducing potential of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli extracts were measured to investigate the antioxidant potential as reported by Djendi et al. [17]. Briefly, 5 mL of sodium phosphate buffer (6.6 pH and 0.2 M) was mixed with 1 mL ethanol extract of cauliflower, broccoli, red cabbage, white cabbage and Chinese cabbage followed by the addition of 5 mL of 1% potassium hexacyanoferrate solution. Whole mixture was then incubated at 50 °C for 20 min in a water bath. After this, 5 mL of 1% trichloroacetic acid was added in the mixture and centrifuged at 3000 rpm and 5 °C in a refrigerator centrifuge (CHM-17; Kokusan Deriki, Tokyo, Japan) for 10 min. Almost 5 mL from the upper layer of supernatant was separated and diluted with 250 µL distilled water. After adding 1 mL of 0.1% aqueous solution of FeCl₃ to the mixture, the absorbance was recorded at 700 nm by a double beam spectrophotometer (Hitachi U-2001, Hitachi, Tokyo, Japan). BHT was used as a reference standard.

2.9. Antibacterial Activities

Antimicrobial activity of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli extracts was determined by using a disc and well diffusion susceptibility method against the two bacterial strains (Gram positive Bacillus cereus and Gram negative Escherichia coli) as reported previously [14]. The medium plates were seeded with the 12–48-h-old cultured microbial inocula (0.5 McFarland used as a reference standard (2 × 10⁷ CFU/mL) was homogenously spread over the agar surface in the Petri plate by using a sterile cotton swab. The stock solution of each extract (40 mg/mL) was prepared in DMSO (dimethyl sulfoxide) solvent. The wells (8 mm in diameter) were made into the agar plate and the 20 µL extract solutions were poured into the relevant wells. An antibiotic named gentamycin (20 µL) was used as a positive control while the DMSO (20 µL) was used as a negative control. Plates were incubated for 24 h at 37 °C and zone of inhibition was examined in mm.

2.10. Cell Culture

The human A549 cancer cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM) enriched with 10% fetal bovine serum (FBS), 100 µg/mL streptomycin and 100 IU/mL penicillin. The antiproliferative cells were grown in the CO₂ incubator with 5% supply of CO₂ at 37 °C [18].

MTT Assay

Anti-proliferative activity of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli extracts was determined by MTT assay as reported by Rasul et al. [18]. The MTT assay is a reliable, sensitive and colorimetric assay and is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. Briefly, 96-wells were
seeded with the A549 cells and after 12–18 h, 0.5 µL from the stock solution, i.e., 40 mg/mL of extracts, were added and diluted up to 100 µL and incubated for 48 h before the addition of 10 µL MTT (5 mg/mL). The absorbance was noted at 490 nm on an ELISA plate reader (Thermo Fisher Scientific, Waltham, MA USA). The cell inhibition percentage was calculated by using the following formula:

\[
\text{Percentage cell inhibition} = 100 \times \left( \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control}} \right)
\]

2.1. Statistical Analysis

Three samples of each plant species were collected and analyzed individually in triplicate and data were represented as the mean values ± standard deviation (SD). The statistical analysis of presented data was carried out by the analysis of variance (ANOVA) using STATISTICA 5.5 (Stat Soft Inc., Tulsa, OK, USA) software and the probability value of \( p \leq 0.05 \) showed a statistical significance difference.

3. Results and Discussion

3.1. Extracts Yield (g/100 g)

Extracts yield, obtained by Soxhlet extraction method of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli is given in the Table 1. Cauliflower showed the maximum yield (12.8 g/100 g of dry matter) while Chinese cabbage showed the minimum yield (11.6 g/100 g of dry matter). The variation in the extract yield of different Brassica species was found to be non-significant (\( p \geq 0.05 \)).

<table>
<thead>
<tr>
<th>Assays</th>
<th>Cauliflower</th>
<th>Broccoli</th>
<th>Red Cabbage</th>
<th>White Cabbage</th>
<th>Chinese Cabbage</th>
<th>Butylated Hydroxytoluene (BHT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (g/100 g)</td>
<td>12.8 ± 0.64 (^a)</td>
<td>12.6 ± 0.63 (^a)</td>
<td>12.7 ± 0.63 (^a)</td>
<td>11.9 ± 0.58 (^a)</td>
<td>11.6 ± 0.59 (^a)</td>
<td>—</td>
</tr>
<tr>
<td>TPC (mg/g dry material)</td>
<td>29.9 ± 1.7 (^c,d)</td>
<td>27.2 ± 1.6 (^c)</td>
<td>32.8 ± 1.9 (^d)</td>
<td>16.4 ± 0.9 (^b)</td>
<td>9.7 ± 0.6 (^a)</td>
<td>—</td>
</tr>
<tr>
<td>TFC (mg/g dry material)</td>
<td>20.9 ± 1.2 (^c)</td>
<td>18.6 ± 1.1 (^c)</td>
<td>23.7 ± 1.1 (^d)</td>
<td>9.9 ± 0.6 (^b)</td>
<td>7.7 ± 0.4 (^a)</td>
<td>—</td>
</tr>
<tr>
<td>DPPH IC(_{50}) (µg/mL)</td>
<td>3.1 ± 0.18 (^c)</td>
<td>3.4 ± 0.20 (^c)</td>
<td>2.3 ± 0.13 (^b)</td>
<td>3.7 ± 0.22 (^d)</td>
<td>4.6 ± 0.27 (^a)</td>
<td>1.5 ± 0.09 (^a)</td>
</tr>
</tbody>
</table>

The values are mean ± standard deviation of three independent observations. Different letters in superscript show significant differences among selected Brassica species.

3.2. Quantification of Phenolic acids and Flavonoids by HPLC

Phenolic profile of different extracts of Brassica species were identified by RP-HPLC method discussed in Table 2 and Figure 1. Cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli extracts showed a broad variation in polyphenolic compounds. Catechine and quercetin were the major flavonoids while gallic acid, \( p \)-coumaric acid, chlorogenic acid and benzoic acid were the major phenolic acids found in different Brassica species. Red cabbage extract contained the highest gallic acid contents (113.21 mg/100 g DW) whereas Chinese cabbage showed the lowest contents of gallic acid (3.00 mg/100 g DW). However, \( p \)-coumaric acid, chlorogenic acid and benzoic acid were also major phenolic compounds found in red cabbage extract (53.29, 25.7, 27.21 mg/100 g DW) correspondingly. \( P \)-hydroxy benzoic acid was detected in all extracts of selected species of Brassica genus cauliflower, broccoli, red cabbage, white cabbage and Chinese cabbage (18.35, 0.08, 3.22, 0.61 and 0.04 mg/100g DW), respectively. Ellagic (3.21 mg/100g DW) was only detected in red cabbage. Catechine was found in high concentration in red cabbage extract (151.8 mg/100 g DW). Different Brassica vegetables contained different amounts of various phenolic acids and flavonoids and the variations were found to be significant (\( p \leq 0.05 \)).
All the species of *Brassica* have sufficient amounts of phenolic acid and flavonoids that are potential antioxidants. Podsedek et al. [19] reported that *Brassica* vegetables are good sources of phenolic acids and flavonoid compounds and their derivatives, such as red cabbage, consists of different acylated groups (sinapic acid and ferulic acid), whereas the white cabbage contains more than 20 phenolic compounds, such as caffeic acid and p-coumaric acid. Another report published by Upadhyay et al., [20] showing different results than our findings, explained that only four phenolic acids were detected in red cabbage rutin (102.14), sinapic (30.20), salicylic acid (6.58) and cinnamic acid (38.23); green cabbage contains gentisic acid (2.50), rutin (1.34), sinapic acid (3.52) and cinnamic acid (0.65); broccoli contains gentisic acid (83.17), sinapic acid (7.37) and cinnamic acid (0.37); while the cauliflower extract contains gentisic acid (35.50), rutin (39.07), vanillic acid (3.60), sinapic acid (4.66) and cinnamic acid (1.45); and five phenolic acids were detected in white cabbage. The variations may be due to the difference in drying parameters, seasonal and geographical conditions and extraction technique [16].

<table>
<thead>
<tr>
<th>Compounds</th>
<th>mg/100 g of Dry Plant Material</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cauliflower</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>18.35 ± 1.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>p-hydroxyl benzoic acid</td>
<td>0.09 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Catechin</td>
<td>2.94 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.55 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.02 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>0.04 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>-</td>
</tr>
<tr>
<td>p-coumaric acid</td>
<td>-</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>7.44 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.06 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>0.05 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>0.05 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quercetin</td>
<td>4.11 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values are mean ± standard deviation of three independent observations. Different letters in superscript show significant differences among selected *Brassica* species.

### 3.3. Quantification of Sugars by HPLC

Free sugars, such as glucose, fructose, maltodextrose and maltose, were reported in selected species of *Brassica* genus, such as cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli extracts, are shown in Table 3. Sugar contents (glucose, fructose, maltodextrose and maltose) were varied according to the *Brassica* species and are ranged from 1.48 to 86.73%. In this study, maltodextrose and fructose were found to be the predominant sugars while maltose and glucose were present in the lowest concentrations in cauliflower, broccoli, red cabbage, white cabbage and Chinese cabbage. Among all sugar contents identified, maltodextrose content was found to be the highest in red cabbage extract (86.73%) while the lowest content of maltodextrose was found in white cabbage (52.52%). Statistical analysis revealed significant (*p* ≤ 0.05) variation in sugar profiles of selected species. Our results are in agreement with the results reported by [21] who reported the presence of glucose, fructose and sucrose in broccoli and cauliflower. In another report [22], a comparison was made between the free sugar level of white cabbage and broccoli which showed the presence of glucose, fructose and sucrose in white cabbage and broccoli. Another report [19] was also published on cabbage showed the presence of glucose, sucrose and fructose. Our results are contrary to the previous reports because sometimes genetic influence takes part in the results, changing of free sugars depending on the climatic and seasonal variation. Inflorescence also affects the variation in the free sugars and sucrose is considered to be the responsible sugar for the sweeter taste [22].
Figure 1. Typical HPLC chromatogram, showing the separation of phenolic acids and flavonoids in red cabbage extract.

Table 3. Sugar composition of selected *Brassica* species.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cauliflower</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>83.31 ± 4.99</td>
</tr>
<tr>
<td>Maltose</td>
<td>2.32 ± 0.14</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.89 ± 0.17</td>
</tr>
<tr>
<td>Fructose</td>
<td>11.47 ± 0.68</td>
</tr>
</tbody>
</table>

The values are mean ± standard deviation of three independent observations. Different letters in superscript show significant differences among selected *Brassica* species.

3.4. Evaluation of Antioxidant Activities

3.4.1. Total Phenolics and Total Flavonoids Contents

The data regarding the inter-varietal variation in the amount of TPC and TFC are discussed in Table 1. Total phenolic contents were expressed in mg/g of dry material measured as gallic acid equivalent (GAE). Total phenolic contents were ranged from 9.7 to 32.8 mg/g of dry weight of plant material measured as GAE. Maximum TPC were observed in red cabbage extract (32.8 mg/g of dry weight of plant material measured as...
GAE) while the minimum TPC were observed in Chinese cabbage extracts (9.7 mg/g of dry weight of plant material measured as GAE). Total flavonoid content, expressed in mg/g of dry material, was measured as catechine equivalent (CE). TFC were ranged from 7.7 to 23.7 mg/g of dry weight of plant material measured as CE. Maximum TFC were also found in red cabbage extract (23.7 mg/g of dry weight of plant material measured as CE) while the minimum was found in Chinese cabbage extracts (7.7 mg/g of dry weight of plant material measured as CE) in comparison with the five selected varieties of Brassica genus as shown in the Table 1. Statistical analysis exhibited that the different extracts of selected species of Brassica genus exert a potential effect ($p < 0.05$) on total phenolic contents and total flavonoids contents. Many studies confirmed that the composition and amount of phenolics and flavonoids contents varies among different species and within the sub-cellular and tissues level [23]. Our results are contrary to the results reported earlier on the TPC and TFC. A previous report published by Podsedek et al. [19,24], reported the TPC (29.13 and 21.38) and TFC (17.44 and 12.33) for red cabbage and white cabbage extract, respectively. Another report, published by Li et al. [25], showed that TPC of Chinese cabbage, broccoli, white cabbage and cauliflower extract were 0.21, 1.06, 0.35 and 0.57 mg/g dry weight, respectively. The difference in the TPC and TFC in our results and in the previous published reports might be due to the difference in extraction temperature, handling techniques, agro-climatic, geographical and seasonal condition in which they are grown [20].

3.4.2. Radical Scavenging Activity (DPPH)

Free radical scavenging activity of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli extracts increased in a concentration dependent manner and the concentration of extracts showed 50% scavenging (IC$_{50}$) as shown in Table 1. Radical scavenging activity (IC$_{50}$) of cauliflower, broccoli, red cabbage, white cabbage and Chinese cabbage extracts was ranged from 2.3 to 4.6 µg/mL. The minimum IC$_{50}$ value was obtained by red cabbage extract (2.3 µg/mL) and showed an excellent radical scavenging activity while the maximum IC$_{50}$ value was obtained by Chinese cabbage extract (4.6 µg/mL) and showed lower radical scavenging capacity. The synthetic antioxidant, BHT, showed a better radical scavenging activity with the lowest IC$_{50}$ value (1.5 µg/mL). Significant variations ($p < 0.05$) in radical scavenging activity were observed among selected species of Brassica genus. Red cabbage showed higher TPC and TFC and showed better radical scavenging activity [25]. Previous results showed that white cabbage, cauliflower, broccoli, Chinese cabbage and red cabbage extracts have IC$_{50}$ values of 1.64, 2.71, 3.85, 1.32 and 70.52 µg/mL, respectively [24,25].

3.4.3. Reducing Potential

Antioxidant activity of different species of Brassica genus is also determined in terms of reducing power and results are reported in Figure 2. The reducing potential of cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli extracts was determined up to 0–10 mg/mL extract concentration. The antioxidant activity in terms of reducing power increases with respect to the increasing concentration. On comparing the reducing potential of all these vegetables, red cabbage showed maximum reducing power while the Chinese cabbage showed minimum reducing potential. The high reducing power of red cabbage might be ascribed to the fact that reducing power is strongly correlated with the antioxidant activities, i.e., there is a good association between phenolic contents and reducing power [20]. As the red cabbage showed strong total phenolic contents, thus it also exhibited the maximum reducing power. Our results are contrary to the previous reports published by [19] who reported the good correlation of reducing power between cabbage and cauliflower. Another report published by [26] explained the reducing power of fermented Chinese cabbage.
Escherichia coli (Gram negative) is presented in Table 4. All the species exhibited moderate antibacterial activity (Figure 3). All the extracts showed higher activity against the B. cereus strain. Red cabbage extract showed the highest activity against B. cereus (16.3 mm) followed by cauliflower (15.0 mm) and broccoli (14.4 mm). In the positive control, gentamicin showed highest activity of the extracts (18.1 mm), against E. coli, white cabbage extract showed highest activity, i.e., 14.4 mm, and broccoli showed the least. Our results are comparable with the published reports that explained that red cabbage extract showed good antibacterial activity [27,28].

### Table 4. Antibacterial activity of ethanolic extracts of selected species of Brassica.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Bacillus cereus (mm)</th>
<th>Escherichia coli (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cauliflower</td>
<td>15.0 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.0 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Broccoli</td>
<td>14.4 ± 0.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;6 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Red Cabbage</td>
<td>16.3 ± 0.71&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>12.3 ± 0.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>White Cabbage</td>
<td>10.5 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.4 ± 0.55&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chinese Cabbage</td>
<td>8.23 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.7 ± 0.41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>18.1 ± 0.91&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.21 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values are mean ± standard deviation of three independent observations. Different letters in superscript show significant differences among selected Brassica species.

Figure 2. Reducing power of ethanol extract of selected Brassica species.

3.5. Antibacterial Activity

Antibacterial activity of cauliflower, broccoli, red cabbage, white cabbage and Chinese cabbage extracts against two bacterial strains, i.e., Bacillus cereus (Gram positive) and Escherichia coli (Gram negative) is presented in Table 4. All the Brassica species exhibited moderate antibacterial activity (Figure 3). All the extracts showed higher activity against the B. cereus strain. Red cabbage extract showed the highest activity against B. cereus (16.3 mm) followed by cauliflower (15.0 mm) and broccoli (14.4 mm). In the positive control, gentamicin showed highest activity of the extracts (18.1 mm), against E. coli, white cabbage extract showed highest activity, i.e., 14.4 mm, and broccoli showed the least. Our results are comparable with the published reports that explained that red cabbage extract showed good antibacterial activity [27,28].

Figure 3. Typical plates showing the inhibition zones of ethanol extract of selected Brassica species.
3.6. Anti-Proliferative Activity of Extracts

Anti-proliferative efficacy of cauliflower, broccoli, red cabbage, white cabbage and Chinese cabbage extracts against human A549 lung cancer cell line were evaluated and results are presented in the Table 5. All the extracts showed >50% inhibition, except Chinese cabbage extract at 40 mg/mL extract concentration against the human A549 cancer cell line. Red cabbage extract showed the highest anti-proliferative effect (58.19%) followed by cauliflower extract (54.12%). There are only few studies that have reported on the anti-proliferative activity against the human cancer cell line A549 on the selected Brassica vegetables. In comparison with the previous reports, ref. [29] explained the anti-proliferative activity of cauliflower, broccoli and cabbage from which broccoli showed the highest anti-proliferative activity with 30% cell inhibition. Our results are comparable to the results reported by [24] who explained that the aqueous extract of white cabbage showed 46.4% cytotoxicity and explained that the Brassicaceae vegetables showed therapeutic properties due to the presence of organic sulfur containing compounds, sulforaphane and isothiocyanates [30]. Brassicaceae vegetables may show therapeutic properties due to the presence of organic sulfur containing compounds and isothiocyanates [31].

Table 5. Anti-proliferative activity of ethanol extract of selected Brassica species against human A549 lung cancer cell line.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>% Inhibition (40 mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cauliflower</td>
<td>54.12 ± 3.3 b,c</td>
</tr>
<tr>
<td>Broccoli</td>
<td>53.16 ± 2.3 b,c</td>
</tr>
<tr>
<td>Red Cabbage</td>
<td>58.19 ± 1.6 c</td>
</tr>
<tr>
<td>White Cabbage</td>
<td>51.93 ± 2.9 b</td>
</tr>
<tr>
<td>Chinese Cabbage</td>
<td>30.3 ± 1.6 a</td>
</tr>
</tbody>
</table>

The values are mean ± standard deviation of three independent observations. Different letters in superscript show significant differences among selected Brassica species.

4. Conclusions

It can be concluded from the results, that all five varieties of Brassica genus studied (cauliflower, red cabbage, Chinese cabbage, white cabbage and broccoli) are rich sources of polyphenols. Among all B. oleracea vegetables, red cabbage (Brassica oleracea var. Capitata F. rubra) proved to be a good source of potential dietary antioxidants and thus showed high antioxidant activity. HPLC analysis showed the variation in the phenolic acid and flavonoid contents in all the Brassica species analyzed. The Brassicaceae species are also proved to have moderate anti-proliferative and antibacterial potential. Brassica vegetables are excellent source of polyphenols and may be used for pharmaceutical applications and it may be used as the food supplements.

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