COMPARATIVE STUDY BETWEEN CELERY LEAVES AND BROCCOLI FLOWERS FOR THEIR CHEMICAL COMPOSITION AND AMINO ACIDS AS WELL AS PHENOLIC AND FLAVONOID COMPOUNDS

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ABSTRACT: The objective of such research was to study the chemical composition as well as phenolic and flavonoid compounds in dried celery leaves and broccoli flowers. Celery leaves contained moisture 88.72% fresh weight but in dried weight total carbohydrates, crude protein, total lipids, crude fiber and total ash contents were 36.8%, 19.47%, 2.1%, 19.85% and 20.98% respectively. While broccoli flowers contained moisture 89.43% fresh weight but in dried weight total carbohydrates 44.8%, crude protein 24.79%, total lipids 5.8%, crude fiber 15.29% and total ash contents were 44.8%, 24.79%, 5.8%, 15.29% and 7.67% respectively. Celery leaves contained 17 amino acids among them aspartic acid, glutamic acid, valine, alanine, leucine and phenylalanine were the major ones comparing with 17 amino acids in broccoli flowers which were glutamic acid, aspartic acid, alanine, arginine, valine and leucine in high amounts. Total phenolic and flavonoid compounds in celery leaves were 30.3 and 18.5 mg/g comparing with 38.4 and 22.5 mg/g of them in broccoli flowers respectively. HPLC results showed that celery leaves contained 22 of phenolic compounds, among them as well as pyrogallol, E-vanillic, ellagic, benzoic, chlorogenic, ferulic and P-OH-benzoic acids were the major active constituents comparing with 23 of phenolic compounds in broccoli flowers which contained as well as pyrogallol those benzoic, P-OH-benzoic, salycilic, E-vanillic and chlorogenic acids were the major of phenolic compounds. Celery leaves contained 17 of flavonoids the highest amounts of them were rosmarinic, luteolin, hesperidin and apig.6-rhamnose 8-glucose comparing with 21 of flavonoids in broccoli flowers which accounted the highest values for luteo.6-arbinose 8-glucose, hesperidin and rosmarinic acid. Phenolic and flavonoid compounds were detected and determined by HPLC.

Key words: Celery – Broccoli – Amino acids – phenolics – Flavonoids – Reducing power.

INTRODUCTION

Celery (Apium graveolens) is a herbal member of the family Apiaceae. It is grown as leafy vegetable crop and medicinal herb. In Egypt, celery is cultivated in two seasons "winter and summer". Celery cultivates as a vegetable crop for the thick, succulent, green and blanched leaf stalks which are esteemed as salad and to a lesser extent as a cooked vegetable. Celery pungent fruits are used in salads, soups, vegetable dishes, meat dishes, celery salt and fruits (Halim et al., 1990).

Medicinal plants provide an important sources of raw materials for pharmaceutical, cosmetic and food industries. Celery has been considered as a medicinal plant since it has been used as an aphrodisiac, anthelmintic, antispasmodic, carminative, diuretic, against asthma bronchitis and swollen glands as well as it provides temporary relief from rheumatism and lumbago. It used also as laxative, sedative, stimulant, and tonic and contains many of chemical compounds such as phenolics, flavonoids and vitamins such as vitamin C and B complex as well as mineral elements (Antuono et al., 2002).
Broccoli (Brassica oleracea L. var. Italica) is a member of the Brassicaceae family as a wild form of this family. Broccoli is an Italian vegetable native to the Mediterranean region (Abou El-Magd et al., 2006). Broccoli is an important vegetable crop and has high nutritional and good commercial value. Broccoli is becoming increasingly popular as fresh vegetable and as a significant source of nutritional antioxidants, such as vitamins and carotenoids, as well as biologically active dietary components, such as flavonoids, hydrocinnamic acid, and sulphur containing compounds, such as the glucosinolates (Price et al., 1998). Broccoli also is considered as a significant source of sulforaphane glucosinolates, a phytochemical compound that is believed to have strong anticarcinogenic properties. Broccoli contains other compounds exhibiting anti-cancer effects, that is protocatechuic acid, chlorogenic acid and carotenoids (Yoldas et al., 2008).

The aim of the present studies was to investigate the chemical composition and amino acids as well as phenolic and flavonoid compounds as a comparative study.

MATERIALS AND METHODS
1- Plant collection and identification.
Fresh leaves of celery plants (Apium graveolens L.) and broccoli flowers (Brassica oleracea L.) were obtained from the Agriculture Research Center in Giza, Egypt, in winter, (February 2015). The plants were identified in Horticulture Department, Faculty of Agriculture, Minoufia University. Plant samples were washed and air-dried for 24 hours, then dried at 50 °C. The dried samples were grinded into fine powder and kept in refrigerator for analysis.

2- Determination of chemical composition.
2-1: Determination of moisture content :-
Plant samples were oven dried at 105° C for 24 h by using electrical oven. The samples were weighed before and after drying and the moisture content was calculated, according to AOAC, (2000).

2-2: Determination of ash:-
Ash content was determined by ashing sample at 550°C for 6 hours according to AOAC, (2000).

2-3: Determination of crude fiber:-
Crude fiber was determined according to AOAC, (2000), that 2.0 g of sample was placed in a round bottom flask, 50 ml of 0.255 N H₂SO₄ was added and the mixture was boiled under reflux for 30 minutes. The hot solution was filtered under suction. The insoluble matter was washed several times with hot water until samples were acid free. Thereafter, samples were transferred into a flask containing 50 ml of hot 0.313 N NaOH solution. The insoluble residues were washed with hot water until base free, then dried to a constant weights at 100°C and were cooled in a desiccator and weighed (X1). The weighed samples were incinerated in a muffle furnace at 525°C for two hours, cooled in a desiccator and re-weighed (X2). The crude fibre was calculated as follows equation :
\[
\text{Crude fibe (\%)} = \frac{X_1 - X_2}{X_1} \times 100
\]
Where X1-X2 = the loss in weight by ashing.

2-4: Determination of total carbohydrate:-
A known weight (0.2 gm.) of dried samples were completely hydrolyzed for 6 hours with HCl ( 1 N ) on boiling water bath under reflux condenser. The solutions were then filtered and the filtrates were clarified by the leading and deleading method using lead acetate solution (137 gm / L) and the excesses of lead salts was precipitated using N/3 disodium hydrogen phosphate solution. The extracts were transferred into a measuring flasks (50 ml.). The combined
filtrates were completed to the mark with distilled water.

The sugars were determined according to the method of Dubois et al. (1956) as follows:

An aliquot of (1 ml.) of the sugars solution was quantitatively transferred into a test tube and treated with 1 ml. 5 % aqueous phenol solution followed by 5 ml. of concentrated sulphuric acid added by a fast delivery pipette. The blank experiment was carried out using 1 ml. of distilled water instead of the sugar solution. The absorbance of yellow-orange color was measured using spectrophotometer at wavelength 490 nm. A standard curve was prepared using known concentrations of glucose. The established curve was used to convert the colorimeter absorbance into milligrams of glucose.

2-5: Determination of crude protein:

Total nitrogen (TN) was determined (on dry weight basis) according to the modified micro-Kjeldahl method as described by the Association of Official Analytical Chemists, (AOAC, 2000). The crude protein contents were calculated using the conversion factor 6.25.

Protein % = 6.25(TN)

2-6: Extraction and determination of crude lipid:

A known weight of samples (10 g) was extracted with n-hexane for 6 hours, in Soxhlet apparatus. The solvent was evaporated and the residue was dried to a constant weight and the percentage of total lipid was calculated, according to AOAC, (2000).

2-7: Determination of amino acids:

Amino acids were determined by A.O.A.C, (2012), performic oxidation methods:

Sample equal to (10 mg) of protein was weighed in a conical flask and 5.0 ml. of performic acid was added, the flask was closed and placed in ice bath for 16.0 h, 5 ml sodium disulfite and 25 ml HCl 6.0 N were added to the oxidized mixture, the flask was placed in an oven at 110 C for 24 h, the flask was then opened using rotary evaporator to reduce the volume to 5-10 ml under vacuum at 60 °C. Adjust the pH to 2.20 with sodium hydroxide solution. Suitable volume of sodium citrate buffer (pH 2.20) was added to hydrolyse sample. After all soluble material were completely dissolved, the sample was ready for analysis. The system used for the analysis were tested using a Biochrom 30 amino acid analyzer (Biochrom Ltd., Cambridge, UK) according to manufacturer's instructions and using validated clinical protocols, including deproteinization with sulfosalicylic acid and quantitation using S-2-aminoethylcystine as internal standard. Reaction temperature between 20°C and 40°C and there are 3 injection modes full loop, partial loop and micro autosampler and we use 200μL volume of samples loop supplied as standard.

2-8: Determination of phenolic compounds:

The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg gallic acid equivalents (GAE)/g dry weight. 10 mL of samples were extracted in methanol. 0.5 mL of each sample and standard were introduced into test tubes and mixed with 2.5 mL of a 10 fold dilute Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The tubes were covered tightly and allowed to stand for 30 minutes at room temperature before the absorbance which was read at 760 nm spectrometrically. (Kim et al., 2003).

2-9: Determination of total flavonoids compounds:

The total flavonoids were determined using the method reported by Dewanto et
Ashoush, et al., (2002). Briefly, an aliquot of 250 μL of each extract or a standard solution was mixed with 1.25 mL of deionised water followed by 75 μL of a 5% NaNO₂ solution. After 6 min, 150 μL of 10% AlCl₃ · 6H₂O solution was added to each mixture. After 5 min, 0.5 mL of 1 M NaOH was added, and the total volume was adjusted to 3.0 mL with deionised water. Catechin was used as a standard using absorbance at 510 nm, which was corrected using a blank, the results were expressed as mg of catechin equivalents (CE)/g dry weight.

2-10: Fractionation and quantitative determination of phenolic compounds by HPLC.
Phenolic compounds were fractionated and determined by HPLC according to the method of Goupy et al., (1999) as follow: 5 g of samples were extracted by methanol and centrifuged at 10000 rpm for 10 min and supernatant was filtered through a 0.2 μm millipore membrane filter then 1-3 ml was collected in a vial and we use 200 μl for injection in HPLC Hewilet Packared (series 1050) equipped with autosampling injection, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1050). Hewlett Packard using a column Alltima C18, 5mm (150mm x 4.6mm Alltech). The column temperature was maintained at 35 °C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Phenolic acid standards from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculate phenolic compounds concentration by the data of Hewllet Packared software. The data were reported with convergence limit in triplicate.

2-11: Fractionation and quantitative determination of flavonoids by HPLC.
HPLC analyses were performed with Dionex Ultimate 3000 liquid chromatograph (Germany) with four solvents delivery system quaternary pump (LPG 3400 SD) including a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20 μl loop and Chromeleon 6.8 system manager as data processor. The separation was achieved by a reversed-phase acclaim TM 120 C18 column (5 μm particle size, 4.6 x 250 mm). A modified method of Zuo et al. (2002) was used where the mobile phase contains 1% aq. acetic acid solution (Solvent A) and acetonitrile (Solvent B), the flow rate was adjusted to 0.7 ml/min, the column was thermostatically controlled at 28 °C and the injection volume was kept at 20 μl. A gradient elution was performed by varying the proportion of solvent B to solvent A. The gradient elution was changed from 10 % to 40% B in a linear fashion for duration of 28 min, from 40 to 60 % B in 39 min and from 60 to 90 % B in 50 min. The mobile phase composition comes back to initial condition (solvent B: solvent A: 10: 90) in 55 min and allowed to run for another 10 min, before the injection of another sample. Total analysis time per sample was 65 min. HPLC chromatograms were detected using a photo diode array UV detector at three different wavelengths (272, 280 and 310 nm) according to absorption maxima of analysed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. The data were reported with convergence limit in triplicate.

RESULTS AND DISCUSSION.
1: Chemical composition contents of celery leaves and broccoli flowers:
The obtained results in Table (1) indicate that celery leaves contain moisture 88.72 % fresh weight but total carbohydrates 36.8 %, crude protein 19.47 %, total lipids 2.18 %, crude fiber 19.85 %,and total ash 20.98 % in dry weight. Our results are in line with those of Leung et al., (1968), Souci et al.,
Comparative study between celery leaves and broccoli flowers for ...............

(2000), Syed and Rajeev, (2012), Amnah and Alsuaibani, (2013a) and Al Jawfi et al., (2012), they found that celery leaves and stalks contained moisture 80.30 - 93.5% , crude protein 12 - 19.1% , total lipid 1.6 - 3.14% , crude fibers 14 - 20% , total carbohydrates 35 - 39% and total ash 14.2 - 19.9% respectively.

While broccoli flowers contained moisture 89.43 % fresh weight but total carbohydrates 44.8 %, crude protein 24.79 %, total lipids 5.8 %, crude fiber 15.29 %.and total ash 7.67 % in dry weight. These results are in agreement with those of Kahlon et al., (2005), Borowski et al., (2008) and Awad et al., (2012), they showed that gross chemical composition contents of broccoli were as follow : total carbohydrate 4.0 – 51.2 % , crude protein 24.1 – 33.2 % , crude fiber 12.77 – 21.1 % , total lipids 4.38 – 5.97 % , total ash 7.11 – 10.3 % ( on dry matter ) and moisture 88.32 – 91.4 % (on fresh matter).

2: Total phenolic compounds and total flavonoids of celery leaves and broccoli flowers .

Data in Table (2) showed that total phenolic and total flavonoid compounds in broccoli flowers were higher than that in celery leaves, whereas total phenolics in broccoli flowers was 38.4 mg/g and total flavonoids was 22.5 mg/g. These results are in the same line with those obtained by Grover et al., (2002) and Amnah and Alsuaibani, (2013 b) they found that total phenolics and flavonoids were 37.38 and 19.96 mg/g respectively. While the values were 30.3 mg/g and 18.5 mg/g total phenolics and total flavonoids respectively in celery leaves. These data are in agreement with those obtained by Elliot, (1999) and Jung et al., (2011) which showed that phytochemical contents in celery were 28.17 - 34.6 mg/g for total phenolics and 16.4 – 19.6 mg/g for flavonoids.

3: Amino acid analysis of celery leaves and broccoli flowers :

Data presented in Table (3) showed that non essential amino acids of celery leaves were aspartic acid was detected as the major amino acid which recorded 23.37 % followed by glycine, and serine 4.22 %, 3.03 % respectively while glutamic acid and alanine recorded 15.47 % , 5.84 % respectively . But for essential amino acids valine was the major amino acid which recorded 7.09 % followed by leucine, phenylalanine, isoleucine, lysine, threonine and methionine which recorded 5.62 % , 5.52 %, 4.11 %, 4.11 %, 3.62 % and 1.3 % respectively . For semi-essential, amino acids tyrosine, proline, arginine, cysteine and histidine recorded 4.22 %, 4.0 %, 3.78 %, 2.49 % and 2.16 % respectively. These results are in the same line with those obtained by Ballmer et al., (2000) who showed that celery leaves contained amino acids such as arginine 3.75 %, histidine 2.25 %, isoleucine 4.20 %, leucine 5.52 %, lysine 4.5 %, methionine 1.8 %, phenylalanine 5.43 %, threonine 3.55 %, tyrosine 4.31 %, valine 7.20 %, aspartic acid 23.62 %, alanine 5.74 %, glutamic acid 13.97 %, glycine 5.83 %, proline 6.67 %, serine 1.66 %.

Table (1): Chemical composition of celery leaves and broccoli flowers .

<table>
<thead>
<tr>
<th>Plants</th>
<th>Celery leaves %</th>
<th>Broccoli flowers %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>88.72</td>
<td>89.43</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>36.8</td>
<td>44.8</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>19.47</td>
<td>24.79</td>
</tr>
<tr>
<td>Total Lipids</td>
<td>2.18</td>
<td>5.8</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>19.85</td>
<td>15.29</td>
</tr>
<tr>
<td>Total Ash</td>
<td>20.98</td>
<td>7.67</td>
</tr>
</tbody>
</table>

These data are in agreement with those obtained by Elliot, (1999) and Jung et al., (2011) which showed that phytochemical contents in celery were 28.17 - 34.6 mg/g for total phenolics and 16.4 – 19.6 mg/g for flavonoids.
Table (2): Total phenolic compounds and total flavonoids content of celery leaves and broccoli flowers (mg/g dry weight).

<table>
<thead>
<tr>
<th>Plants</th>
<th>Total phenolics (mg/g)</th>
<th>Total flavonoids (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celery leaves</td>
<td>30.3</td>
<td>18.5</td>
</tr>
<tr>
<td>Broccoli flowers</td>
<td>38.4</td>
<td>22.5</td>
</tr>
</tbody>
</table>

Table (3): Amino acid analysis of celery leaves and broccoli flowers.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Celery leaves %</th>
<th>Broccoli flowers %</th>
<th>Amino Acid</th>
<th>Celery leaves %</th>
<th>Broccoli flowers %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid (ASP)</td>
<td>23.37</td>
<td>11.05</td>
<td>Isoleucine (ILE)</td>
<td>4.11</td>
<td>3.84</td>
</tr>
<tr>
<td>Threonine (THE)</td>
<td>3.67</td>
<td>3.56</td>
<td>Leucine (LEU)</td>
<td>5.62</td>
<td>5.81</td>
</tr>
<tr>
<td>Serine (SER)</td>
<td>3.03</td>
<td>4.26</td>
<td>Tyrosine (TYR)</td>
<td>4.22</td>
<td>3.23</td>
</tr>
<tr>
<td>Glutamic acid (GLU)</td>
<td>15.47</td>
<td>22.25</td>
<td>Phenylalanine (PHE)</td>
<td>5.52</td>
<td>4.82</td>
</tr>
<tr>
<td>Proline (PRO)</td>
<td>4.0</td>
<td>5.81</td>
<td>Histidine (HIS)</td>
<td>2.16</td>
<td>2.39</td>
</tr>
<tr>
<td>Glycine (GLY)</td>
<td>4.22</td>
<td>4.21</td>
<td>Lysine (LYS)</td>
<td>4.11</td>
<td>5.06</td>
</tr>
<tr>
<td>Alanine (ALA)</td>
<td>5.84</td>
<td>8.01</td>
<td>Arginine (ARG)</td>
<td>3.78</td>
<td>6.89</td>
</tr>
<tr>
<td>Valine (VAL)</td>
<td>7.09</td>
<td>6.56</td>
<td>Cystine (CYS)</td>
<td>2.49</td>
<td>1.08</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.3</td>
<td>1.17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The amino acids composition of broccoli flowers are tabulated in Table (3). From the data in such table, it could be seen that non essential amino acids were glutamic acid was the major amino acids which recorded 22.25 % followed by aspartic acid , alanine, serine and glycine which were 11.05 %, 8.01 %, 4.26 % and 4.21 % respectively. As for essential amino acids, valine was the major amino acids which recorded 6.56 % followed by leucine, lysine, phenylalanine, isoleucine, threonine and methionine which recorded 5.81 %, 5.06 %, 4.82 %, 3.84 %, 3.56 % and 1.17 % respectively. For semi-essential amino acids arginine, proline, tyrosine, cysteine and histidine recorded 6.89 %, 5.81 %, 3.23 %, 2.39 % and 1.08 % respectively. These data are in agreement with those obtained by Hu et al., (2011) who found that broccoli amino acid content was as follows methionine 1.35 %, lysine 5.32 %, cystine 1.2 %, threonine 3.28 %, leucine 5.7 %, isoleucine 3.98 %, arginine 6.25 %, histidine 3.41 %, phenylalanine 4.95 %, valine 7.19 %, aspartic acid 10.35 %, glutamic acid 20.9 %, alanine 9.04 %, proline 6.3 %, glycine 5.74 % and tyrosine 5.04 %.

From data in such table it has been found the amino acids of high amounts in celery leaves were aspartic acid, glutamic acid and aspartic acid, while that of high values in broccoli flowers were glutamic acid, aspartic acid,
Comparative study between celery leaves and broccoli flowers for alanine, arginine and valine. On the other hand, the other amino acids recorded results of convergent average.

4: Quantitative analysis of phenolic compounds in celery leaves and broccoli flowers by HPLC.

Phenolic compounds in celery leaves and broccoli flowers were analyzed by High Performance Liquid Chromatography (HPLC), and the concentrations of all tested phenolic compounds were given in Table (4).

From this table it was found that celery leaves contained pyrogallol as the main phenolic compound which reached 1037.74 ppm and showed high level of e-vanillic, ellagic, benzoic, chlorogenic, ferulic acid, P-OH-benzoic acid as well as caffeine which recorded 612.62, 392.63, 267.34, 218.79, 163.19, 125.78 and 100.61 ppm respectively.

Table (4): Quantitative analysis of phenolic compounds in celery leaves and broccoli flowers by HPLC.

<table>
<thead>
<tr>
<th>Phenolic compounds ( ppm )</th>
<th>Celery leaves</th>
<th>Broccoli flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogallol</td>
<td>1037.74</td>
<td>2898.52</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>16.51</td>
<td>2.10</td>
</tr>
<tr>
<td>4-Amino-benzoic acid</td>
<td>18.87</td>
<td>38.14</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>43.17</td>
<td>83.79</td>
</tr>
<tr>
<td>Catechin</td>
<td>48.24</td>
<td>78.51</td>
</tr>
<tr>
<td>Catechol</td>
<td>20.55</td>
<td>28.61</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>218.79</td>
<td>150.79</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>44.16</td>
<td>72.03</td>
</tr>
<tr>
<td>P-OH-benzoic acid</td>
<td>125.78</td>
<td>183.04</td>
</tr>
<tr>
<td>Caffeine</td>
<td>100.61</td>
<td>57.74</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>36.57</td>
<td>21.28</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>26.28</td>
<td>12.03</td>
</tr>
<tr>
<td>P-coumaric acid</td>
<td>16.62</td>
<td>18.02</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>163.19</td>
<td>55.11</td>
</tr>
<tr>
<td>Iso-ferulic acid</td>
<td>27.87</td>
<td>0.33</td>
</tr>
<tr>
<td>E-vanillic acid</td>
<td>612.62</td>
<td>161.95</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>392.63</td>
<td>45.34</td>
</tr>
<tr>
<td>Alpha-coumaric acid</td>
<td>18.44</td>
<td>1.89</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>267.34</td>
<td>270.76</td>
</tr>
<tr>
<td>Coumarin</td>
<td>49.37</td>
<td>1.13</td>
</tr>
<tr>
<td>3,4,5 tri-methoxy-cinnamic acid</td>
<td>24.25</td>
<td>29.34</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>------</td>
<td>161.97</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>3.68</td>
<td>3.76</td>
</tr>
</tbody>
</table>
These results are in agreement with those of Erkan, (2012) who found that phenolic compounds of celery were as follow: protocatechuic acid 43.07 ppm, catechol 18.69 ppm, P- coumaric acid 17.07 ppm, catechin 49.28 ppm, caffeic acid 34.05 ppm, vanillic acid 603.43 ppm, pyrogallol 1012.34 ppm, ferulic acid 172.87 ppm and gallic acid 18.43 ppm.

Analysis of phenolic compounds in broccoli flowers showed that pyrogallol reached 2898.52 ppm, followed by benzoic acid, P-OH-benzoic acid, salicylic acid, e- vanillic acid and chlorogenic acid which were 270.76, 183.04, 161.97, 161.95 and 150.79 ppm respectively.

Results of phenolic compounds analysis are nearly similar to those reported by Figueiredo et al., (2015) who found that phenolics compound contents were as follow: cinnamic 4.5 ppm, benzoic acid 265.8 ppm, pyrogallol 2845.2 ppm, ellagic acid 50.8 ppm, gallic acid 4.2 ppm, chlorogenic acid 139.9 ppm, caffeine 61.3 ppm and salycilic acid 158.4 ppm.

5: Quantitative analysis of flavonoids in celery leaves and broccoli flowers by HPLC.

Flavonoids in celery leaves and broccoli flowers were analyzed by High Performance Liquid Chromatography (HPLC), and concentrations of all tested flavonoids are given in Table (5).

Table (5): Quantitative analysis of flavonoids in celery leaves and broccoli flowers by HPLC.

<table>
<thead>
<tr>
<th>Flavonoids ( mg/100g )</th>
<th>Celery leaves</th>
<th>Broccoli flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteo. 6-arbinose 8-glucose</td>
<td>93.19</td>
<td>114.59</td>
</tr>
<tr>
<td>Luteo. 6-glucose 8-arbinose</td>
<td>5.48</td>
<td>6.55</td>
</tr>
<tr>
<td>Apig. 6-rhamnose 8-glucose</td>
<td>124.24</td>
<td>4.67</td>
</tr>
<tr>
<td>Apig. 6-glucose 8-rhamnose</td>
<td>13.03</td>
<td>8.79</td>
</tr>
<tr>
<td>Naringin</td>
<td>-----</td>
<td>16.84</td>
</tr>
<tr>
<td>Luteolin</td>
<td>134.63</td>
<td>35.93</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>103.94</td>
<td>87.91</td>
</tr>
<tr>
<td>Rutin</td>
<td>13.39</td>
<td>2.54</td>
</tr>
<tr>
<td>Quercetin-3-o-glucoside</td>
<td>-----</td>
<td>3.37</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>997.03</td>
<td>50.93</td>
</tr>
<tr>
<td>Apig.7-O-neohespiroside</td>
<td>6.07</td>
<td>-----</td>
</tr>
<tr>
<td>Kaemp.3,7-dirhamoside</td>
<td>19.12</td>
<td>6.86</td>
</tr>
<tr>
<td>Apig. 7-glucose</td>
<td>9.54</td>
<td>1.17</td>
</tr>
<tr>
<td>Quercetin</td>
<td>13.89</td>
<td>7.69</td>
</tr>
<tr>
<td>Quercetin</td>
<td>-----</td>
<td>1.63</td>
</tr>
<tr>
<td>Kaem.3,(2-p-comaroyl) glucose</td>
<td>-----</td>
<td>11.21</td>
</tr>
<tr>
<td>Naringenin</td>
<td>-----</td>
<td>3.85</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>9.42</td>
<td>5.85</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>1.73</td>
<td>0.38</td>
</tr>
<tr>
<td>Rhamnetin</td>
<td>1.25</td>
<td>0.96</td>
</tr>
<tr>
<td>Apigenin</td>
<td>0.84</td>
<td>0.51</td>
</tr>
<tr>
<td>Acacitin</td>
<td>102.04</td>
<td>19.67</td>
</tr>
</tbody>
</table>
From this table, it was found that celery leaves contained rosmarinic as the main flavonoid which reached 997 mg/100g with high level of luteolin, apig.6-rhamnose 8-glucose, hesperidin, acacetin and luteo.6-arbinose 8-glucose which were amounted of 134.63, 124.24, 103.94, 102.04 and 93.19 mg/100g respectively and low level of apig.7-glucose, hesperetin, apig.7-O-neohesperiose, kaempferol, rhamnetin and apigenin which had values of 9.54, 9.42, 6.07, 1.73, 1.25 and 0.84 mg/100g respectively.

Results of flavonoids analysis are nearly similar to those reported by Benhammou et al., (2007) who studied the main flavonoids of celery leaves and found that celery leaves contained kaempferol 2.1 mg, luteolin 132.9 mg, rutin 15.3 mg, rosmarinic 964.7 mg, apigenin 1.92 mg, hesperidin 110.1 mg, acacetin 100.4 mg and rhamnetin 2.3 mg/100g.

Analysis of flavonoids in broccoli flowers showed that the main flavonoid was luteo.6-arbinose 8-glucose reached 114.59 mg/100g as well as high level of hesperidin, rosmarinc and luteolin which were 87.91, 50.93 and 35.93 mg/100g respectively, followed by acacetin, naringin, hesperetin, naringenin, rhamnetin, kaempferol and apigenin which were 19.67, 16.84, 5.85, 3.85, 0.96, 0.38 and 0.51 mg/100g respectively.

These results is agreed also with those of Galan et al., (2004) who indicated that flavonoids found in broccoli flowers were naringin 18.3 mg, apigenin 1.8 mg, luteo.6-arbinose 8-glucose 112.8 mg, hesperidin 91.9 mg, rosmarinic acid 49.2 mg, luteolin 38.7 mg and quercetin 9.3 mg/100g.

Conclusion.

Celery leaves contained 17 amino acids as arginine, proline and histidine. Broccoli flowers contained 17 amino acids. Essential amino acids were valine, lysine and methionine. Non essential amino acids were glutamic acid, aspartic acid and alanine. While semi-essential amino acids were cystene, arginine and proline. Celery leaves were found to contain 22 of phenolic compounds, among them pyrogallol, e.vanillic acid, ellagic acid, benzoic acid, chlorogenic acid, ferulic acid and caffeine were the major active constituents comparing with 23 of phenolic compounds in broccoli flowers which contained pyrogallol, benzoic acid, chlorogenic acid, and salicylic acid those were the major of constituents phenolic compounds. Celery leaves contained 17 of flavonoids, the highest values of them were rosmarinic, luteolin, hesperidin and apig.6-rhamnose 8-glucose comparing with 21 of flavonoids in broccoli flowers from which the highest amounts were of luteo.6-arbinose 8-glucose, hesperidin and rosmarinic. Broccoli flowers showed high contents of total phenolic compounds and flavonoids comparing with celery leaves.

REFERENCES


Amnah, M. and A. Alsuhaibani (2013 b). Effect of Broccoli on the Antioxidant Activity of Experimental Rats Ingested...
Ashoush, et al.,


Ballmer, W., M. Besler, S. Hoffmann, S. Vieths and W. Brunello (2000). The present data collection reviews detailed information on the prevalence and symptoms of celery allergy as well as cross-reactivities, and molecular biological and allergenic properties of the major celery allergens in tabular form. Internet Symposium on Food Allergens .2(3):145-167.


Comparative study between celery leaves and broccoli flowers for 

by spinach, kale, brussels sprouts, 
broccoli, mustard greens, green bell 
pepper, cabbage and collards. Food 
Chem. 100: 1531–1536. 

Antioxidant capacity of phenolic 
phytochemicals from various cultivars of 

Food composition table for use in Africa. 
FAO, Rome, Italy. pp 306. 

Price, K.R., F. Casuscelli, I.J. Colquhoun 
and content of flavonol glycosides in 
broccoli flowers (Brassica olearacea) and 
their fate during cooking. J. Sci. Food 
Agric. 77: 468 –472. 

Souci, S.W., W. Fachmann and H. Kraut 
(2000). Food composition and nutrition 
tables 6th edn. (eds) Deutsche 
Forschungsanstalt für 
Lebensmittelchemie, Garching b. 
M‘unchen, Medpharm Scientific 
Publishing, Stuttgart, Germany (Boca 
Raton, London, NewYork,Washington, 

on the Pharmacognotical and 
Pharmacological Characterization of 
Apium Graveolens Linn. Indo Global J. 
Pharmaceut. Sci. 2(1): 36-42. 

Yoldas, F., S. Ceylan, B. Yagmur and N. 
fertilizer on yield quality and nutrient 
content in broccoli. J. Plant Nut. 31: 1333 
–1343. 

Simultaneous Determination of 
Catechins, Caffeine and Gallic acids in 
Green, Oolong and Black tea using 
HPLC with a Photodiode Array Detector. 
Talanta, 57: 307-316.
دراسة مقارنة بين أوراق الكرفس وأزهار البروكلي من حيث التركيب الكيميائي والأحماض الأمينية والمركبات الفينولية والفلافونويدات

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قسم الكيمياء الحيوية الزراعية - كلية الزراعة - جامعة المنوفية (1)
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تمت دراسة التركيب الكيميائي لأوراق الكرفس وأزهار البروكلي ومكوناتها من الأحماض الأمينية وكذلك المركبات الفينولية والفلافونويدات ويمكن تلخيص نتائج هذه الدراسة فيما يلي:

أولاً التركيب الكيميائي:
1- أوراق الكرفس: تحتوي على 88.72% رطوبته في العينية الطازجة في حين أن العينات الجافة تحتوى على 36.8% كربوهيدرات، 19.47% بروتين كلي، 2.18% دهون كلي، 19.85% ألياف، 20.98% رماد كلي كما يوجد بها 3.03 مجم/كليه من فينولات كليه، 18.5 مجم/كليه من فلافونويدات كليه.
2- أزهار البروكلي: تحتوي على 89.4% رطوبته في العينية الطازجة في حين أن العينات الجافة تحتوى على 44.8% كربوهيدرات، 2.79% بروتين كلي، 8.29% دهون كلي، 15.29% ألياف، 7.67% رماد كلي كما يوجد بها 3.84 مجم/كليه من فينولات كليه، 2.2 مجم/كليه من فلافونويدات كليه.

ثانياً الأحماض الأمينية:
تم تقديم الأحماض الأمينية عن طريق جهاز تقدير الأحماض الأمينية (Amino acid analyser) وكانت النتائج:
1- أوراق الكرفس: تحتوي على 17 حمض أميني كانت كمية الأحماض الأمينية الأساسية منها 31.42% أهمها الفاليوم 7.09%، الباراسيدين 5.62%، الأيزوليوسين 4.11% والأحماض الأمينية الغير أساسية 68.58% وكان أهمها حمض الأسبارت 23.37%، الجليسين 4.22%، السيرين 3.03% ، حمض الجلوتاميك 15.47%، الأليين 5.84%.
2- أزهار البروكلي: تحتوي على 15 حمض أميني وكانت كمية الأحماض الأمينية الأساسية منها 30.82% أهمها الألفاcion 6.56%، الباراسيدين 5.81%، الأيزوليوسين 3.84% والأحماض الأمينية الغير أساسية 69.18% وكان أهمها حمض الجلوتاميك 22.25%، الجليسين 4.21%، السيرين 4.26%، حمض الأسبارت 11.05%، والأليين 8.01%.

ثالثاً تقدير الفلافونويدات:
تم تقدير المركبات الفلافونويدية بواسطة جهاز (HPLC) والذى أظهر النتائج التالية:
Comparative study between celery leaves and broccoli flowers for ..............

1- أوراق الكرفس : تحتوي على 17 مركب أهمها حمض الروسمارينيك 997.03 مجم|100جم , لوتيلولين 134.63 مجم|100جم , هيسبيريدين 103.94 مجم|100جم , أكاسيتين 102.04 مجم|100جم .
2- أزهار البروكلين : تحتوي على 21 مركب أهمها ليوتيولين-6-أرابينوز-8-جلوكوز 114.59 مجم|100جم , هيسبيريدين 91.91 مجم|100جم , حمض الروسمارينيك 50.93 مجم|100جم , لوتيلولين 35.93 مجم|100جم .

رابعا تقدير المركبات الفينولية.

تم تقدير المركبات الفينولية بواسطة جهاز (HPLC) والذي أظهر النتائج التالية :

1- أوراق الكرفس : تحتوي على 22 مركب فينولي أهمها بيروجالول 1037.74 جزء في المليون , حامض ايلاجيك 392.63 جزء في المليون , حامض كلوروجينيك 218.79 جزء في المليون , حامض بينزويك 267.34 جزء في المليون , حامض فيروليك 163.19 جزء في المليون , كافين 100.61 جزء في المليون , كاتيشين 48.24 جزء في المليون .
2- أزهار البروكلين : تحتوي على 23 مركب أهمها بيروجالول 2898.52 جزء في المليون , حامض بينزويك 270.76 جزء في المليون , حامض ساليساليك 161.97 جزء في المليون , حامض كلوروجينيك 150.79 جزء في المليون , حامض برتوكتاشويك 83.79 جزء في المليون , كاتيشين 78.51 جزء في المليون .

وقد تم التعرف على المركبات الفينولية والفلافونويدات وتذكيرا عن طرق جهاز HPLC.