Polyphenol content and antioxidant capacity in organically and conventionally grown vegetables

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1. Introduction

Vegetables are becoming highly important in the food diet of Malaysians. The per capita consumption of vegetables in 1982 to 2001 has increased from 27.25 kg to 40.58 kg, with significant increase in the vegetables brassica, cucumber, cabbage, water spinach, and Chinese spinach, and is expected to continue to rise due to improving....

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living standards and increasing health concerns among consumers[3].

The Ministry of Health Malaysia recommends five servings of fruits and vegetables of approximately 400 g[2], with three servings for vegetables per day. In a survey conducted to investigate patterns of fruits and vegetables consumption among Malaysian adults in Selangor, subjects were found to consume more brassica vegetables than other vegetable categories. They were Chinese mustard (Brassica juncea), round cabbage (Brassica rapa ssp. pekinensis), cauliflower (Brassica oleracea var. botrytis subvar. cauli/broccoli), Chinese cabbage (B. rapa var. chinensis), broccoli (Brassica oleracea B. oleracea), Chinese white cabbage (Brassica rapa), kale [Brassica albioglabra (B. alboglabra)], pak choy (Brassica rapa var. chinensis), and Brussels sprout (B. oleracea)[3].

Various studies have confirmed that vegetables are high in polyphenols such as phenolic acids, flavonoids, and anthocyanins, and that these natural antioxidants are effective in avoiding oxidative stress related--diseases such as cancer, cardiovascular diseases, and aging-related disorders[4-7]. While consumers are becoming increasingly aware of the health benefits of vegetable consumption, the increasing demand for organic food comes with the perception that organic products are healthier due to higher levels of phytochemicals and nutritional quality of produces. The increasing demand for organic food comes with the perception that organic products are healthier due to higher levels of phytochemicals and nutrients contained in them[8-10]. However, there is still no evidence to support this claim. Although there are many studies on polyphenol content and antioxidant activity of vegetables, only a few studies compared organically with conventionally grown vegetables has been brought into discussion. There has been increased interest in organically grown food in the past decade due to concerns for the environment caused by conventional agriculture and nutritional quality of produces. The increasing demand for organic food comes with the perception that organic products are healthier due to higher levels of phytochemicals and nutrients contained in them[8-10]. However, there is still no evidence to support this claim. Although there are many studies on polyphenol content and antioxidant activity of vegetables, only a few studies compared organically with conventionally grown plants.

This study aimed to investigate the differences of polyphenol content and antioxidant activity among organically and conventionally grown vegetables. This study was conducted by evaluating the polyphenol content and antioxidant capacity of three common leafy vegetables, kailan (B. alboglabra), bayam (Amaranthus spp.) and sawi (Brassica parachinensis) of both conventional and organic types used in this study were obtained from local supermarket and local wet market in Kuala Lumpur and Kuantan, respectively. Each vegetable (250 g) was randomly sampled from the market shelves.

2.3. Extract preparation

Each of the vegetable types were washed with water, chopped into small pieces, transferred into respective beakers added with absolute ethanol, and left to shake on a shaker for 72 h at room temperature. The solvent was then separated from the plant residue by straining. The filtrate was collected and stored at room temperature while the residue was re-extracted twice, each time with fresh solvent. Finally, all the filtrates were combined and evaporated under reduced pressure at 60 °C using a rotary evaporator to obtain the crude extracts. The crude extracts were weighed and stored at 4 °C until further analysis.

2.4. Determination of polyphenol content

2.4.1. Total phenolic content (TPC) assay

TPC of the vegetable extracts was determined according to the method described with slight modifications[11]. Stock solutions (1 mg/mL) of extracts and standard solutions of gallic acid (0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL) were prepared. Extracts or gallic acid standard solution (0.3 mL) was pipetted into a cuvette. Diluted FC reagent (1.5 mL) was then added and mixed. The mixture was left for 3 min before adding 1.5 mL of sodium carbonate (75 g/L) solution and left for 60 min. The absorbance was read at wavelength 765 nm using a UV spectrophotometer and ethanol was used as the blank. TPC was calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts (mg GAE/g).

2.4.2. Total flavonoid content (TFC) assay

TFC of the vegetable extracts was determined using the aluminum chloride colorimetric method described by Chang et al.[12], but with slight modifications. Stock solution (1 mg/mL) of extracts was prepared. Quercetin was dissolved in 80% ethanol to make standard solutions (0.025, 0.050, 0.075, and 0.100 mg/mL) to plot a standard curve. Aliquots of 0.5 mL of diluted extract or standard solution were mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 0.1% potassium acetate, and 0.1 mL of 0.1% sodium carbonate. After reacting for 10 min, the absorbance was read at 540 nm against a blank.

2.5. Statistical analysis

Data were analyzed using SPSS 19.0 for Windows. Statistical significance was considered at P<0.05. Tukey’s test was used to determine the significance of differences between groups. Finally, all the filtrates were combined and evaporated under reduced pressure at 60 °C using a rotary evaporator to obtain the crude extracts. The crude extracts were weighed and stored at 4 °C until further analysis.
1 mol/L potassium acetate, and 2.8 mL of distilled water in the cuvette. The mixture was left at room temperature for 30 min. The absorbance was read at wavelength 415 nm. For the blank, 10% aluminium chloride was substituted with distilled water of the same amount. TFC was calculated and expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g).

2.4.3. Total anthocyanin content (TAC) assay

TAC of the vegetable extracts was determined colourimetrically following the method described with slight modifications[13]. Stock solutions of 10 mg/mL of extracts were prepared. Extract solution (3 mL) was pipetted into a cuvette. The intensity of the extract colour was measured at wavelength 520 nm using UV-VIS spectrophotometer. Ethanol was used as a blank. TAC was calculated and expressed as milligrams per 100 g (mg/100 g) using the following equation:

\[
\text{TAC} = \frac{\text{Absorbance of sample} \times \text{DF} \times 100}{m \times E}
\]

Where, DF stands for dilution factor; m means weight of sample used to make stock solution; E refers to extinction coefficient (55.9).

2.5. Determination of antioxidant capacity

Antioxidant capacity of the extracts was determined using DPPH assay as described by Azim Almey et al. with slight modifications[14]. Stock solution (1 mg/mL) of extract was diluted to concentrations of 0.10, 0.20, 0.30, 0.40, 0.60, and 0.80 mg/mL in methanol. Methanolic DPPH solution was prepared by dissolving 6 mg of DPPH in 100 mL methanol. The methanolic DPPH solution (2 mL) was added to 1 mL of each extract solution of different concentrations and the mixture was left for 30 min and the absorbance was read at wavelength 517 nm. Control was prepared by mixing 1 mL of methanol with 2 mL of DPPH solution. Methanol was used as a blank while trolox was used as a standard. Antioxidant capacity based on the DPPH free radical scavenging ability of extracts was calculated using the following equation:

\[
\% \text{ inhibition} = \frac{1 - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%
\]

2.6. Statistical analysis

Assays for each extract were performed in triplicate and results were expressed as means±SD of three measurements. Data were analyzed using SPSS version 12.0.1. Significant differences between means of organic and conventional extracts were analyzed by independent sample t-test while correlation between TPC, TFC, TAC and DPPH inhibition values were determined by correlation test.

3. Results

3.1. Percentage yield of solvent extracts

Table 1 shows the percentage yield of ethanol extracts of organic and conventional kailan, bayam and sawi.

<table>
<thead>
<tr>
<th>Vegetable extracts</th>
<th>Average extract weight (g)</th>
<th>Average percentage yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional kailan</td>
<td>3.57</td>
<td>2.38</td>
</tr>
<tr>
<td>Organic kailan</td>
<td>6.13</td>
<td>2.85</td>
</tr>
<tr>
<td>Conventional bayam</td>
<td>2.50</td>
<td>1.47</td>
</tr>
<tr>
<td>Organic bayam</td>
<td>1.06</td>
<td>0.63</td>
</tr>
<tr>
<td>Conventional sawi</td>
<td>3.50</td>
<td>2.33</td>
</tr>
<tr>
<td>Organic sawi</td>
<td>4.50</td>
<td>2.14</td>
</tr>
</tbody>
</table>

3.2. Phytochemical contents of extracts

3.2.1. TPC

Figure 1 shows the mean of TPC in the extracts of three leafy vegetables of organically and conventionally cultivars. The differences in phenolic content in organically and conventionally grown vegetable extracts were found to be statistically insignificant for all the leafy vegetables (P>0.05). The organically grown sawi and bayam extracts showed higher phenolic content as compared to conventionally grown sawi and bayam while organically grown kailan extract shown lower phenolic content compared to conventionally grown kailan extract.

3.2.2. TFC

Figure 2 shows the mean of TFC in the extracts of three leafy vegetables of organically and conventionally cultivars. The differences in flavonoid content were found to be statistically insignificant for bayam of both cultivars (P>0.05), whereas there is significant difference in flavonoid content in the organically and conventionally grown kailan and sawi (P<0.05). The flavonoid content of organically grown sawi extract is much higher compared to conventionally
grown sawi extract, while organically grown bayam and kailan extracts show lower flavonoid content compared to organically grown bayam and kailan.

3.2.3. TAC

Figure 3 shows the mean of TAC in the extracts of three leafy vegetables of organically and conventionally cultivate. There is significant difference in terms of TAC between organically and conventionally grown kailan \( (P<0.05) \). However, there is no significant difference between organically and conventionally grown bayam as well as sawi \( (P>0.05) \). All the organically grown vegetable extracts showed lower TAC than the conventionally grown ones.

3.2.4. Antioxidant capacity

The activity of antioxidants of the vegetable extracts was evaluated using DPPH assay, whereby the extracts were tested for their ability to scavenge DPPH free radicals. Trolox was used as positive control to test its DPPH-scavenging activity. The strength of the antioxidant activity between the vegetables extracts and positive control was determined by IC\(_{25}\) values obtained via extrapolation from the graph of percentage inhibition versus concentration. The IC\(_{25}\) values of the vegetable extracts and positive control are presented in Table 2. The lower IC\(_{25}\) value means the higher scavenging activity.

![Graph of percentage of inhibition versus concentration of vegetable extracts](image)

**Table 2**

IC\(_{25}\) of the organic and conventional vegetables extracts.

<table>
<thead>
<tr>
<th>Vegetable extracts</th>
<th>IC(_{25}) (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional kailan</td>
<td>0.90±0.12</td>
</tr>
<tr>
<td>Organic kailan</td>
<td>0.99±0.10</td>
</tr>
<tr>
<td>Conventional bayam</td>
<td>0.59±0.15</td>
</tr>
<tr>
<td>Organic bayam</td>
<td>1.21±0.72</td>
</tr>
<tr>
<td>Conventional sawi</td>
<td>0.55±0.14</td>
</tr>
<tr>
<td>Organic sawi</td>
<td>1.01±0.87</td>
</tr>
<tr>
<td>Standard trolox</td>
<td>0.16±0.00</td>
</tr>
</tbody>
</table>

*: Significant difference \( (P<0.05) \).

Trolox is shown to have the lowest IC\(_{25}\) value hence the highest scavenging activity. There is significant difference between the the organically and conventionally grown vegetable extracts and positive control trolox \( (P<0.05) \). Conventionally grown bayam and sawi extracts have lower IC\(_{25}\) value compared to their organically ones, while conventionally grown kailan extract has lower IC\(_{25}\) value than organically grown kailan extract. There is no significant difference between the organically and conventionally grown kailan extracts \( (P>0.05) \) but there is significant difference between organically and conventionally extracts of bayam and sawi \( (P<0.05) \).

The percentage inhibition of DPPH free radical by extracts of organically and conventionally grown vegetables and positive control trolox, are demonstrated in Figure 4. Based on the graph, trolox demonstrates the higher scavenging activity compared to organically and conventionally grown kailan, bayam, and sawi extracts. Based on statistical analysis, there is significant difference between each of the organically and conventionally grown vegetable extracts and positive control and significant difference between organically and conventionally grown extracts of bayam \( (P<0.05) \). However, there is no significant difference between organically and conventionally grown vegetables extracts of kailan and sawi \( (P>0.05) \).
grown types were used to compare the antioxidant properties which is demonstrated in Figure 5. Antioxidant activity is higher in all the conventionally grown vegetable extracts compared to their organically grown vegetable extracts counterparts. Statistical analysis showed that there was no significant difference between the the organically and conventionally grown vegetable extracts of kailan and sawi ($P>0.05$), but organically and conventionally grown bayam extracts had significant difference ($P<0.05$).

Figure 5. A comparison of percentage inhibition of organically and conventionally grown vegetable extracts at lowest concentration 0.1 mg/mL. *: Significant difference ($P<0.05$).

3.2.5. Correlation between polyphenol and antioxidant activity

Correlation between TPC, TFC, TAC and IC$_{25}$ of the ethanol extracts of the organically and conventionally grown vegetable extracts based on the DPPH radical scavenging activity assay showed no relationship among them. However, there is a positive relationship between TAC and IC$_{25}$ of conventionally grown vegetable extracts.

4. Discussion

The purpose of this study was to compare the polyphenol content and the antioxidant activity of three organic and conventional leafy green vegetables. The vegetables chosen in this study were kailan (B. alboflagbra) and sawi (B. parachinensis), which are from varieties of Brassica genus, and bayam (Amaranthus spp.), which is from Amaranthus genus.

Vegetables are excellent sources of antioxidants, which have been proven to play a role as protective agents against chronic diseases. Plants produce many types of compounds called secondary metabolites that mediate interactions between plants and their environment, help defend against pathogens and herbivores, as well as attract pollinators and seed dispersers for reproduction[15,16]. According to Grace[16], these secondary metabolites are classified into three groups, one of them is phenolic or also known as polyphenols. Phenolics, which are aromatic metabolites that have one or more acidic phenolic hydroxyl groups, are further classified into hydroxycinnamic acids, flavonoids, anthocyanins and tannins.

Brassica vegetables have been found to reduce the risk of cardiovascular diseases and gastrointestinal cancer. They are considered a good source of bioactive phytochemicals and have been found to contain phenolic compounds that have antioxidant and free radical scavenging properties[17,18]. Vegetable amaranth is a useful natural antioxidant and their leaves are found to be better source of protein, minerals, such as calcium, iron, phosphorus and carotenoids than most vegetables[19]. Hence, vegetables like brassica and amaranth are of interest in epidemiological and biological studies. Plants, being an important source of natural antioxidant, might be used to develop new drugs to treat many disorders.

In this study, there was no significant difference in TPC between all three organically and conventionally grown vegetables. Among the three types of vegetables, organically and conventionally grown sawi extracts had the highest TPC, followed by kailan and bayam. A study by Ismail et al. found that bayam exhibited higher phenolic content than kailan[20]. This could suggest that the TPC levels may vary from one vegetable to another. According to Cartea et al.[21], factors such as environment and methods of analysis used can influence phenolic compounds. Hence, comparing phenolic content among crops under different conditions and with different analytical methods will give inaccurate results. Different TPC levels could be due to procedures and standards used to express total phenolic contents. FC reagent used is not specific to phenolic compounds and could be attributed to other components that react with the reagent such as ascorbic acid[22,23]. Isabelle et al. reported that ascorbic acid was also an effective scavenger and since it is able to reduce FC reagent, it is essential to subtract ascorbic acid content from TPC to acquire a more accurate TPC[23].

Findings in this study showed that there was significant difference in TFC between the organically and conventionally grown sawi extract, whereby the organically grown type had higher TFC value than the conventionally grown one, and was also the highest among the other organically grown vegetable types. Flavonoids are among the major group of polyphenols in Brassica vegetables[21,24]. Previous studies that compared main phenolics in B. oleracea vegetables reported that the highest phenolic and flavonoid contents were found in broccoli and kalian varieties[25]. Lin and Harnly reported the detection of more than 50 flavonoids in 17 vegetables from Brassica species other than B. oleracea including choy sum or sawi[18]. These could support the findings of the difference in flavonoid content between the Brassica (kalian and sawi) and Amaranthus (bayam) vegetables.

Difference in flavonoid content between the organically and conventionally cultivar could be due to the extraction, hydrolysis times and temperatures or even climate, geography or agronomics practice[26]. Season and
environment can affect flavonoid content in crops for example, increased amounts of light can increase total flavonoid synthesis; the combination of interaction with high light and low temperature and vice versa could be responsive to flavonoid compounds in plants[27].

TAC can be quantified using pH–differential method, whereby absorbance at two different pH values is measured spectrophotometrically, as the anthocyanin pigments go through structural transformations with pH change[28]. TAC can also be determined colorimetrically by measuring absorptivity of the solution of extract diluted with the extracting solvent at a single wavelength. TAC has maximum absorption at wavelength 490 to 550 nm, which is far from the absorption bands of other phenolics[29]. According to Wrolstad et al.[28], regardless of which method was used to quantify anthocyanin, an absorptivity coefficient, which was absorption of a 1% solution measure through a 1 cm path or also known as molar absorption coefficient, was needed to determine the amount of TAC present. The total pigment content can be expressed as cyaniding-3–glucoside, which is the most abundant anthocyanin in plants, if the pigment identity is not known.

TAC was significantly different between the organically and conventionally grown kailan extracts. Conventionally grown kailan extract had the highest TAC value among all the vegetable extracts, whereas TAC value between the other vegetables extracts was relatively similar. Test for anthocyanin content done by previous studies on high bush blueberries in New Jersey and red oranges from Italy showed the higher levels of anthocyanins in organically grown cultivars, while a test done on syrah grapes from France reported that the conventionally grown grapes had higher anthocyanin levels[30]. Thus, it could suggest that anthocyanin levels in fruits and vegetables depend on variety and species. Anthocyanin content is also likely to vary due to extraction method and storage. According to Harborne[31], anthocyanins, which are usually in vascular plants, may be replaced by pigment called betacyanins, which is very similar superficially but differs chemically. Hence, it is not clear if the analysis detected anthocyanin pigments already present or some other compounds.

DPPH is a stable free radical that has a deep–purple colour, which changes to yellow when undergoes reduction by donating hydrogen to an antioxidant compound it reacts with[14]. Antiradical activity of antioxidants is determined using DPPH, which has absorption at wavelength 515 to 520 nm. Azlim Almey et al. states that the antioxidant activity of antioxidants, and the speed of reaction between DPPH and antioxidant is dependent on the decrease in absorbance of DPPH solution[14], IC50 values, which represent the volume of extract required to reduce the absorbance of DPPH radical by half, are calculated and are used to measure antioxidant assay. Lower IC50 value indicates higher antioxidant activity in the extract.

In this study, 50 percent inhibition was not achieved even at 1 mg/mL, so IC50 was used for estimation instead of IC100. The results from the DPPH radical scavenging activity assay showed that all the conventionally grown vegetable extracts had lower IC50 value compared to the organically grown vegetable extracts, which indicates better free radical scavenging activity. The results also showed the highest antioxidant capacity in conventionally grown sawi followed by conventionally grown bayam and kailan extracts, with significant differences in scavenging activity between conventionally and organically grown bayam extracts. Faller and Fialho reported in their study that conventionally grown broccoli stems had higher antioxidant capacity than its organically grown counterpart, while the organically grown broccoli leaves had slightly higher antioxidant capacity than conventionally grown ones[32]. Khanam et al. reported the higher antioxidant capacity measured by DPPH assay in pok choi than in green Amaranthus[33]. Most of the leafy vegetables used in their study had lower antioxidant capacities, when using the DPPH assay compared to the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay. This could be due to some compounds that show ABTS scavenging activity might have higher antioxidant capacity. Thus, this may suggest that different antioxidant assays may affect total antioxidant activity values.

In the present study, the correlation between the polyphenol content and antioxidant activity in the organically and conventionally grown vegetable extracts was evaluated. Although it was thought that the organically vegetable extracts would have higher polyphenol content compared to conventionally grown vegetable extracts, there was not much significant difference between either types of cultivation. There was no correlation between TPC and antioxidant activity, between TFC and antioxidant activity, and between TAC and antioxidant activity of organically and conventionally grown vegetables. However, there was a correlation between TAC and IC50 of the conventionally grown vegetable extracts.

The negative correlation could be because the extract may not necessarily have the antioxidants incorporated in the TPC, and while Folin Ciocalteu assay gives an estimate of TPC contained in an extract, free radical scavenging assay is not specific to polyphenols that may react differently in DPPH assay[14]. Difference of phenolic content on the other hand could be due to difference in variety and farming practice. In a study conducted by Zhao et al.[34], to compare phenolic acids in organically and conventionally grown pac choi, factors such as nutrient availability, pest attack and use of insecticide most likely affect the high concentrations of total phenolics in the organically grown pac choi. Lima et al. also found variations among the species of plant they studied and only a few of the organically grown showed the higher flavonoid content[35]. The higher phenol content in plants analysed could have relation with plant stress, with the possibility of the presence of polyamines as stress indicators.
Food analysed could have variations between them in terms of total phenol and flavonoid contents, both of which could have been influenced by cultivation methods, like organically and conventionally grown[35]. High–yield crop cultivars, chemical fertilizers and pesticides, irrigation, and mechanization are used in conventional agricultural practice. The concern of this agricultural method is regarding the negative biological and environmental consequences and related long–term sustainability. Organic agricultural practices on the other hand, do not let crops be genetically engineered, irradiated, fertilized with sewage sludge and treated with synthetic pesticides or herbicides. Instead, disease–resistant cultivars are often used, and plant nutrients are supplied through crop rotation, cover crops, and animal manure[36].

Asami et al. obtained results that total phenolic were higher in sustainably grown crops compared to organically grown ones, where sustainably grow crops treated with synthetic fertilizers and pesticides to combat pathogens and hence the pathogenic pressure causing synthesis of total phenolics[36]. However, these findings are not in agreement with the study by Young et al. who found that the total phenolic content of organically grown pak choi was higher than the conventionally grown ones, and may be related to increased pest attacks[37]; the organically grown pac choi samples produced higher total phenolic level in response to insect attack which suggested that this biotic stress factor could have contributed to the increasing levels of TPC in organically grown vegetables. Zhao et al. also reported that organic production methods influenced levels of phenolic acid compounds in Brassica rapa L. chinensis because of the organic fertilization which caused higher phenolic content than conventional fertilization[34].

The quantitative measurements of the extracts of three different vegetables of organically and conventionally grown vegetables showed that the polyphenol contents were relatively similar. There were relationships between the polyphenol content and antioxidant capacity of the vegetable extracts. The conventionally grown vegetables generally were higher in polyphenol and antioxidant activity than organically grown ones and there was no clear difference between organic and conventional vegetables in terms of polyphenolic content and antioxidant activity. Overall, findings of this study indicate that each type of vegetable possesses different antioxidant contents and activity, and agricultural practice is most likely to have contributed to the levels of phytochemicals contents.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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