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Research Article | Advances in Nutrition and Food Science | ANAFS-158
ISSN: 2641-6816

# Comparative Study on Antioxidant Activity of *Brassica rapa var. parachinensis* Leaves and Seeds

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**Received Date:** November 15, 2019; **Accepted Date:** January 09, 2020; **Published Date:** February 06, 2020

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## Abstract

The aim of this study was to assess the antioxidant activities of *B. rapa* var. *parachinensis* leaves and seeds. The aqueous extraction of leaves and seeds were analyzed for total amount of phenolic, flavonoids, ascorbic acid, chlorophylls, carotenoids, 2, 2-diphenyl-1-picryl-hydrazil (DPPH) radical scavenging activity and reducing power capacity. The total amount of biochemical compounds showed different variety between leaves and seeds. The content of vitamin C was incredibly higher in seeds while the quantity of chlorophyll, carotenoids and phenolic contents was higher in leaves. DPPH scavenging activity of seed extraction displayed higher percent of inhabitation in comparison with leaves. The reduce power of seed and leaves at 10 mg extract were0.46±0.024 and 1.03±0.058, respectively. The data were analyzed by one-way ANOVA multiple comparison test followed by Duncan's multiple range test.

**Keywords:** Antioxidants, *Brassica*, Disease, Vegetables assay

## Introduction

Regular consumption of vegetables and fruits as sources of natural antioxidants which consist of many different antioxidant components such as vitamins C and E, carotenoids, and phenolic compounds have been recognized reduce the risk of chronic diseases **[1].** Studies approved that an antioxidant rich diet has a very positive health impact in the long run **[2 3].** Antioxidants play a very important role in the body defense system against ROS **[4]**. Halliwell (2007) **[5]** reported that an antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule.

Antioxidants can scavenge free radicals and protect the human body from the oxidative stress that eventually, protect human body from cardiovascular disease, cancer, high blood pressure, diabetes and obesity. Cruciferous vegetables, in particular those included into the *Brassica* genus are good sources of a variety of nutrients and health-promoting phytochemicals **[6].**

Several epidemiological studies have been reported that there is an inverse connection between consumption of *Brassicaceae* and risk of cancer **[7-9]**. Frequent consumption of cruciferous vegetables such as broccoli, cauliflower, leaf mustard, cabbages, radishes, turnips, and kale reduce the risk of developing chronic disorders, including cardiovascular diseases and in protecting against cancer **[10, 11]**, stroke **[12]**, diabetes **[13]**, and even cancer **[14, 15]**.

The antioxidant activities of some *Brassica* vegetables such as cabbage, broccoli, cauliflower, kale, turnip have been studied extensively. However; no study had been conducted on antioxidant components and antioxidant activity of *Brassicarapa*var. *Parachinensis* which is the main and common food at some countries specially Malaysia. Therefore, this present study was undertaken to measure and compare the composition and antioxidant activity of *B. rapavar. Parachinensis* leaves and seeds **[5]**.

## Material and Methods

### Plant Materials

The plant was grown in the mixture soil with the ratio 3 top soil and 1 river soil at Taman Pertanian University, University Putra Malaysia from January – August 2014. Mature seeds were collected according to method by Delmas et al., 2013.

### Preparation of Plant Extract

The amount of 100 mg expanding leaves and mature seeds were taken for each replicate. Samples were heated in 1 ml 80% ethanol for 20 minutes. The supernatant was collected and the residue was re-extracted until the samples turned to white.

### Measurement of Total Chlorophyll

The extraction and measurement of total chlorophyll was conducted according to method reported by Lichtenthaler and Wellburn (1985) **[16]**. The chlorophyll was measured spectrophotometrically at 470nm, 645nm and 663nm. Total chlorophyll and carotenoids were calculated according to the formula:

$$\text{Total chlorophyll} = 20.2_{A645} + 8.02_{A663}$$
$$C_a = 12.21_{A663} - 2.81_{A646}$$
$$C_b = 20.13_{A646} - 5.03_{A663}$$
$$\text{Carotenoids} = (1000_{A470} - 3.37_{Ca} - 104_{Cb})/227$$

### Measurement of Total phenolics

The content of total phenolic was measured spectrophotometrically using the Folin-Ciocalteau method **[17]**. Sample extraction(5 µl)) was added to tubes containing 120 µl ultrapure water, 12.0 µl of Folin-Ciocalteau reagent and 47 µl of saturated $Na_2CO_3$ (20g $Na_2C0_3$ in 100 ml distilled water). The mixture was mixed, followed by incubation for 1 hour at room tempreture and measured spectrophotometrically at 760nm. The total phenol content of pant parts was expresses as miligrams of tannic acid per equivalent per 0.1 gram of fresh sample (mg TAE/0.1g FW) from a cilibration curve.

### Total Flavonoid

The measurement of total flavonoid was conducted with using aluminum chloride colorimetric assay **[18]**. The amount of 2% $AlCl_3$ in 2 ml ethanol was added to 2 ml extraction, mixed well and left in darkness for 1 hour. Then, the mixture was measured spectrophotometrically at 420 nm and prepared reagent was used as the blank. The total flavonoid content is expressed as milligram of quercetin (QE)/0.1 g fresh weight.

### Ascorbic Acid

Vitamin C content was determined by the titrimetric method as described in the Association of Official Analytical Chemists (AOAC,1995).Fresh plant (0.1 g)was extracted with 10 ml of 1% phosphate citrate buffer (28.39g $Na_2HPO_4$ was solved in 19.21g citric acid in 1 liter) using through chilled pestle and mortar. The homogenous extraction was filtered through filter paper (Whatman filter paper no. 1). The extraction was added to 1 ml of 1.7 mM 2, 6-Dicholorophenol Indophenol sodium (DCPIP) and mixed well followed by incubation at room temperature for 15 minutes. Ascorbic acid was measured spectrophotometrically at 520 nm and 3 ml of DCIP-reagent as blank. Vitamin C content was expressed as mg ascorbic acid (AAE) equivalent/0.1 g of plant on a fresh weight basis.

### Antioxidant Assay

### Extraction Method for Antioxidant Activity

Leaves and seeds (0.2 g) was harvested and extracted in 10 ml of deionized water. The mixture was left at room temperature for 1 h in the dark place with occasional agitation. The aqueous extract was obtained by filtering the mixture through filter paper (Whatman No. 1) and the filtrate was assayed for antioxidant activity.

### DPPH Radical Scavenging Activity

The antioxidant activity was measured according to the method described by Wong et al. (2006) **[19]**. The initial absorbance of 3 ml of 0.1 m$M$ methanolic DPPH reagent was measured at 515nm. Leaves and seeds extraction (40 µl) was added to DPPH reagent and kept at room temperature. The differences in absorbance were measured after 30 minutes at 515 nm. The antioxidant capacity based on the DPPH free radical scavenging ability of the extract was expressed as µmole Trolox equivalent per gram and aascorbic acid was used a positive control. The percentage of the DPPH radical scavenging is calculated using the equation as given below:

$$\left(\frac{Abs_{t=0} - Abs_{t=30}}{Abs_{t=0}}\right) \times 100$$

Abs $_{t=0}$ is the absorbance of DPPP at time 0
Abs $_{t=30\ min}$ is the absorbance of DPPP at time 30 min of incubation

### Reducing Power Assay

The reducing power of crude extract was measured according to the method by Oyaizu (1986) **[20]**. Crude extracts dissolved in 1 m of distilled water and mixed with 2.5 ml of 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 ml of 1%

(w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Following this, 2.5 mL of 10% (w/v) trichloroacetic acid were added and the mixture was centrifuged at 1750 x G for 10 min. A 2.5 ml aliquot of the upper layer was combined with 2.5 ml of distilled water and 500 µL of 0.1% (w/v) $FeCl_3$. The absorbance of the reaction mixture was measured at 700nm. The capacity of samples extract to reduce the ferric-ferricyanide complex to the ferrus-ferricyanide complex of prussian blue was determined by measuring spectrophotometrically the absorbance at 700nm after incubation. Increased absorbance of the reaction mixture indicates greater reduction capability **[21]**.

### Analysis of Data

All data were analyzed using SPSS Windows version 21. T-test at significant level, $p<0.05$ was carried out to determine the significance difference between leaves and seeds. Tukey multiple comparison test was applied to determine which means are statistically difference if the ANOVA was significant.

## Results and Discussion

### Total Phenolic Content

Phenolic compounds, containing one or more acidic hydroxyl residues attached to an aromatic arene (phenyl) ring, are one of the most active constituents that contribute to the antioxidant activities of plant foods **[22]**. Therefore, it is great to measure phenolic content and determine its contribution to the antioxidant activity of plant materials. The total phenolic content of leaves and seeds was found to be 0.027±0.0005 and 0.016±.0012, respectively **(Table 1)**. The total phenolic content was calculated using the following linear equation based on calibration curve of Tannic acid (y=13.283x, R2=0.997). Then, the phenolic contents are around two times higher on seeds in comparison with leaves. Studies on phenolic are increasing specially in the food industry because they delay oxidative degradation of lipids and thereby improve the quality and nutritional value of food will be improved **[23]**.

### Vitamin C Content

Vitamin C or ascorbic acid is a water-soluble vitamin that can be found ubiquitous in fresh fruits and vegetables. Vitamin C has many nutritional and clinical benefits for human health. Vitamin C is a bioactive compound that is involved in healing wounds, iron metabolism, tyrosine metabolism, conversion of folic acid to folinic acid, synthesis of lipids and protein, resistance to infections, and cellular respiration. Moreover, vitamin C is an important antioxidant because ofsuppressive effects on oxidation of other compounds over donation of its electrons **[24]**. We observed that seeds have higher ascorbic acid contents ($0.22^b$±0.019 mg/0.1g) as compared to leaves ($0.022^a$±0.004 mg/0.1g). It is obvious from the present study that total amount of ascorbic acid is 10 times higher on seeds in comparison with leaves **(Table 1)**. The values obtained from seeds were higher than total amount of ascorbic acid in Savoy cabbage (0.049.06 ± 7.52 mg/0.1g) that was reported by Fernandez-Leon et al., (2014) **[25]**.

### Total Chlorophyll and Carotenoid Content

The total chlorophyll and carotenoid contents of *B.rapa var. parachinensis* leaves and seeds are presented in **(Table 1)**. Leaves contain significantly higher amount in total chlorophyll, carotenoid than seeds (t- test, $p<0.05$). Carotenoids have many physiological functions. Carotenoids are precursors of vitamin A and also contribute to the prevention of cancer and cardiovascular diseases. Epidemiological studies proved that people, who have been taken high level of β-carotene in their daily diets, significantly reduced the risk of lung cancer. In addition, carotenoids are included as one of the most important free radical scavengers that have efficient antioxidant activity **[26].**

### Total Flavonoid

The antioxidant activity of flavonoids and also its benefits as human nutrition and health care are importantly considerable **[27]**. The total flavonoid content of leaves and seeds shown in **(Table 1)**. There is no significance difference of total flavonoid between leaves and seeds (t-test, $p>0.05$).It is well-known that compounds such as flavonoid, which contain hydroxyl functional groups, are responsible for antioxidant effect in the plants.

| Part of plant | Total Chlorophyll (mg/0.1g) | Carotenoid (mg/0.1g) | Phenolic (mg/0.1g) | Flavonoid (mg/0.1g) | Ascorbic Acid (mg/0.1g) |
|---|---|---|---|---|---|
| Leaves | $49.96^a$±2.26 | $31.120^a$±126.1 | $0.027^a$±0.0005 | $3.04^a$±0.16 | $0.022^a$±0.004 |
| Seeds | $1.15^b$±0.24 | $8.877^b$±6.52 | $0.016^b$±.0012 | $3.10^a$±0.05 | $0.22^b$±0.019 |
| Values followed by the same letter in the same column are not significantly different according to Independent t-test at $p<0.05$. | | | | | |

**Table 1:** Bioactive compounds between leaves and seeds of *B. rapa var. parachinensis.*

**Antioxidant Activities between Leaves and Seed of *B. Rapa Var. Parachinensis***

**DPPH Radical Scavenging Activity**

The DPPH assay is one of the most widely preferable methods to measure the radical scavenging activity of plant extracts. This assay is rapid, relatively simple, and easily standardized **[28]**. DPPH has a stable nitrogen-centred free radical that exhibit a violet colour in methanol solution. When DPPH free radicals do reaction with suitable reducing agents (for example, antioxidants), the solution loses its violet colour depends on the number of electrons taken up **[29]**. In present study, the radical scavenging activity of aqueous extraction of leaves and seeds, and ascorbic acid at five concentrations (0.5, 1, 2, 5 and10_mg/l) was tested by DPPH method **(Figure 1, 2)**. All the extracts demonstrated a capacity to scavenge the DPPH radical in the test run. However, the extracts of seeds have relatively high DPPH scavenging activity comparing with extracts from leaves (ANOVA, $p<0.05$).Studies on the capacity of *Brassica* seed as antioxidants are very limited while this study discovered that the seed extraction of *B. rapa var. parachinensis* have dramatically higher capacity (> 80% at 10mg/assay) as a DPPH radical scavenger than leaves. Therefore, seeds have higher content of antioxidant compounds with greater potential than leaves.

Seeds of tronchuda cabbage showed pro-oxidant activity for concentrations higher than 1.9 µg/ml, due to the high amount of ascorbic acid which exhibit higher antioxidant potential than its leaves **[30]**. The antioxidant capacity of tronchuda cabbage seeds higher than internal and external leaves. By measuring the DPPH activity, 2000 µg/ml of external leaves need to inhibit 95% and more than 4000 µg/ml of internal leaves need to inhibit 95%, compared with seed only need 400 µg/ml to inhibit more than 95%. This is not surprising, since seeds often contain the highest amount of lipids than any plant tissue, with high content of polyunsaturated fatty acids. The existence of high levels of phenolic compounds, and organic acids, namely ascorbic acid, in tronchuda cabbage seeds, indicate that these compounds protect storage lipids from oxidation, as observed with tocopherols **[31]**, contributing to the viability of seeds and their rapid germination once oxygen demand during germination is high **[32, 33]**. The crude extract of mustard seeds showed more than 50% inhibition at 50µg/100µL assay **[34]**. Comparison studies also found that the antioxidant capacity of mustard seed higher (57% inhibition) than fenugreek seed (50.4 %) and poppy seed (44.0%) at 50µg/100µL assay. In the plant studied seeds displayed more than 66.0 % inhibition at the 2mg/ml assay tested that confirms others.

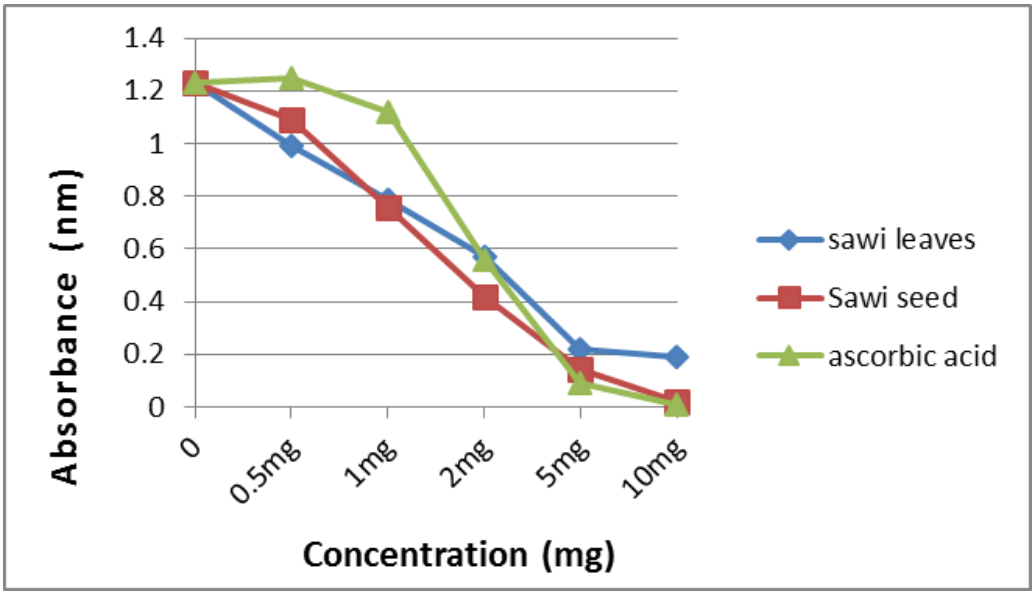


**Figure 1:** Scavenging effect of crude extracts of leaves and seed on 2,2-diphenyl-1-picrylhydrazyl radical as measured by changes in absorbance at 517nm.

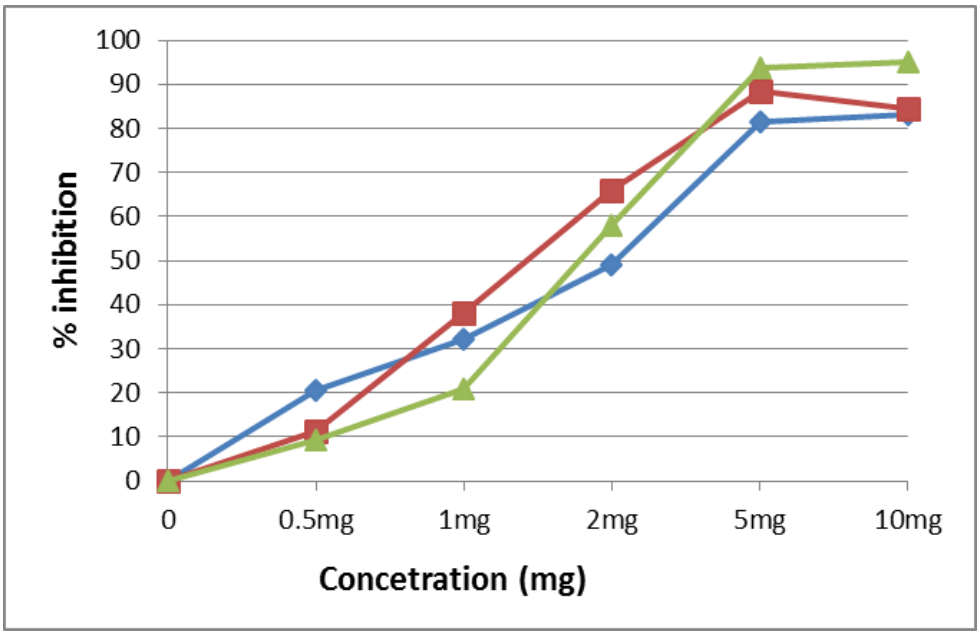


**Figure 2:** DPPH radical scavenging activity of leaves and seed of *B.rapa* var. *parachinensis*. Ascorbic acid used as positive controls. Each point averaged from triplicate measurements with standard errors falling within the data points.

**Reducing Power**

The reducing power between leaves and seed of *B. rapa*var. *parachinensis* and the reference compound (ascorbic acid) were determined. All extracts showed some degree of electron donation capacity in a concentration-dependent manner **(Figures 3)**. The capacity of leaves was significantly lower (t-test, $p<0.05$) than seed. In addition, from **(Table 2)** shows that the reducing power of seed (1.03) significantly higher (t-test, $p<0.05$) than leaves (0.46) for 10 mg of extract in test. According to Ishtiaque et al. (2013) **[34]**, the reducing power of mustard seed are higher (0.53) than fenugreek seed (0.47) and poppy seed (0.425) at 100µg/100uL. Methanol extractions of rapeseed (B. napus L) also have high reducing power (1.6) at 2.0mg/ml assay tested. Thus, *B. rapa*var.*parachinensis* seed has the high reducing power ability among the plants which studied with 0.78 reducing power at 1mg/ml assay tested.

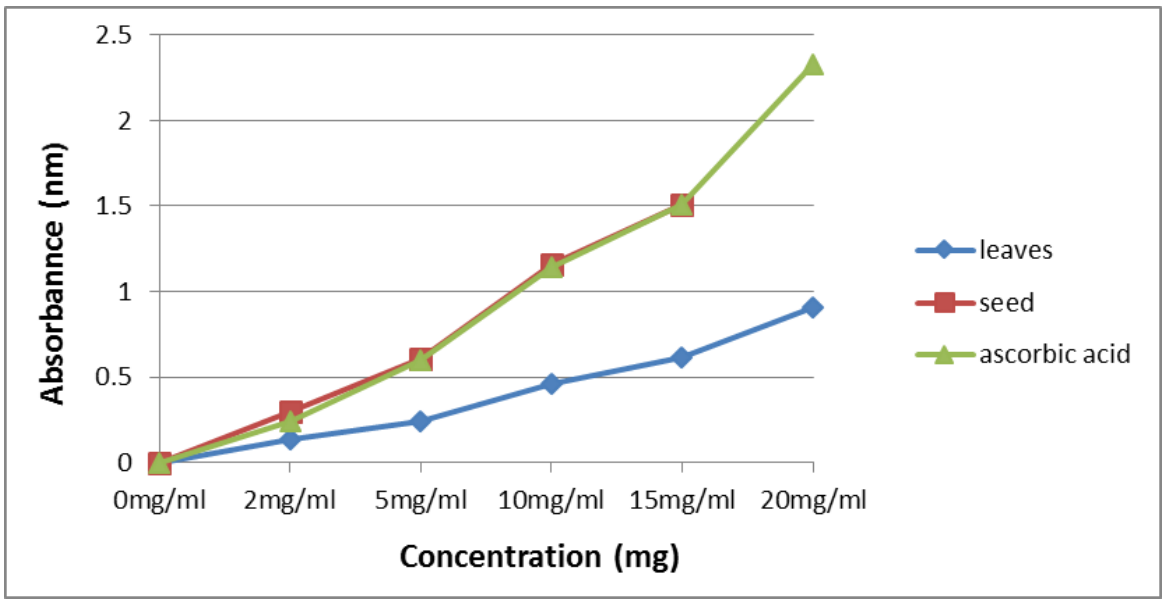


**Figure 3:** Reducing power of crude extracts of *B. rapa* var. *parachinensis* leaves and seeds.

| *B. rapa* var. *parachinensis* | Reducing Power (10 mg/ml extract in test) |
|---|---|
| **Leaves** | $0.46^{a} \pm 0.024$ |
| **Seeds** | $1.03^{b} \pm 0.058$ |
| All the values are presented in mean ± SE (n=5). There was 10 mg of indicated extract in each sample. Standard ascorbicacid used and absorbance in 700 nm was 1.142 ± 0.000. | |

**Table 2:** Comparison of Reducing Power between leaves and seed of *B. rapa* var. *Parachinensis.*

## Conclusion

We evaluated the antioxidant activities and nutritional propertiesof leaves and seed of *B. rapa*var*parachinensis*. The results obtained in this study suggest that leaves and seeds of this plant constitute a good source of health-promoting compounds, namely, carotenoids, flavonoid, phenolic compounds, and ascorbic acid. It should be emphasized that leaves and seeds of this plant supply distinct phenolic. This could be of great relevance when biological activities are considered and deserves further studies. Nevertheless, the antioxidant capacity of leaves and seeds follows the order: seeds > leaves. The distinct chemical composition of these plant materials has a great influence in their antioxidant properties. Thus, the commercialization of standardized aqueous extracts of leaves and seeds to be used as antioxidants may be regarded as a possibility for both food and pharmaceutical industries. Seeds have a potential as a source of natural antioxidant in the food industry and pharmaceutical.

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***Citation:*** *Ghanad M, Nulit R, Go R, Seok CY (2020) Comparative Study on Antioxidant Activity of Brassica rapa var. parachinensis Leaves and Seeds.*