

## Antioxidant Properties of Two Varieties of Bitter Gourd (*Momordica charantia*) and the Effect of Blanching and Boiling on Them

Choo, W. S. \*, Yap, J. Y. and Chan, S. Y.

School of Science, Monash University Sunway Campus, Jalan Lagoon Selatan, 46150 Bandar Sunway, Selangor, Malaysia

### ABSTRACT

The antioxidant properties of whole fruits and fruits without seeds and pith of two varieties of *Momordica charantia* (commonly known as bitter gourd), var. minima and var. maxima were investigated. The antioxidant content was investigated using ascorbic acid and total phenolic contents whereas the antioxidant activity was investigated using 2,2-diphenyl-1-picryl hydrazyl radical scavenging activity and ferrous ion chelating activity. The results showed that the fruits without seeds and pith and whole fruits of the two varieties of *Momordica charantia* exhibited different antioxidant content and activities. The ascorbic acid content ranged from 8.12 mg/100g to 24.46 mg/100g whereas the total phenolic content ranged from 1.47 mg GAE/100g – 27.23 mg GAE/100g. The antiradical power ranged from 4.67 to 5.94 and the ferrous ion chelating activity using the fruit extract concentration of 0.34 g/mL ranged from 10.6% to 89.3%. The effect of blanching and boiling on the antioxidant properties of fruits without seeds and pith of *Momordica charantia* var. maxima and minima was also investigated. Blanching and boiling of fruits without seeds and pith of *M. charantia* var. maxima and var. minima induced changes in the antioxidant content differently. The radical scavenging activity of the fruits without the seeds and pith of *M. charantia* var. maxima and var. minima increased as a result of blanching and boiling but their ferrous ion chelating activity became undetectable. There was no clear correlation between the antioxidant content and the antioxidant activities.

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#### E-mail addresses:

choo.wee.sim@monash.edu (Choo, W. S.),

jyyap3@student.monash.edu (Yap, J. Y.),

sycha46@student.monash.edu (Chan, S. Y.)

\* Corresponding author

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## INTRODUCTION

*Momordica charantia* L. Cucurbitaceae is commonly known as bitter gourd, bitter melon or balsam pear (Marr *et al.*, 2004; Krawinkel & Kedig 2006). It is a member of the cucurbit family commonly grown in tropical and subtropical countries. The cultivated *M. charantia* is divided into two groups, which are fruits with a diameter less than 5cm known as var. minima and fruits with a diameter more than 5cm in diameter known as var. maxima (Reyes, 1994). *M. charantia* is grown for its edible fruit in African, Asian and South American countries including the Caribbean (Basch *et al.*, 2003). The immature fruits of *M. charantia* can be prepared in several ways and the seeds and pith are usually discarded before cooking. Besides frying and cooking, they can be dehydrated, pickled or canned. Pre-treatment such as blanching or soaking in salt water is done to reduce the bitter taste. The fruits, flowers and young shoots can also be used as flavorings (Marr *et al.*, 2004; Morgan & Midmore, 2002). The whole fruit of *M. charantia* (including the seeds and pith) can be used to produce juice as a health drink. According to Morgan & Midmore (2002), the fruits are a good source of vitamin C and also provide vitamin A and B, phosphorus, calcium, potassium and iron. The mineral and vitamin concentration of *M. charantia* is superior to that of other members of Cucurbitaceae pear (Marr *et al.*, 2004). *M. charantia* possesses some medicinal properties such as anti-diabetic, anti-tumor (Budrat & Shotipruk, 2009; Fang & Ng, 2011), anticancer, anti-inflammatory,

antiviral, cholesterol lowering effects (Budrat & Shotipruk, 2009). The antidiabetic properties of *M. charantia* are due to charantin, vicine, polypeptide-p and other bioactive components such as antioxidants (Krawinkel & Kedig 2006). *M. charantia* is used as a topical internal or external treatment of wounds for management of worms and parasites infection (Grover & Yadav, 2004; Wu & Ng, 2008).

Alteration in the total antioxidant content and activity due to different processing methods is of scientific importance as it has a direct impact on dietary nutrition. Blanching is a treatment of vegetables to inactivate enzymes such as polyphenol oxidase, catalase, peroxidase, lipogenase, and chlorophyllase (Ahmad & Shivhare, 2006). Blanching is also used as surface disinfectants to destroy microorganisms and cleans the dirt of the vegetables. It makes vegetables more compact, bright in colors and also hinder the loss of vitamins (Zheng & Lu, 2011). On the other hand, boiling is a treatment that softens vegetables by breaking down the cell walls, so as to make it consumable (Yao & Ren, 2011).

The main objectives of this study were (i) to determine the ascorbic acid and total phenolic contents of whole fruits and fruits without seeds and pith of the two varieties of *M. charantia*, namely *M. charantia* var. maxima and var. minima, (ii) to evaluate their antioxidant capacity (free radical scavenging activity and ferrous ion chelating activity), and (iii) to examine the effects of thermal treatments (boiling and blanching) on the antioxidant properties of

the fruits without seeds and pith of the two varieties of *M. charantia*.

## MATERIALS AND METHODS

### *Materials*

Young, emerald green fruits of *M. charantia* var. minima and var. maxima were used in this study. The fruits were obtained from a local supermarket in Selangor, Malaysia. All chemicals and solvents were of reagent-grade level and purchased either from Sigma-Aldrich (U.S.A.) or Merck (Germany).

### *Sample Preparation*

The sample preparation was carried out according to the method of Lim *et al.* (2007) with some modifications. Whole fruits and fruits without seeds and pith for the two varieties of *M. charantia* were prepared by washing the fruits and wiping them to dryness. Seventy grams of samples were cut and crushed to a paste-like state with 25 mL of water for 2 mins (with intermittent stops to minimize heating) using a Waring blender. The homogenized sample was transferred into a 250 mL volumetric flask and top up to the mark with 50% ethanol. The mixture was mixed for 15 mins and then filtered under suction. Centrifugation was carried out a  $1500 \times g$  to obtain a clear supernatant liquid, which was used for subsequent assays. The extracts were stored at  $-20^{\circ}\text{C}$  and all tests were performed within a week. All assays were carried out in triplicate from different fruit samples.

### *Thermal Treatment*

Blanching and boiling of the fruits of the two varieties of *M. charantia* were carried out according to the modified methods of Yao & Ren (2011) and Myojin *et al.* (2008). Fruits of *M. charantia* were washed and wiped dry. The fruits were cut lengthwise to remove the seeds and pith, and sliced into 1 cm thickness. Thermal treatment of the fruits was carried out by immersing the fruit slices in boiling water for an assigned time period [blanching (4 mins); boiling (10 mins)] and drained for 1 min. Four minutes of blanching was needed to inactivate the peroxidase in the fruits. The fruit slices were then transferred to ice water bath (ratio of ice to water was 1:4) for 2 mins to halt the heating process and drained for 1 min. An uncooked sample with no treatment was used as a control. Sample extraction was then carried out according to the sample preparation method mentioned previously.

### *Ascorbic Acid Content*

The ascorbic acid content was determined by the iodine titration method (Suntornsuk *et al.*, 2002) or the RP-HPLC method: Waters Symmetry C-18 column ( $3.9 \times 100$  mm, 5  $\mu\text{m}$  particle size), mobile phase 5% acetic acid, flow-rate 0.5 mL/min and 254 nm detection wavelength. Both methods gave similar results to within 5%.

### *Total Phenolic Content*

Total phenolic content was determined according to the method of Lim *et al.* (2007). Extract (0.3 mL) was placed into test tubes followed by the addition of 1.5 mL of Folin-

Ciocalteu's reagent (diluted 10 times with water) and 1.2 mL of sodium carbonate (7.5% w/v). The test tubes were covered with parafilm, vortexed and allowed to stand for 30 mins. The absorbance was measured at 765 nm against a reagent blank. If the sample absorbance exceeded 1, the sample was diluted appropriately to give reading less than 1. A standard calibration curve was prepared by using gallic acid. Total phenolic content was expressed as gallic acid equivalents (GAE) in mg per 100g of fruits. As ascorbic acid contributes to the formation of blue molybdenum-tungsten complex, absorbance originating from it was corrected by measuring an ascorbic acid calibration curve.

#### *Free Radical Scavenging Activity using 2,2-Diphenyl-1-Picryl Hydrazyl (DPPH)*

The free radical scavenging activity was determined according to the method of Suja *et al.* (2005) with some modifications. Sample (1 mL), each with different concentrations was added into 2 mL of 0.02g/L DPPH solution in ethanol. Absorbance at 517 nm was taken after allowing the solution to stand for 30 mins. The amount of sample needed to decrease the initial DPPH concentration by 50% (EC<sub>50</sub>) was calculated graphically. The anti-radical power of extract was calculated as the reciprocal of EC<sub>50</sub>.

$$\% \text{ remaining radical} = \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

#### *Ferrous Ion Chelating Assay*

The ferrous ion chelating assay was determined according to the method of Lim *et al.* (2009). Two millimolar of iron sulphate and 5 mM of ferrozine were prepared and diluted for 20 times. Extract (1 mL), each with different concentrations was mixed with 1mL of diluted iron sulphate, followed by 1mL of diluted ferrozine. The tubes were mixed well and allowed to stand for 10 mins at room temperature. The ability of the sample to chelate ferrous ions was calculated and expressed as: Chelating effect (%) =  $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\%$

#### *Statistical Analysis*

Data were interpreted by one-way analysis of variance (ANOVA) with Duncan's multiple range test using SAS software package (SAS Institute Inc, Cary, NC, USA). The statistical significance was evaluated at p<0.05 level.

## **RESULTS AND DISCUSSION**

### *Ascorbic Acid Content*

The fruits without seeds and pith for the two varieties of *M. charantia* were higher in ascorbic acid contents as compared to those of whole fruits (Table 1). Since the same amount of *M. charantia* was used for the ascorbic acid analysis, this indicates that the ascorbic acid content in the flesh of *M. charantia* fruits was higher than that in the seeds and pith. The ascorbic acid contents of fruits without seeds and pith of *M. charantia* in this study were lower than those reported by Iqbal *et al.* (2006) [85mg/100g fruit] and Myojin *et al.* (2008) [79.7mg/100g

fruit] but were higher than those reported by Somsu et al. (2008) [3.8-8.8 mg/100g]. The variety of *M. charantia* fruits used by these authors, however, was not mentioned. This difference is most likely due to the strong influence by genotype differences and external factors such as environmental conditions, maturity stage, harvest and post-harvest practices. The ascorbic acid content of *M. charantia* fruit in this study was higher than those of banana ( $4.9 \pm 0.6$  mg/100g) and mangosteen ( $5.8 \pm 0.8$  mg/100g) but were lower than those of guava ( $144 \pm 60$  mg/100g), papaya ( $108 \pm 16$  mg/100g) and orange ( $67 \pm 9$  mg/100g) [Lim et al., 2007]. Hence, fresh *M. charantia* fruits can be a source of ascorbic acid.

The blanched and boiled samples of *M. charantia* var. maxima and var. minima showed higher ascorbic acid content as compared to the uncooked samples (Table 2). This is most likely due to the inactivation of ascorbic acid oxidase by heat induced during blanching and boiling. Heating prior to matrix disruption retains ascorbic acid effectively, as heat will first inactivate enzymes, thus avoiding the situation where enzymes are being transferred to the other parts of the fruits during matrix disruption, particularly ascorbic acid oxidase which takes part in ascorbic acid degradation (Munyaka et al., 2010). Boiled samples had significantly lower ascorbic acid content as compared to that of blanched samples (Table

TABLE 1  
Ascorbic acid content of uncooked *M. charantia* var. maxima and var. minima

|   | Ascorbic acid content (mg/100g) |
|---|---------------------------------|
| <i>M. charantia</i> var. minima, whole fruit                  | $8.12 \pm 0.14^c$               |
| <i>M. charantia</i> var. minima, fruit without seeds and pith | $13.79 \pm 2.22^b$              |
| <i>M. charantia</i> var. maxima, whole fruit                  | $8.44 \pm 1.56^c$               |
| <i>M. charantia</i> var. maxima, fruit without seeds and pith | $24.46 \pm 0.24^a$              |

<sup>abc</sup> Values with different superscript letter within a column indicate significant difference at  $p < 0.05$

TABLE 2  
Ascorbic acid content, total phenolic content, EC<sub>50</sub> and anti-radical power of fruits without seeds and pith of *M. charantia* var. maxima and var. minima after thermal treatment

|                                 | Ascorbic acid content (mg/100g) | Total phenolic content (mg GAE/100g) | EC <sub>50</sub> (mg/mL) | Anti-radical power |
|---------------------------------|---------------------------------|--------------------------------------|--------------------------|--------------------|
| <i>M. charantia</i> var. minima |                                 |                                      |                          |                    |
| Uncooked                        | $20.91 \pm 0.80^b$              | $9.33 \pm 0.77^{bc}$                 | $0.43 \pm 0.04^a$        | $2.44 \pm 0.16^d$  |
| Blanched                        | $29.53 \pm 1.87^a$              | $10.42 \pm 0.96^b$                   | $0.05 \pm 0.01^c$        | $20.93 \pm 1.59^b$ |
| Boiled                          | $23.33 \pm 2.96^b$              | $5.80 \pm 2.30^{cd}$                 | $0.06 \pm 0.01^c$        | $18.23 \pm 1.35^c$ |
| <i>M. charantia</i> var. maxima |                                 |                                      |                          |                    |
| Uncooked                        | $11.98 \pm 1.48^c$              | $14.74 \pm 2.46^a$                   | $0.38 \pm 0.04^b$        | $2.63 \pm 0.36^d$  |
| Blanched                        | $27.11 \pm 0.32^a$              | $7.60 \pm 2.53^{bcd}$                | $0.04 \pm 0.00^c$        | $24.09 \pm 1.35^a$ |
| Boiled                          | $22.32 \pm 0.63^b$              | $5.16 \pm 2.99^d$                    | $0.06 \pm 0.00^c$        | $17.35 \pm 0.22^c$ |

Values<sup>abcd</sup> with different superscript letters within a column indicate significant difference at  $p < 0.05$

2). This is in accordance with the study of Somsub *et al.* (2008) on the effect of boiling and blanching on the vitamin C content of *M. charantia* although the variety was not mentioned. Boiling, being a more intense heat treatment than blanching, caused a greater degradation of ascorbic acid.

#### *Total Phenolic Content*

The total phenolic contents of uncooked, whole fruits of *M. charantia* were higher than those of fruits without seeds and pith (Table 3). Since the same amount of *M. charantia* was used for the total phenolic content analysis, this indicates that the seeds and pith had a higher amount of phenolic compounds compared to the flesh of the fruit. The total phenolic contents of *M. charantia* fruits in this study were lower than those of Lin & Tang (2007) [ $143.6 \pm 8.4$  mg GAE/100g] and Ng *et al.* (2011) [ $582.9 \pm 47.9$  mg GAE/100g]. Again, the variety of *M. charantia* fruits used by these authors was not mentioned. The difference in the total phenolic content was due to the different sample preparation method by these authors. Lin & Tang (2007) and Ng *et al.* (2011) determined the use of total phenolic content in powder form and lyophilized form, respectively, whereas liquid extract was used in this study. The total phenolic content of *M. charantia* fruit was comparable to those of dragon fruit ( $21 \pm 6$  mg/100g) and papaya ( $28 \pm 6$  mg/100g) but was lower than those of guava ( $138 \pm 31$  mg/100g), star fruit ( $131 \pm 54$  mg/100g) and orange ( $75 \pm 10$  mg/100g) [Lim *et al.*, 2007]. Blanching and boiling process

showed different effects on the total phenolic content of the two varieties of *M. charantia* fruits (Table 2). Decreased phenolic content was found after blanching and boiling of the fruits without seeds and pith of *M. charantia* var. maxima. The decrease in phenolic compounds after blanching and boiling was in accordance with the studies of Myojin *et al.* (2008), Amin *et al.* (2006), Wen *et al.* (2010) and Miglo *et al.* (2008). These researchers reported that the phenolic compounds in the vegetables studied were sensitive to heat and the heat treatment caused a significant loss of phenolic content which leached into the water. There were no significant differences in the total phenolic content of fruits without seeds and pith of *M. charantia* var. minima after blanching and boiling compared to the uncooked samples (Table 2). This may be due to the phenolic compounds in *M. charantia* var. minima were more heat stable.

#### *Free Radical Scavenging Activity*

The concentration of a sample required to scavenge 50% of DPPH is termed as EC<sub>50</sub>. The lower the EC<sub>50</sub>, the better it was able to scavenge the radicals. The increased amount of antioxidant in a given volume of fruit extract is responsible for the increased reduction of the DPPH solution (Lim *et al.*, 2007). Phenolic compounds and ascorbic acid can act as free radical scavenger. Table 4 shows the EC<sub>50</sub> and anti-radical power of *M. charantia* var. minima and maxima. The whole fruits of *M. charantia* var. minima had higher anti-radical power compared to the fruits without seeds and pith of the same

variety of *M. charantia*. With no significant differences in ascorbic acid contents (Table 1), this is most likely due to the much higher total phenolic contents in the whole fruits with the seeds and pith (Table 3) contributing significantly to the anti-radical power. Wu & Ng (2008) reported that the free radical scavenging activity of a wild variety of *M. charantia* fruit was contributed mainly by the presence of phenolic compounds. Kubola & Siriamornpun (2008) reported a positive correlation between the radical scavenging activity and phenolic content in water extract of leaf, stem and fruit fractions of *M. charantia*. However, the EC<sub>50</sub> and antiradical power of whole fruits and fruits without seeds and pith of *M. charantia* var. maxima did not show any significant difference even though the total phenolic contents of whole fruits were significantly higher ( $p > 0.05$ ) than those of fruits without seeds and pith (Table 3). This may be due

to its much lower ascorbic acid content (Table 1).

Table 2 shows that the anti-radical power of the blanched and boiled samples was higher than that of the uncooked samples. Comparing the total phenolic content in Table 2, the scavenging activity was not proportional to the phenolic compounds, which is not in accordance with the study of Myojin *et al.* (2008) and Ng *et al.* (2011) who reported a positive correlation between the radical scavenging activity and phenolic content of the blanched and boiled samples of *M. charantia*, respectively. On the other hand, comparing the ascorbic acid content with the anti-radical power in Table 2, it can be suggested that the radical scavenging activity of *M. charantia* in this study was mainly contributed by the ascorbic acids with minor contribution from the phenolic compounds. Ascorbic acid acts as a free radical scavenger and its reaction was faster

TABLE 3  
Total phenolic content of uncooked *M. charantia* var. maxima and minima

|   | Total phenolic content (mg GAE/100g) |
|---|--------------------------------------|
| <i>M. charantia</i> var. minima, whole fruit                  | 14.82 ± 5.27 <sup>b</sup>            |
| <i>M. charantia</i> var. minima, fruit without seeds and pith | 5.92 ± 1.40 <sup>c</sup>             |
| <i>M. charantia</i> var. maxima, whole fruit                  | 27.23 ± 1.87 <sup>a</sup>            |
| <i>M. charantia</i> var. maxima, fruit without seeds and pith | 1.47 ± 0.13 <sup>d</sup>             |

<sup>abcd</sup> Values with different superscript letter within a column indicate significant difference at  $p < 0.05$

TABLE 4  
EC<sub>50</sub> and anti-radical power of uncooked *M. charantia* var. minima and maxima

|  | EC <sub>50</sub> (g/mL)   | Anti-radical power        |
|--|---------------------------|---------------------------|
| <i>M. charantia</i> var. minima, whole fruit                   | 0.17 ± 0.03 <sup>b</sup>  | 5.94 ± 0.84 <sup>a</sup>  |
| <i>M. charantia</i> var. minima, fruits without seeds and pith | 0.21 ± 0.02 <sup>a</sup>  | 4.78 ± 0.36 <sup>b</sup>  |
| <i>M. charantia</i> var. maxima, whole fruit                   | 0.21 ± 0 <sup>a</sup>     | 4.67 ± 0.11 <sup>b</sup>  |
| <i>M. charantia</i> var. maxima, fruits without seeds and pith | 0.18 ± 0.02 <sup>ab</sup> | 5.52 ± 0.70 <sup>ab</sup> |

<sup>ab</sup> Values with different superscript letter within a column indicate significant difference at  $p < 0.05$

compared to other scavenging molecules such as polyphenols (Scalzo 2008; Zhang & Hamauzu, 2003). These results indicate that the contribution of phenolics and ascorbic acids to each sample's radical scavenging activity varied markedly from one to another, depending on their levels in each sample and/or synergistic effect of antioxidants.

#### *Ferrous Ion Chelating Assay*

Primary antioxidants scavenged radicals to inhibit chain initiation and break chain propagation whereas secondary antioxidants suppress the formation of radicals and protect against oxidative damage (Lim *et al.*, 2007). Ascorbic acid could act as primary or secondary antioxidant (Akoh & Min, 2008). *In vivo*, ascorbic acid donated hydrogen atoms as a primary antioxidant. Ascorbic acid was also capable of scavenging radicals directly by converting hydroperoxides into stable products. In foods, ascorbic acid act as a secondary antioxidant with multiple functions such as scavenge oxygen, shift the redox potential of food systems to the reducing range, acts synergistically with chelators and regenerate primary antioxidants (Bauernfeind & Pinkert, 1970). By forming a stable iron (II) chelate, an extract with high chelating power reduced the free ferrous ion concentration thus decreasing the extent of Fenton reaction which caused many diseases (Halliwell & Gutteridge, 1990). The chelating activity of whole fruits and fruits without seeds and pith for *M. charantia* var. maxima and var. minima is shown in Fig.1. It was observed

that a gradual increment of chelating activity as the concentration of the fruit extract increased. The highest % chelating activity of whole fruits and fruits without seeds and pith of *M. charantia* var. maxima (0.34 g/mL) was 86.8% and 84.8%, respectively. The highest % chelating activity of whole fruits and fruits without seeds and pith of *M. charantia* var. minima (0.34 g/mL) was 89.3% and 10.6%, respectively. No study has been reported on the ferrous ion chelating activity on *M. charantia*, thus no comparison can be made. According to Lim *et al.* (2009), ferrous ion chelating activity did not correlate with the total phenolic content. In this study, ferrous ion chelating activity did not correlate with total phenolic content of whole fruits of the two varieties of *M. charantia*. It seems that ascorbic acids also do not contribute much to the ferrous ion chelating activity of fruits without seeds and pith of both varieties of *M. charantia*. The ferrous ion chelating activity was most likely due to chelating ligands in the fruits (Lim *et al.*, 2009). There was no ferrous ion chelating activity detected in the blanched and boiled samples (0.20 g/mL) [Table 5] although ascorbic acids and phenolic compounds were still detected in these samples (Table 2).

#### **CONCLUSION**

The results obtained in this study demonstrated that the whole fruits and fruits without seeds and pith of two varieties of *M. charantia* showed different levels of ascorbic acid (8.12 mg/100g – 24.46 mg/100g) and phenolic compounds (1.47 mg GAE/100g –



27.23 mg GAE/100g). Different magnitude of the free radical scavenging activity (primary antioxidant activity) and ferrous ion chelating activity (secondary antioxidant activity) of the two varieties of *M. charantia* was also demonstrated. The antiradical power ranged from 4.67 to 5.94 and the ferrous ion chelating activity using the fruit extract concentration of 0.34 g/mL ranged from 10.6% to 89.3%. The fruits of *M.*

*charantia* can be made into juice as a drink with primary and secondary antioxidant activities. The primary antioxidant activity of the fruits without the seeds and pith of *M. charantia* var. maxima and var. minima increased as a result of blanching and boiling but their secondary antioxidant activity became undetectable.

TABLE 5  
 Ferrous ion chelating (FIC) activity (%) of fruits without seeds and pith of *M. charantia* var. maxima and var. minima (0.20 g/mL) after thermal treatment

|          | FIC activity (%) |              |
|----------|------------------|--------------|
|          | Maxima           | Minima       |
| Uncooked | 44.47 ± 0.56     | 73.59 ± 3.92 |
| Blanched | Not detected     | Not detected |
| Boiled   | Not detected     | Not detected |

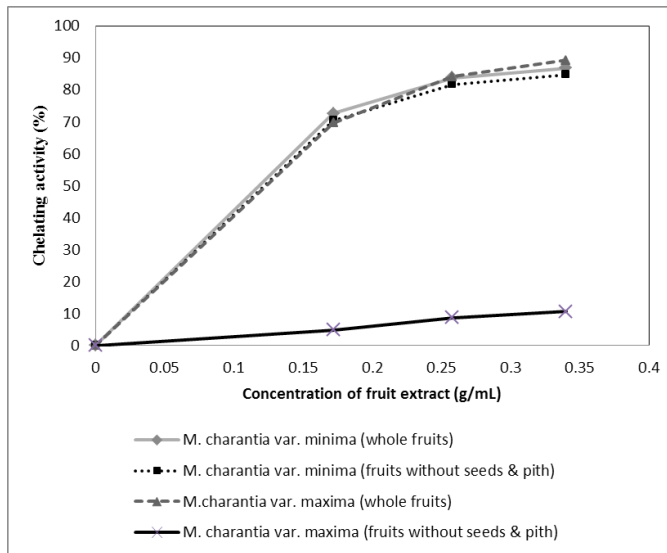


Fig.1: Ferrous ion chelating activity (%) of uncooked *M. charantia* var. maxima and minima

Note: The concentration of fruit extract was calculated by taking the weight of sample and divided it with the final volume of extract obtained.

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