Rose, a Potential Nutraceutical: An Assessment of the Total Phenolic Content and Antioxidant Activity

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Abstract: *Rosa hybrida* L. was reported to contain high total phenolic content and antioxidant activity. The scarce information on antioxidant properties of Malaysian cultivated *R. hybrid* L. had lead to the present study, which aimed to determine the effect of different solvent extraction on the total phenolic content and antioxidant activity of roses of different colours. All the 23 *R. hybrida* L. cultivars' petals extracted with 70% ethanol had significantly higher 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity compared to the water extraction. The five cultivars (03, 203, 205, 402 and M203) that comprise the highest DPPH scavenging activity were subjected to various antioxidant assays. Cultivar M203 showed highest total phenolic content (TPC) at all concentration. Cultivar M203 and 402 gave higher DPPH radical scavenging ability (EC_{50}=107.08 µg/ml) and 2,2-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) radical cation scavenging ability (EC_{50}=258.13 µg/ml). In ferric reducing antioxidant power assay, cultivar M203 has the highest trolox equivalent value at 200, 300 and 500 µg/ml concentrations while in β-carotene bleaching assay, cultivars 03, 205, and M203 (at the concentration of 500 µg/ml) showed higher antioxidant activity than synthetic antioxidant (BHA). Strong positive correlations were found between TPC and antioxidant activities, hence, suggesting that the high antioxidant activity of selected *R. hybrida* L. petals might be mainly contributed by the phenolic compounds. In general, cultivar M203 showed the best antioxidant activity with nutraceutical potential.

Keyword: Scavenging ability, solvent extraction, reducing power, *Rosa hybrida* L., total phenolic.

INTRODUCTION

Free radical is a deficient electron at the outermost electron shell, which is unstable. It is highly reactive and easily reacting with other atom and molecule [1-3]. The common free radicals are hydrogen peroxide and superoxide, which will cause oxidation process in the human body, chemical and food system [4-6] by damaging the biological components such as amino acid, nucleic acid and fatty acid [7, 8]. Hence, suggesting that diseases are strongly linked to free radicals and supplementation of the antioxidant through dietary and antioxidant supplement could greatly reduce the free radicals and oxidative stress inside the human body.

Antioxidant is a compound that is able to inactivate and reduce the amount of reactive free radical from damaging the cell, tissue, and non-living components [7, 9]. Antioxidants work by minimizing and protecting the damaging effect against free radicals through chelating, quenching, reducing and scavenging process [9, 10]. Since antioxidant plays an important role to the consumer's health [9, 11], therefore natural antioxidant is more preferable and was used to replace the synthetic antioxidant in view of the unwanted side effect [4, 5, 12].

Natural antioxidants are obtained through the consumption of vegetables, fruits and tea. These common daily foods contain natural antioxidants such as vitamins (A, C and E), phenolics, and carotenoids [5, 13-15]. Among these natural antioxidants, phenolic compound is a secondary plant phytochemical product specifically used to reduce the amount of free radical [9, 16]. Therefore, natural plant rich in phenolic compound is promoted and widely use as food additive to prevent oxidation and improve quality and nutritional value of foods, and as traditional medicine to maintain a healthy body [5, 9, 12, 16].

Rosa hybrida L, or commonly recognised as rose is one of the member of the Rosaceae family. The Rosaceae family is a large family with numerous species comprising of ornamental plant and food crops. Pellegrini [13] and Ercisli [17] reported that members of Rosaceae family contained high antioxidant capacity compared to other plant family. As a member of the Rosaceae family, *R. hybrida* L. was reported to contain high amount of phenolic content, resulting in high antioxidant activities, as well as anticancer, antiallergy and analgesic potential [18-20]. In Malaysia, the *R. hybrida* L. cultivars are mainly cultivated in Cameron Highlands, Pahang, Malaysia. The antioxidant activity of these cultivars, however, might vary with those cultivated and reported in other countries due to the environment and cultivation conditions that differ from each other, resulting in the variation of the secondary metabolite produced by the plant. Therefore, the

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present study aimed to investigate the effect of water and 70% ethanol extraction on the phenolic content and antioxidant activity of *R. hybrida* L. across a variety of colours that are cultivated in Cameron Highlands, Pahang.

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and reagents were of analytical grade. Chloroform, sodium acetate anhydrous, sodium carbonate anhydrous and Tween 40 (polyoxyethylenesorbitan monopalmitate) were purchased from Fisher Scientific (UK). Gallic acid, iron (III) chloride anhydrous, linoleic acid and 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) were purchased from Acros Organics (USA/ Belgium). Folin-Ciocalteu reagent, glacial acetic acid, hydrochloric acid, methanol, and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Merck (Germany). 2,2'-Azino-di[3-ethyl-benzthiazoline sulfonate] (ABTS) was purchased from Calbiochem (Germany). β-Carotene, butylated hydroxyanisole (BHA) 2,2-diphenyl-1-picryhydrazyl (DPPH) and potassium peroxdisulfate were purchased from Sigma-Aldrich (Germany). The distilled water used was of milipore grade (Milipore, USA).

Sample Preparation and Extraction

Different cultivated *R. hybrida* L. cultivars were purchased from Boon Sing Trading Sdn Bhd, Cameron Highlands, Pahang. The rose petals were plunked and washed with running tap water. The washed cultivar petals were placed on a clean white paper and dried at room temperature for one month. The dried samples were ground to powder using a miller (MF 10B, IKA® Werke, Germany) with 1.0 mm mesh size, vacuum packaged into a nylon-linear low density polyethylene film, stored at ambient temperature and away from direct sunlight prior to analysis.

The powdered rose petal was extracted with water and 70% ethanol at room temperature for 24 hours on an orbital shaker (LSI-1, Daihan Labtech, Korea) at 150 rpm. About 0.5 g of powdered rose powder was extracted with 25 ml solvent and the filtrates were directly used for DPPH radical scavenging assay. The five cultivars that gave the highest DPPH radical scavenging activity with the best solvent employed were then subjected to different antioxidant activity tests that include DPPH, ABTS, FRAP, and β-carotene linoleic acid bleaching assay, as well as total phenolic analysis. The antioxidant activities were evaluated as relative activities against synthetic antioxidant, BHA.

DPPH Radical Scavenging Activity

Radical scavenging activity by antioxidant in the rose petal extract was evaluated using DPPH radicals based on the method as described by Lim and Murtijaya [21] with slight modification. The DPPH radical solution was freshly prepared by dissolving 14.2 mg DPPH in 100 ml methanol. Accurately, 2 ml of methanolic DPPH radical solution was added to 1 ml of sample and the mixture was shaken vigorously for 3 second and left to stand at room temperature in the dark for 30 min. Absorbance was measured against the blank reagent at 517 nm (UviLine 9400, Secoman, France). All the measurements were carried out in triplicate. The radical scavenging activity was calculated according to the equation as follows:

$$Radical\ Scavenging\ Activity\,(\%) = \left[1 - \left(\frac{Abs_{sample}}{Abs_{control}}\right)\right] \times 100$$

ABTS Radical Cation Scavenging Activity

Determination of ABTS radical cation scavenging activity of rose cultivar crude extract was performed according to the method by Zhang *et al.* [22] with some modifications. The ABTS radical cation reagent was prepared by adding 38.4 mg of ABTS powder and 6.6 mg of $K_2S_2O_8$ to 5 ml distilled water in a covered universal bottle with cap. The mixture was kept in the dark at room temperature for 12–16 hours to allow the completion of radical generation. After that, 95% ethanol was used to adjust the absorbance of the $ABTS^{\bullet+}$ reagent to 1.500–1.600 at 734 nm (UviLine 9400, Secoman, France). A total of 1.8 ml of $ABTS^{\bullet+}$ reagent was added to 100 ml of cultivar crude extract. The mixture was allowed to stand at room temperature for 5 min after the addition. Absorbance was measured against the blank reagent at 734 nm. All determinations were performed in triplicate. The radical scavenging activities were calculated according to the equation as follows:

$$Radical\ Scavenging\ Activity\,(\%) = \left[1 - \left(\frac{Abs_{sample}}{Abs_{control}}\right)\right] \times 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing activity of mushroom extract was estimated based on the method as described by Wong *et al*. [5] with slight modification. The FRAP reagent was freshly prepared by mixing acetate buffer (pH3.6),

10 mM TPTZ solution in 40 mM HCl and 20 mM iron (III) chloride solution in proportions of 10:1:1 (v/v) respectively. The freshly prepared FRAP working reagent was warmed in 37˚C water bath (WNB 14, Memmert, Germany). Then, 1.5 ml of the FRAP reagent was added to 50 µl of rose crude extract. The absorbance was measured at 593 nm against the blank (UviLine 9400, Secoman, France) after 4 min. FRAP value was calculated and expressed as mg Trolox equivalent antioxidant capacity (TEAC) per g of crude extract. Linearity range of the calibration curve was 5–200 µg/ml (R^2 = 0.99).

β-Carotene-Linoleate Bleaching Assay

The antioxidant activity of mushroom extract was determined by β-carotene-linoleate model system as described by Ordoñez *et al.* [23] with slight modification. Firstly, β-carotene solution was prepared by dissolving 0.2 mg of β-carotene into 1 ml of chloroform. Then, 1 ml of the freshly prepared β-carotene solution was added to round bottom flask containing 0.02 ml of linoleic acid and 0.2 ml of Tween 40. After evaporation of chloroform at 50˚C (Rotary R-200, Buchi, Switzerland) for 10 min, 50 ml of oxygenated deionized water was added and the mixture was shaken vigorously to form an emulsion (β-carotene-linoleate emulsion). An aliquot of 2 ml of the reagent mixture was added to 250 µl of rose crude extract. The mixture was then shaken vigorously to form a liposome solution, and the test tubes were allowed to incubate in the water bath (WNB 14, Memmert, Germany) at 50˚C. The zero time absorbance (Abs_0) was measured at 470 nm and the absorbance was taken at time intervals (Abs_t) of 10 min for 60 min. Every sample was extracted in triplicate. Antioxidant activity percentage (AA %) was calculated using the following equation:

$$AA\% = [1 - (A_0 - A_t / A_{00} - A_{0t})] \times 100\%$$

- A_0 was initial absorbance reading of emulsion with sample at time 0;
- A_{00} was absorbance reading of the beginning incubation without sample;
- A_t was absorbance reading of emulsion with sample at time of t (10, 20, 30, 40, 50 and 60 min);
- A_{0t} was absorbance reading of emulsion without sample at time of t.

Total Phenolic Content (TPC) Analysis

The TPC analysis was performed using Folin-Ciocalteu method as described by Lim and Murtijaya [21]. A 300 µl of sample was mixed with 1.2 ml of 10 fold diluted Folin-Ciocalteu reagent followed by 1.5 ml of 7.5% sodium carbonate solution was added to the mixture. The mixture was allowed to stand at room temperature in the dark environment for 30 min. Absorbance was measured against the blank reagent at 765 nm. Gallic acid was used for the calibration curve with a concentration range of 5–150 µg/ml (R^2 = 0.99) and analyzed as above. Results were expressed as mg gallic acid equivalent (GAE)/g cultivar crude extract. All experiments were performed in triplicate.

Statistical Analysis

All analyses were performed in triplicate and averaged. Statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences) version 19. One-way analysis of variance (ANOVA) with Tukey's multiple comparisons and Pearson's correlation coefficient were performed. Significant level was defined using $p < 0.05$.

RESULTS AND DISCUSSION

DPPH assay is an assay that is commonly used to evaluate the antioxidant activity of a sample. The DPPH radical scavenging of *R. hybrida* L. cultivars extracted with water and 70% ethanol respectively is shown in Figure **1**. The DPPH radical scavenging activity of water extracted rose petal ranged from 57.96% to 80.83%. The five rose cultivars that gave the highest antioxidant activity were of mix colour cultivar, M203 (80.83%) > red colour cultivar, 102 (79.17%) > white colour cultivar, 03 (79.14%) > pink colour cultivar, 205 (77.12%) > pink colour cultivar > 203 (75.21%). The antioxidant activities of cultivar M203, 102 and 03, however, were not significantly different with one another. The DPPH radical scavenging activity of the rose samples extracted with 70% ethanol ranged from 68.97% to 89.47%. The rose cultivars that showed the highest antioxidant activity were pink colour cultivar, 205 (89.47%) > white colour cultivar, 03 (87.25%) > mix colour cultivar, M203 (86.88%) > pink colour cultivar, 203 (85.90%) > yellow colour cultivar, 402 (85.83%) with the pink colour cultivar (205) showing a significant higher antioxidant activity than the other cultivars. Cultivar 03, M203, 203 and 402 on contrary did not show significant differences in antioxidant activity with one another.

Comparison done between two solvents employed in the extraction in this study clearly showed that 70% ethanol was able to extract the metabolic compounds in rose petal more effectively and efficiently. This could

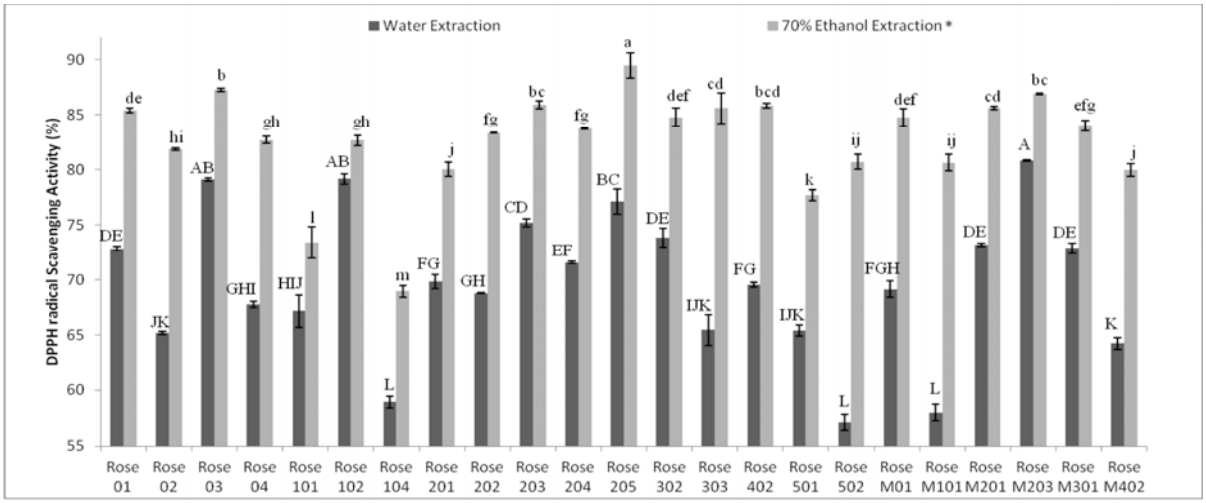

Figure 1: DPPH radical scavenging activity of 23 different *R. hybrida* L. cultivars.

Note: Within each extraction media, bars with different alphabet letters (capital: water extraction, small letter: 70% ethanol extraction) were significantly different ($p < 0.05$).

*70% ethanol extraction was significantly higher than water extraction for all cultivars ($p < 0.05$).

be explained as the differences in the solvent polarity and solubility that has a direct correlation with the hydrophobic and hydrophilic characteristic of the individual antioxidant compounds in a sample [4, 5, 21, 24]. In addition, the organic and volatile solvents are found to be more efficient in degrading the plant cell wall and thus, able to extract higher amount of endocellular materials including the antioxidant compound [21, 25]. Therefore, 70% ethanol was chosen as the extraction solvent for the five selected cultivars (03, 203, 205, 402 and M203) which is later subjected to DPPH radical scavenging assay, ABTS radicals cation scavenging assay, ferric reducing/antioxidant power (FRAP) assay, β-carotene linoleic acid bleaching (BCB) assay and total phenolic content (TPC) assay.

In both extraction, the four cultivars (03, 203, 205 and M203) are either in white colour, pink colour or consists both colours; contain higher DPPH scavenging activity than the red base cultivar in general. Zeng *et al.* [25] on the contrary, found that the fresh red *R. hybrid*a L. cultivar's petal contain higher phenolic content and antioxidant capacity than other colour while Vinokur *et al.* [19] also found that red and white colour air dried *R. hybrida* L. cultivar's petal have the highest total phenolic content and antioxidant capacity, as well as strong correlation between petal colours and anthocyanin content. The red colour cultivars are relatively high in anthocyanin content and thus, results in high antioxidant activity. Although they found red colour rose generally gave higher antioxidant activity, they also found that not all the cultivars that contribute less anthocyanin content exhibit a lower antioxidant activity, suggesting that rose petals contain other phenolic compounds that contribute significantly to the antioxidant activity apart from anthocyanin alone.

The total phenolic content of the five selected *R. hybrida* L. cultivars is shown in Figure **2**. Cultivar M203 has the highest TPC (139.82 ± 0.65 mg GAE per g of extract) followed by cultivar 03 (136.48 ± 0.52 mg GAE per g of extract), cultivar 203 (122.75 ± 0.45 mg GAE per g of extract), cultivar 205 (106.59 ± 0.15 mg GAE per g of extract) and cultivar 402 (101.78 ± 0.17 mg GAE per g of extract). The high amount of phenolic content in the Malaysian cultivated rose also showed a similar pattern when compared to previous rose studies as reported by Vinokur *et al.* [19] and Zeng *et al.* [25]. Various studies carried out on plants had showed that the changes of climatic conditions and ecological environment, indeed results in the variation of secondary metabolite production due to the different biotic and abiotic stress on the plant [26-29]. The variation, however was not observed in the current study, suggesting that rose species, even though cultivated in a different environment conditions, would still produce a great amount of secondary metabolite that constitute high phenolic content, which in turn results in high antioxidant activity.

The DPPH radical scavenging activities and ABTS cation radical scavenging activities of the rose cultivars used in the current study generally increase by the increase of the extract concentration. The average DPPH EC_{50} value of all cultivars is 121.2 µg/ml. In Figure **3**, cultivar M203 had the lowest DPPH EC_{50} value (107.08 µg/ml) and is comparable to cultivar 03

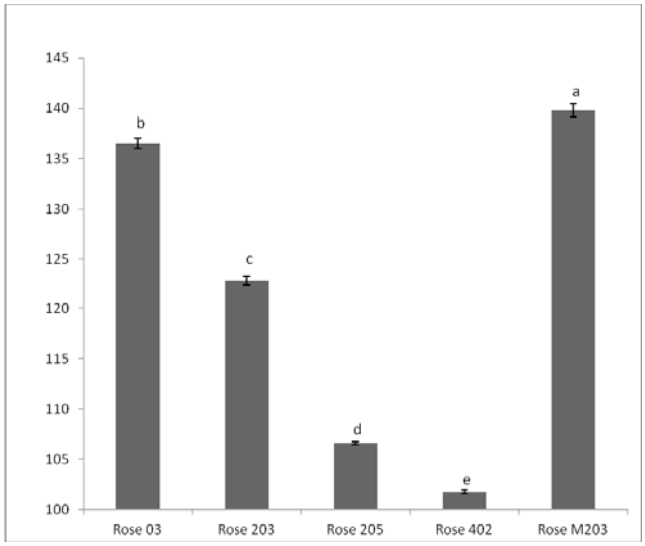

Figure 2: Total phenolic content of five selected *R. hybrida* L. cultivars.

Note: Bars with different letters were significantly different ($p < 0.05$).

(107.80 µg/ml) while cultivar 402 had the highest DPPH EC_{50} value (170.13 µg/mL) and was significantly higher than other cultivars. On the other hand, cultivar 402 also showed lowest ABTS EC_{50} value (258.13 µg/ml) and yet was not significantly lower compared with cultivar 205 (267.42 µg/ml). The average ABTS EC_{50} value of all the cultivars is 293.88 µg/ml. The EC_{50} value of the remaining three cultivars are 203 (285.07 µg/ml) < M203 (319.11 µg/ml) < 03 (339.65 µg/ml) in an increasing order. When it is compared to the positive control (BHA), the same concentration of the

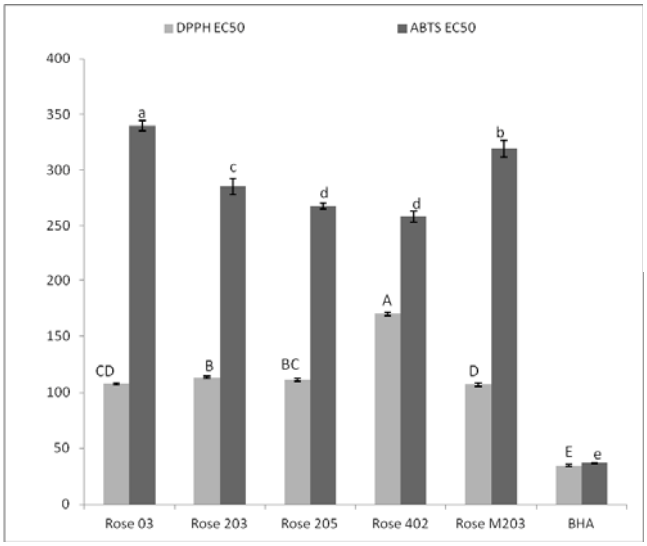

Figure 3: DPPH radical scavenging activity of five selected *R. hybrida* L. cultivars and BHA.

Note: Within each scavenging assay, bars with different alphabet letters (capital: DPPH free radical scavenging assay, small letter: ABTS cation scavenging assay) were significantly different ($p < 0.05$).

crude extract demonstrates a lesser activities. This could be explained as the crude extract contained a mixture of compounds that is potentially affected by other environment factor, resulting in lower scavenging activity as compared to the synthetic antioxidant that is more stable.

The TEAC values of the five selected rose cultivars generally increase with the increase of extract concentration. Among the five selected cultivars, cultivar M203 had the highest TEAC value; 325.73 ± 2.96 mg TEAC per g of extract, followed by cultivar 205; 312.89 ± 6.44 mg TEAC per g of extract, cultivar 203; 385.63 ± 3.56 mg TEAC per g of extract, cultivar 03; 251.55 ± 4.82 mg TEAC per g of extract, and cultivar; 221.40 ± 6.50 mg TEAC per g of extract. Therefore, cultivar M203 contains highest electron donation capacity, while cultivar 402 contains lowest electron donation capacity to reduce the ferric-TPTZ. In this assay, the rose cultivars have different TEAC value at different concentration. Each individual activity was differed by their dose-response characteristics, but the response of the respective antioxidant is linear. Hence, the antioxidant activity in FRAP assay is not affected by concentration [7].

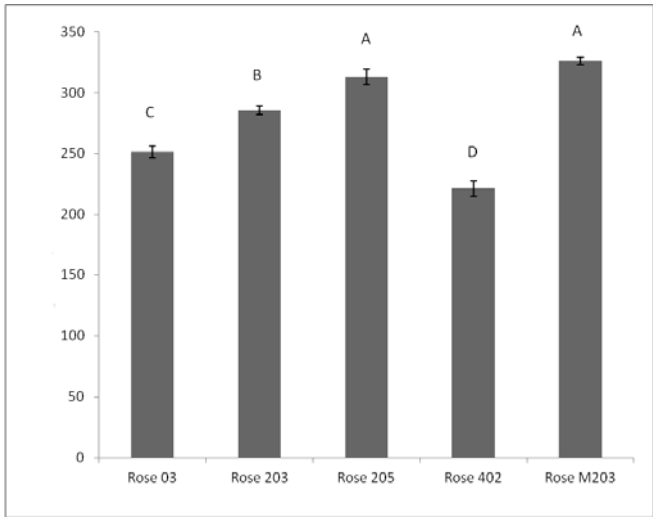

Figure 4: Trolox equivalent antioxidant capacity (TEAC) of five selected *R. hybrid* L. cultivars.

Note: Bars with different letters were significantly different ($p < 0.05$).

The antioxidant effects of five selected cultivars on BCB assay are presented into two forms: the protection ability of 500 µg/ml concentration in an absorbance reading and as the antioxidant activity percentage that shows the effect of scavenging ability at different concentration on the peroxyl free radical. Rose cultivar 203, 205 and 402 had slight decrease of absorbance reading during the incubation period. On contrary,

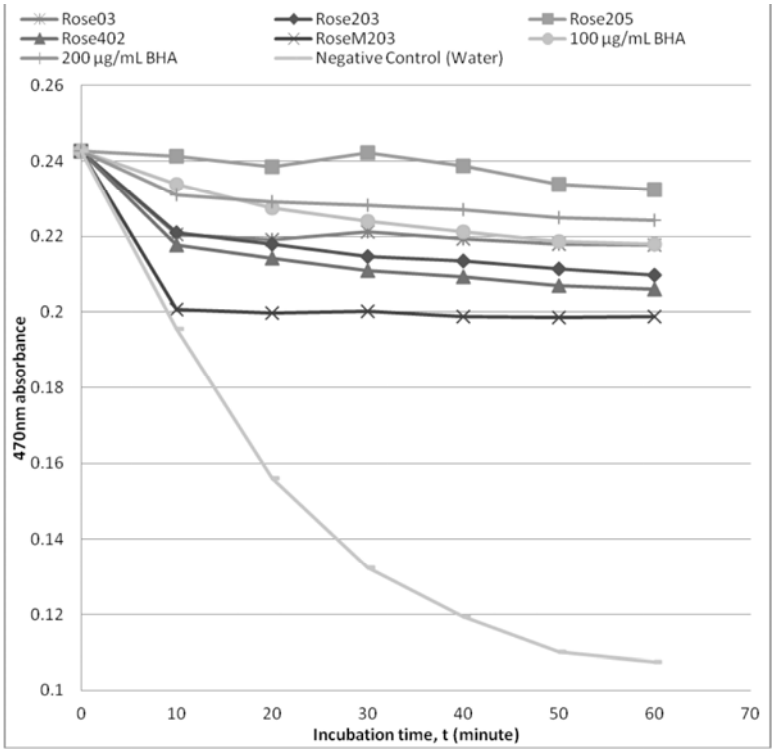

Figure 5: Absorbance reading at 470 nm versus incubation time line graph of five selected *R. hybrid* L. cultivars (500 µg/ml) and BHA.

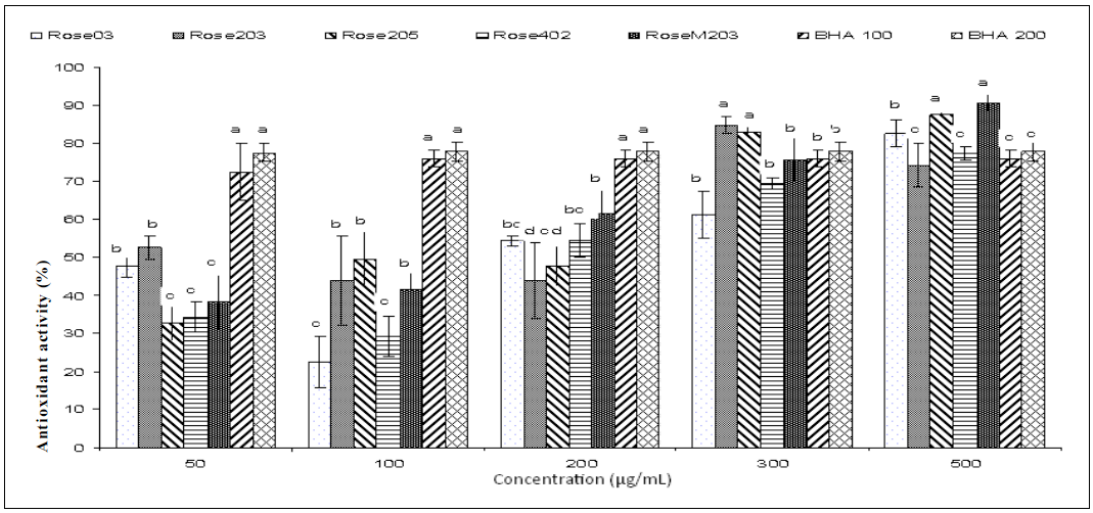

Figure 6: Antioxidant activity (as measured by β-carotene bleaching inhibition) at different concentrations of five selected *R. hybrida* L. crude extract and 100, 200 µg/ml of positive control BHA.
Note: Bars with different letters at respective concentration were significantly different ($p < 0.05$).

cultivar 03 had slight increase of absorbance reading during the incubation period while cultivar M203 showed constant absorbance reading after 10 minutes incubation period. At the 60 minutes of incubation, cultivar 205 showed highest absorbance reading at 500µg/mL concentration, which is higher than the positive control (100 µg/ml and 200 µg/ml) in protecting the β-carotene from being bleached.

The overall results of antioxidant activity percentage indicated that cultivar 205 and M203 had the highest antioxidant activity percentage to protect the β-carotene from being bleached by the linoleic acid at both low and high concentration respectively. Thus, suggesting that β-carotene linoleic acid bleaching assay is dose-dependent. Mariod [30] found that there are no correlation between TPC and BCB. This is due to TPC indicates the presence of lipophilic and

Table 1: Pearson Correlations Between TPC and Different Antioxidant Assays of Five Selected *R. hybrida L. cultivars*

Sample	DPPH	ABTS	FRAP	BCB
03	0.863	**0.999****	**0.999****	0.861
203	0.892	**0.998****	**0.997****	0.717
205	0.884	**0.996****	**0.999****	**0.913***
402	**0.976***	**0.998****	**0.996****	**0.934***
M203	0.864	**0.995****	**0.999****	**0.983****

The correlation was applied between total phenolic content and four different antioxidant assays (DPPH, ABTS, FRAP, and BCB). Data sets with strong correlation (> 0.900) are bolded.
Correlation is significant at *$p < 0.05$; ** $p < 0.01$.

hydrophilic compound. However, BCB is only an indicator of the presence of the lipophilic compounds.

The correlation between TPC and antioxidant assays of the five selected cultivar was analysed using Pearson's correlation analysis and the results are shown in Table **1**. Most of the cultivars have shown strong correlation values between TPC and antioxidant assays. Generally, the high correlation value of the assays done on the five selected *R. hybrida* L. indicate that these roses have excellent hydrogen and electron donation capacity to scavenge free radicals and ability to reduce transition metals. In addition, cultivar 205, 402 and M203 also had high chain breaking ability. Wong [5] found that high correlation between TPC and antioxidant assays possibly imply the presence of high phenolic content. In addition, Vinokur [19] and Zeng [25] also found that rose flower has significant correlation between phenolic content and free radical scavenging activity, which is similar to the results obtained in this study. Consequently, we concluded that the phenolic contents in the rose petals could be the prime component that contributed to the high antioxidant activity. Therefore, these Malaysian cultivated roses are a promising and potential source that could be developed into various nutraceuticals to replace the synthetic antioxidant.

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STATEMENT OF AUTHORS' CONTRIBUTIONS TO MANUSCRIPT

VFV and HSY designed the study; SCP and VFV conducted the research; VFV, SCP, and HSY analyzed data and wrote the manuscript. HSY had primary responsibility for final content. All authors read and approved the final manuscript.

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