Antioxidant Potential of *Carissa carandas* Seed Comparing to The Dietary Supplement Products

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Abstract

Carissa carandas is well known for its various medicinal properties for example, it can be used to treat the liver dysfunction, break fever and to counteract the putrefaction of blood. The purposes of this study were to analyze the antioxidant capacity, which included total phenolic content, ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, total anthocyanin content, proanthocyanins, ascorbic acid content and tyrosinase inhibitory activities determination comparing to dietary supplement products. A seed of *C.carandas* showed the highest value of total phenolic content, FRAP, DPPH and proanthocyanidins that were 256.42 mgGAE/g, 2104.31 μmol$FeSO_4$/g, 285.06 mgAAE/g and 465.69 mgCE/g, respectively. Cranberry extract was found to have higher rate of tyrosinase inhibition (27.74 mgKAE/g) than other samples. In this study, the bilberry extract showed the highest amount of total anthocyanins with 130.60 mg/L(cyanidin-3-glucoside), and contained the greatest amount of vitamin c with 123.93 mg/g. This study revealed that *C. carandas* seeds were rich in natural antioxidant. Our discovery suggested that the extract from *C. carandas* seeds have good commercial potential as a promising antioxidant for using in dietary supplement food as well as cosmetics.

Keywords: *Carissa carandas,* Antioxidant, Dietary supplement product, Anthocyanins, Proantocyanidins

1. Introduction

Nhamdaeng (Thailand), Caramba (Philippines) and Ci huangguo (Chinese) are the local name of *Carissa carandas*. Parts of this plant are used in traditional medicine in Thailand. *C. carandas* leaves can be used as an easily accessible source of natural antioxidants. Unripe fruits can be used to treat the liver dysfunction, break fever and to counteract the putrefaction of blood (Wiart, 2006). In addition, fully-ripe fruits are a good source of anthocyanin and phenolic compound, whereas unripe fruits are a good source of vitamin C (Pewlong *et al*., 2014; Prasad *et al*., 2010). In terms of Bilberry (*Vaccinium myrtillus* L.), it is the rich source of anthocyanins and contains diverse anthocyanins, such as delphinidin and cyanidin glycosides (Scalzo *et al*., 2008). Grape seed extract (GSE) and cranberry extract are recommended for using as a source of the antioxidant. The component of interest is polyphenols, mainly proanthocyanidins which are condensed tannins (Blumberg *et al*., 2013; Weh *et al*., 2016).

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Nowadays, the GSE, cranberry extract and bilberry extract product are expensive for the consumers in Thailand and other countries. Thus, the aim of this study was to investigate the potential of the antioxidant of *C. carandas* seeds comparing to other parts and dietary supplement products. Product development would create an added value to this plant and provide an alternative for the consumers who prefer the health benefits of natural antioxidants.

2. Materials and Methods

The experimental materials of *Carissa carandas* were pulp, bark, seed, fruit and leaf that were collected from Thailand Institute of Nuclear Technology's area in Nakhon Nayok Province, Thailand. The samples of dietary supplement products were cranberry, bilberry and grape seed extracts that were purchased from the supermarket in Bangkok. VISTRA (grape seed extract) and BLACKMORE (cranberry and bilberry extract) were the brands of the dietary supplement products that were selected in this study

2.1 Sample extraction

The fresh samples of *Carissa carandas* were investigated in this study. In term of dietary supplement products, grape seed extract and bilberry extract tablets were ground to powder for an experiment, and cranberry extract capsules were released to get the inside powder for study. Briefly, 0.5 g of samples were extracted with 50 mL of 40% ethanol under shaking condition at 40 rpm in RT for 24 h. The sample suspensions were centrifuged, and the supernatant was filtered through a filter paper No.4, and the filtrate was stored at –20ºC before the analysis.

2.2 Chemical content

2.2.1 Total phenolic content

The total phenolic content was estimated by using the Folin-Ciocalteu assay according to the method developed by Velioglu *et al*. (1998). First, 0.75 mL of 10-fold diluted Folin-Ciocalteu reagent and 100 µL of each sample extract were placed in a test tube. The mixture was mixed and allowed to stand at room temperature for 5 min. Then, 0.75 mL of 6% (w/v) sodium carbonate solution was added. The mixture was homogenized and allowed to stand at room temperature for 90 min. The total phenolic content was determined via the absorbance measurements at 725 nm by using a spectrophotometer(Cintra 10_e, model Cintra Double Beam). The standard calibration curve was plotted by using gallic acid at the concentrations of 0.02–0.1 mg/mL. The total phenolic content was expressed in mg gallic acid equivalent (GAE)/g sample.

2.2.2 Total anthocyanin content

Total anthocyanin content was measured by the pH-differential spectrophotometric method (Wrolstad et al., 2005). Each sample was diluted with potassium chloride (0.025 M) at pH 1.0 and sodium acetate (0.4 M) at pH 4.5. They were allowed to equilibrate for 15 min before a detection by spectrophotometer. The absorbance was measured at 520 nm and 700 nm. The difference in the absorbance at different pH values and wavelengths was calculated as:

$$A = (A520\ nm - A700\ nm)\ pH1.0 - (A520\ nm - A700\ nm)\ pH4.5.$$

The concentration of total anthocyanin pigments was calculated as:

$$\text{Total anthocyanin content (mg/L)} = (A \times MW \times DF \times 1000)/ \varepsilon \times 1$$

Where MW is the molecular weight, DF is the dilution factor, ε is the molar absorptivity, and 1 is for 1 cm path length. The molecular weight (MW = 449.2 g mol^{-1}) and the molar absorptivity (ε = 26,900 L cm^{-1} mol^{-1}) of cyanidin-3-glucoside were used.

2.2.3 Proanthocyanidins

Proanthocyanidins in the samples were determined by vanillin-HCl assay (Sun *et al*., 1998). 1 mL of the sample solution in a test tube, 2.5 mL of 1% vanillin solution in methanol and 2.5 mL of 9 M HCl in methanol were added. The reaction mixture was incubated for 20 min at 30ºC and the absorbance at 500 nm was measured. The standard calibration curve was plotted using catechin at concentrations ranging from 0.05–0.30 mg/mL. The proanthocyanidins was expressed in mg catechin equivalent (CE)/g sample.

2.2.4 Vitamin C content

Ascorbic acid was analyzed by HPLC (Jasco, model PU980, serial NO.D3925845, Japan) with a UV detector. The separation was carried out using a C18, 300x3.98 mm, 10 micron, Phenomene x column and the run time was 15 min. The peak of ascorbic acid was detected at 205 nm. The entire chromatographic separation was performed at an isocratic mobile phase of methanol and 0.05 M potassium dihydrogen phosphate (pH 3.0) at the ratio 3:97 v/v with flow rate 1 mL/min.

2.3 Bioactivity properties

2.3.1 Ferric-ion reducing antioxidant potential (FRAP)

FRAP assay was performed according to the method described by Benzei and Strain (1996). The FRAP reagent was prepared by mixing 16.7 mM $FeCl_3.6H_2O$ and 8.3 mM 2,4,6-tripyridyl-s-triazine (TPTZ) with 250 mM acetate buffer, pH 3.6. A total of 75 µL sample and 225 µL of distilled water were added to 2.25 mL of freshly prepared FRAP reagent in a test tube. The mixture was incubated at room temperature throughout the reaction. The absorbance was read at 596 nm by using a spectrophotometer (Cintra 10_e, model Cintra Double Beam) 30 min after mixing. The antioxidant potential of the samples were analyzed

based on a calibration curve plotted by using $FeSO_4.7H_2O$ at concentrations ranging from 100 to 500 µM.

2.3.2 Free radical scavenging power (DPPH assay)

Determination of free radical scavenging power was performed as previously described by Khattak *et al*. (2008) with slight modifications. 100 µL of each extract was added to 900 µL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol solution (150 µM) and the solution was shaken vigorously. After incubation at room temperature for 15 min in the darkness, the absorbance of each solution was determined at 517 nm. The free radical scavenging power was expressed as ascorbic acid equivalent (AAE)/g sample.

2.3.3 Tyrosinase inhibitory activity

Tyrosinase inhibitory activity was determined by using the method described by of Masamoto et al. (2003) with some modifications. Tyrosinase inhibitory activity assays were performed with L-DOPA as substrate. The solvent of sample (350 µL) was added to the mixture of tyrosinase solution (100U/mL, 350 µL) (Tyrosinase solution was prepared from Tyrosinase from mushroom, Sigma, lot SLBD9504V), sodium phosphate buffer pH 6.8 (0.02 M, 350 µL) and L-DOPA (0.3 mg/mL, 350 µL). After that, the reaction mixture was incubated at 37ºC for 30 min and the absorbance at 450 nm was measured. The calibration curve was plotted using kojic acid at concentrations ranging from 0.02 to 0.10 mg/mL.

2.9 Statistical analysis

The data were reported as the means ± SD of three replicated determinations. Where appropriate, the data were tested by one-way ANOVA using IBM SPSS v.19, followed by Duncan post hoc test. Differences of $p<0.05$ were considered significantly.

3. Results and Discussion

The results of total phenolic content in different parts of *C. carandas* and three dietary supplement products were determined by Folin-Ciocalteu assay that were shown in Figure 1. Pulp, bark, fruit and leaf exhibited the lowest total phenolic content among all samples with 0.93, 5.24, 3.36 and 14.29 mgGAE/g, respectively. Meanwhile, *C. carandas* seed showed the highest total phenolic content with 256.42 mgGAE/g. Interestingly, the total phenolic content was found in *C. carandas* seed much more than the dietary supplement products. Grape seed, cranberry and bilberry extract demonstrated the total phenolic content at 104.96, 114.82 and 10.47 mgGAE/g, respectively.

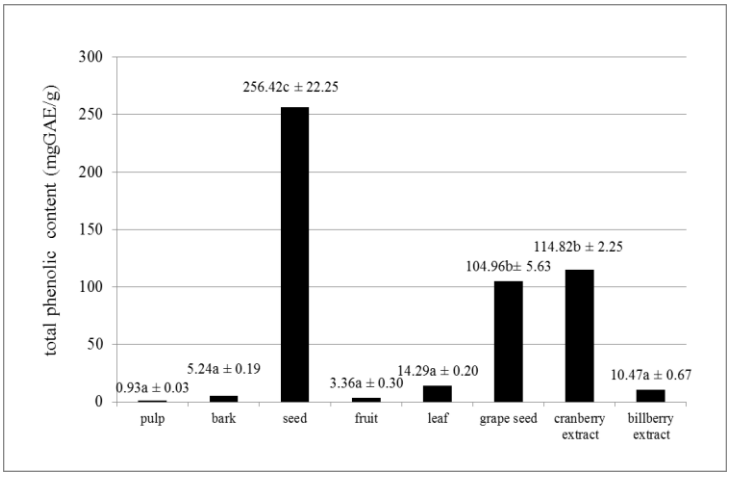

Figure 1 Total phenolic content of different parts of *C. carandas* and three dietary supplement products.

Note: Each value is expressed as mean ± standard deviation (n = 3).

Means with same letters are non-significantly different ($p \geq 0.05$). GAE = Gallic acid equivalent

The Ferric-ion reducing antioxidant potential (FRAP) of the samples was determined by using a modified Fe3+ to Fe2+ reduction assay, whereby the yellow color of the test solution was changed to various shades of blue, depending on the reducing power of the samples. The presence of antioxidants in the samples would cause the reduction of the Fe3+/ferricyanide complex to the Fe2+ form, where Fe2+ was monitored by measuring the absorbance at 596 nm. In Figure 2, *C. carandas* seed showed outstanding ferric reducing ability compared to other extracts. The value of FRAP can be sequenced in the following order: seed > cranberry extract > grape extract > bilberry extract.

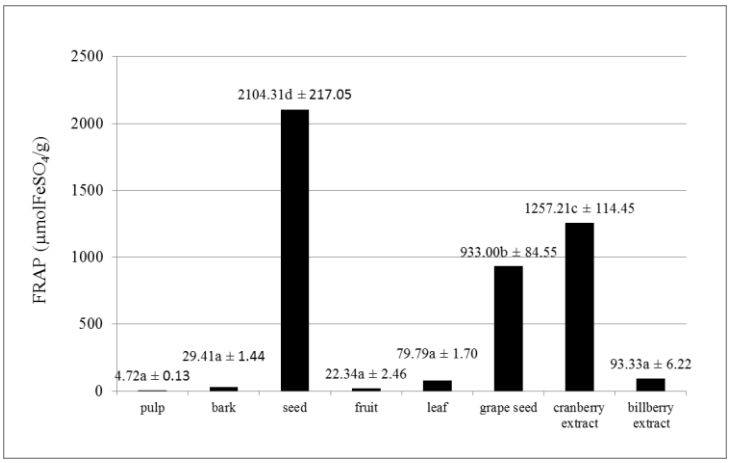

Figure 2 Ferric-ion reducing antioxidant potential (FRAP) of different parts of *C. carandas* and three dietary supplement products.

Note: Each value is expressed as mean ± standard deviation (n = 3).

Means with same letters are non-significantly different ($p \geq 0.05$).

DPPH assay is one of the most widely used methods for screening the antioxidant activity of plant extracts. The assay is based on the measurements of the ability of the antioxidants to scavenge the stable radical DPPH. Figure 3 represented the DPPH radical scavenging activity in different parts of *C. carandas* and three dietary supplement products (grape seed extract, cranberry extract and bilberry extract). This study revealed that the DPPH radical scavenging activity in *C. carandas* seed was highest with 285.06 mgAAE/g and followed by grape seed extract and cranberry extract which were 120.13 and 109.50 mgAAE/g, respectively.

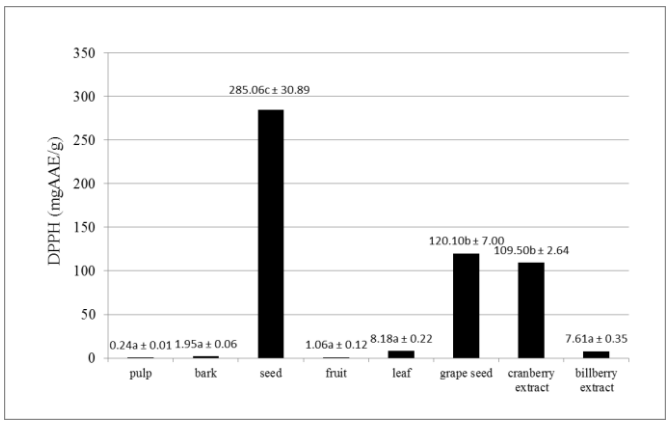

Figure 3 Free radical scavenging power (DPPH assay) of different parts of *C. carandas* and three dietary supplement products.

Note: Each value is expressed as mean ± standard deviation (n = 3).

Means with same letters are non-significantly different ($p \geq 0.05$). AAE = Ascorbic acid equivalent

The tyrosinase inhibitory activities of each sample was determined based on the obstruction of the conversion of substrate of tyrosinase, L-DOPA into dopachrome. In this study showed that cranberry extract was the highest on activities of the tyrosinase inhibition with 23.74 mgKA/g and followed by *C. carandas* seed which exhibited 11.39 mgKA/g.

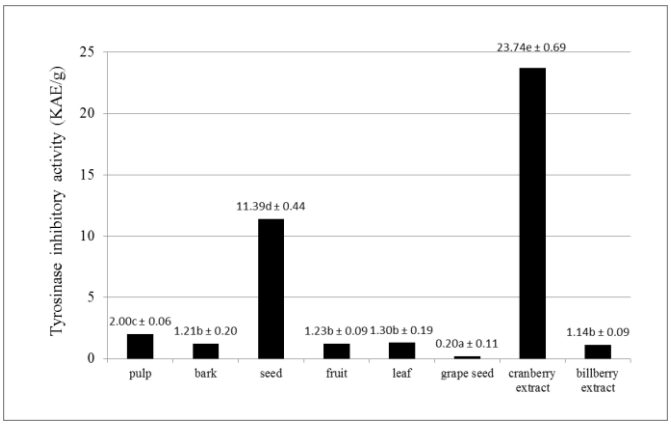

Figure 4 Tyrosinase inhibitory activity of different parts of *C. carandas* and three dietary supplement products.

Note: Each value is expressed as mean ± standard deviation (n = 3).

Means with same letters are non-significantly different $p \geq 0.05$). KAE = Kojic acid equivalent

This study, proanthocyanidins was determined by vanillin-HCl assay. In Figure 5, *C. carandas* seed showed the highest of proanthocyanidins content with 465.69 mgCE/g, followed by grape seed extract with 139.35 mgCE/g.

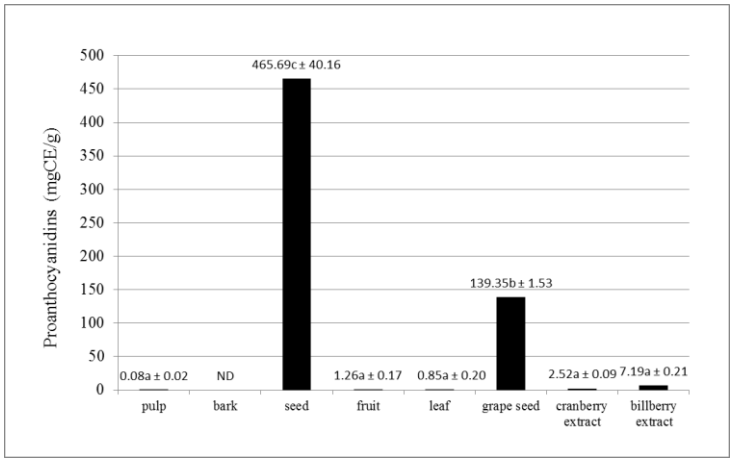

Figure 5 Proanthocyanidins of different parts of *C. carandas* and three dietary supplement products.

Note: Each value is expressed as mean ± standard deviation (n = 3). Means with same letters are non-significantly different ($p \geq 0.05$). CE = Catechin equivalent, ND = not detected

Total anthocyanin content was determined by pH-differential spectrometric method. Total anthocyanin content in pulp and bark of *C. carandas* were non-detectable (Figure 6). Bilberry extract contained the highest amount of anthocyanins with 130.60 mg/L (cyanidin-3-glucoside) followed by *C. carandas* seed with 14.27 mg/L (cyanidin-3-glucoside).

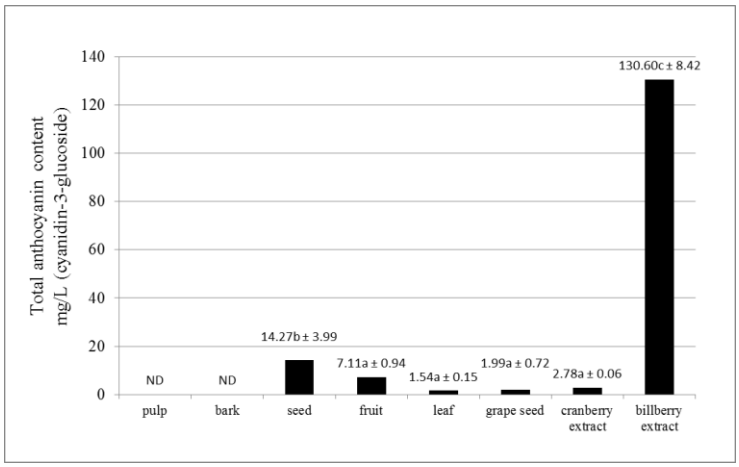

Figure 6 Total anthocyanin content of different parts of *C. carandas* and three dietary supplement products.

Note: Each value is expressed as mean ± standard deviation (n = 3). Means with same letters are non-significantly different ($p \geq 0.05$). ND = not detected

In this study, ascorbic acid content in different parts of *C. carandas* and three dietary supplement products were determined by HPLC method (Figure 7). Cranberry extract showed the highest amount of ascorbic acid with 123.93 mg/g. Meanwhile, there were not detected in the pulp and bark of *C. carandas*. There were found a slight amount of ascorbic acid in seed 1.87 mg/g, fruit 0.52 mg/g, leaf 0.94 mg/g, grape seed extract 0.54 mg/g and bilberry extract 0.46 mg/g.

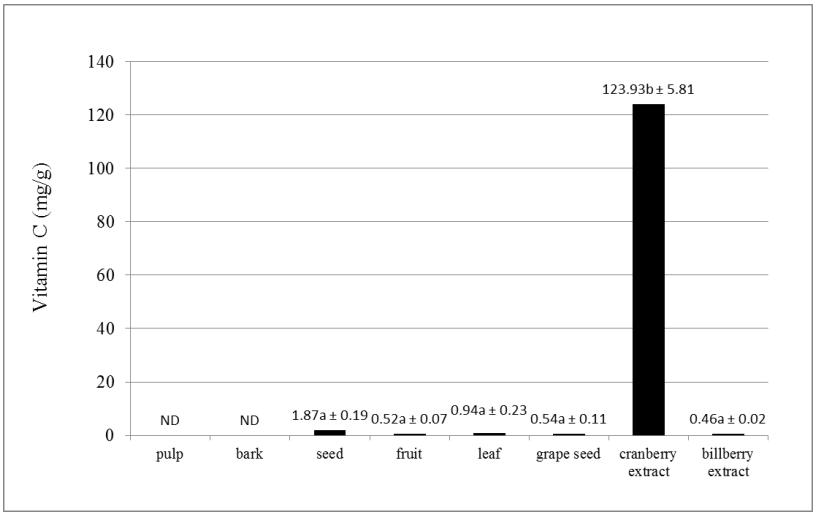

Figure 7 Vitamin C content of different parts of *C. carandas* and three dietary supplement products.

Note: Each value is expressed as mean ± standard deviation (n = 3). Means with same letters are non-significantly different ($p \geq 0.05$). ND = not detected

Contreras-Calderon *et al*. (2011) studied on the antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. They found that the antioxidant capacity and total phenolic content in seeds were greater than those of edible parts of fruit. They conclude that by-products with a high antioxidant potential, could be usefully studied by the food, pharmaceutical or cosmetic industries for the development of diverse products. Likewise, Soong and Barlow (2004) exhibited that the seeds of avocado, jack fruit, longan, mango and tamarind showed a much higher antioxidant activity and phenolic content than the edible portions. In term of apple, Xu *et al*. (2015) studied on phenolic compounds and antioxidant activity in apple seeds of seven cultivars. They revealed that apple seeds showed a higher antioxidant activity than its peels or flesh.

The high of polyphenolic content of *Carrissa carandas* seed was correlated with the high in antioxidant activity as illustrated in Figure 2 and 3. The mechanism of relation between antioxidant agents and antioxidant activities has been described that the antioxidants scavenge free radicals of foods by donating hydrogen to them, and they produce relatively stable antioxidant radicals with low standard reduction potential, and the adequacy of antioxidants to scavenge free radicals relies upon the bond dissociation energy between

oxygen and a phenolic hydrogen, pH related to the acid dissociation constant, and reduction potential and delocalization of the antioxidant radicals (Cao *et al.*, 2007).

4. Conclusion

The present study demonstrated a significantly higher antioxidant capacity of fruit seeds than those of other portions. This study showed that the total phenolic content, FRAP, DPPH and proanthocyanidins in seeds of *C. carandas* were higher than those of *C. carandas* parts and three dietary products, while the highest amount of vitamin c and tyrosinase inhibitory activities were found in the cranberry extract product. Surprisingly, this study indicated that proanthocyanidins content (an important bioactive compound in grape seeds) in *C. carandas* seeds were higher than those in grape seed extract product. Thus, the seed of *C. carandas* appeared to be an interesting material with a potential for development as a healthy product in the future.

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