Research article

Antioxidant Activity of Crude Extract from Raw and Heat-treated *Moringa oleifera*

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Abstract The aim of this research was to investigate the effect of heat treatment on antioxidant properties of crude extract from *Moringa oleifera* (peel, pulp, and seed). Total phenolic content and antioxidant properties based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), and metal chelating activity were investigated. Results revealed that crude extract obtained from raw moringa peel showed the highest total phenolic content and DPPH radical scavenging activity (3.65 mg gallic acid eq./g DW and 1.35 mg Trolox eq./g DW), followed by pulp (3.44 mg gallic acid eq./g DW and 1.25 mg Trolox eq./g DW) and seed (2.80 mg gallic acid eq./g DW and 0.91 mg Trolox eq./g DW), respectively. The highest FRAP were obtained from the raw moringa peel extract (3.62 mg ferrous eq./g DW), whereas the highest metal chelating activity was found in raw moringa pulp extract 0.42 mg EDTA eq./g DW). All samples with heat treatment at 100 °C for 15 min showed lower total phenolic content and antioxidant properties than crude extract from raw moringa samples ($P<0.05$).

Keywords *Moringa oleifera*, heat treatment, antioxidant activity

INTRODUCTION

In recent years, attention has been focused on antioxidants derived from natural sources, due to the fact that antioxidative activity of extracts from plants or animals, in some cases, is similar or higher than that of commonly used synthetic antioxidants such as butylated hydroxytoluene (BHA) and butylated hydroxylanisole (BHT), which are quite unsafe and their toxicity is a problem of concern (Hossain et al., 2008). Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential in various oxidative reaction systems have been discovered. The antioxidant properties include scavenging or quenching of reactive oxygen species (ROS)/free radicals and inhibition of ROS induced oxidation of biological macromolecules such as lipids, proteins, and DNA. Other mechanisms of antioxidant activity include transition metal chelating activity and ferric reducing power (Arabshahi-Delouee et al, 2007).

Moringa oleifera is a widely cultivated tree considered as a multi-purpose plant which can be found in tropical and subtropical climates and contains various phytochemicals such as carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolics (Siddhuraju and Becker, 2003; Upadhyay et al., 2015). Almost all the parts of this plant such as root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been reported as source of different biochemical compounds with anticarcinogenic, antiinflammatory, antidiabetic, antioxidant,

and antimicrobial effects (Chuang et al., 2007; Upadhyay et al., 2015). *M. oleifera* leaf was found to have high source of protein, beta-carotene, vitamin C, iron, potassium, and other nutrients (Jongrungruangchok et al., 2010). Extracts from *M. oleifera* roots and flowers were found to have a significant hepatoprotective effect (Ruckmani et al., 1998).

The effect of food processing procedure on the antioxidant activity of foods are generally the result of different processing. Thus, the evaluation of processing factors influencing the antioxidant activity is imperative to increase or preserve their lability (Nicoli et al., 1999). Several studies have reported the antioxidant potential of *M. oleifera* leaf (Jongrungruangchok et al., 2010; Sreelatha and Padma, 2009; Verma et al., 2009; Vongsak et al., 2013). Only a few studies have reported the antioxidant activity of Moringa peel, pulp, and seed and their ability after heat process. Therefore, this research aimed to investigate the effect of heat treatment on antioxidant properties of crude extract from *Moringa oleifera* (peel, pulp, and seed).

OBJECTIVE

The objective of this study was to investigate the effect of heat treatment on antioxidant properties of crude extract from *Moringa oleifera* (peel, pulp, and seed).

METHODOLOGY

The *Moringa oleifera* pods were purchased from a commercial market in Phathumtani province, Thailand and then separated into *Moringa oleifera* peel, pulp, and seed samples and cut into small pieces (Fig. 1). Each sample was divided into raw and heat-treated samples (in a boiling water bath for 15 min.). All samples were incubated at 60 ºC for 24 hours. The dried samples were powdered and passed through sieve no. 18 and analyzed for moisture content (AOAC, 2000). Each sample was extracted with 50% ethanol at a ratio of 1:20 (w/v). The mixture was then shaken at room temperature for 24 hours with a shaking speed of 150 rpm. Supernatant was collected and filtered through Whatman No.1 filter paper. Then, this crude extract was subjected to total phenolic content and antioxidant activity assays.

(A) **(B)** **(C)**

Fig. 1 Moringa peel (A), pulp (B), and seed (C) samples used in this study

Total phenolic compound was determined by Folin-Ciocalteu method (Kähkönen et al., 1999). Briefly, 500 µL of each sample were mixed well with 2.5 mL of 0.2 M Folin–Ciocalteu reagent, followed by the addition of 2 mL of 7.5% (w/v) sodium carbonate. The mixture was allowed at room temperature for 60 min and absorbance was measured at 7650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg gallic acid equivalents/g dry weight (mg gallic acid eq./g DW).

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity assay (DPPH assay) was performed according to Burits and Bucar (2000) with some modifications. Sample (1 mL) were mixed with 1 mL of 0.1 mM DPPH solution. The reaction tubes were wrapped in aluminum foil and incubated at

room temperature for 10 min in dark. The absorbance was monitored at 517 nm. DPPH assay was expressed as mg Trolox equivalents/g dry weight (mg Trolox eq./g DW).

Ferric reducing antioxidant power assay (FRAP assay) was performed according to Benzie and Strain (1996) with some modifications. Sample (100 µL) were mixed with 1900 µL of FRAP reagent. The absorbance of the reaction mixture was monitored at 593 nm after incubating at 37 ºC for 10 min. FRAP value of each sample was expressed as mg ferrous equivalents/g dry weight (mg ferrous eq./g DW).

Metal chelating activity assay was performed according to Decker and Welch (1990) with some modifications. Sample (100 µL) were mixed with 1,400 µL of distilled water and 100 µL of 2 mM $FeCl_2.4H_2O$. The reaction mixture was incubated at room temperature for 3 min. Then, the reaction mixture was added with 400 µL of 5 mM ferrozine and incubated at room temperature for 10 min. The absorbance was monitored at 562 nm. Metal chelating activity of each sample was expressed as mg EDTA equivalents/g dry weight (mg EDTA eq./g DW).

All the extraction processes and the analyses on each sample were done in duplicate. The experiments followed a completely randomized design (CRD) comprising six crude extract samples. Differences among mean values were established using Duncan Multiple Range Test (DMRT) at $P<0.05$.

RESULTS AND DISCUSSION

Moisture content of raw moringa peel, pulp, and seed powder were 9.13, 9.90, and 9.97, respectively. Whereas, moisture content of heat-treated moringa peel, pulp, and seed powder were 8.65, 8.88, and 8.92, respectively. Total phenolic content and DPPH radical scavenging activity of raw and heat-treated moringa peel, pulp, and seed extracts were shown in Table 1. The results showed that the highest total phenolic content was found in moringa peel (3.65 mg gallic acid eq./g DW), followed by pulp (3.44 mg gallic acid eq./g DW) and seed (2.85 mg gallic acid eq./g DW), respectively. After heat treatment, moringa peel, pulp, and seed samples showed lower total phenolic content than their respective raw samples. Siddhuraju and Becker (2003) reported that the main phenolic compounds in drumstick leaves and its extracts are flavonoid groups such as quercetin and kaempferol. They also contained 3-caffeoylquinic and 5-caffeoylquinic acid. The whole pods are reported to contain nitriles, isothiocyanate and thicarbamates (Faizi et al., 1995) and has antioxidant activity, which is due to the presence of carotenoid compounds (Kumar et al., 2007).

Table 1 Total phenolic content and DPPH radical scavenging activity of raw and heat-treated *Moringa oleifera* peel, pulp, and seed extracts

Sample	Total phenolic content (mg gallic acid eq./g DW)	DPPH radical scavenging activity (mg Trolox eq./g DW)
Raw moringa peel	$3.65^{a} \pm 0.03$	$1.35^{a} \pm 0.02$
Raw moringa pulp	$3.44^{b} \pm 0.25$	$1.25^{b} \pm 0.03$
Raw moringa seed	$2.80^{c} \pm 0.02$	$0.91^{c} \pm 0.04$
Heat-treated moringa peel	$1.38^{d} \pm 0.01$	$0.78^{d} \pm 0.03$
Heat-treated moringa pulp	$1.19^{de} \pm 0.02$	$0.41^{e} \pm 0.04$
Heat-treated moringa seed	$0.91^{e} \pm 0.01$	$0.23^{f} \pm 0.01$

Means of the same column with different superscripts indicate significant difference ($P<0.05$).

The antiradical activity was measured as ability to reduce DPPH radical by crude extract from raw and heat-treated *M. oleifera* (peel, pulp, and seed). As shown in Table 1, raw moringa peel showed the highest DPPH radical scavenging activity (1.35 mg Trolox eq./g DW), followed by pulp (1.25 mg Trolox eq./g DW) and seed (0.91 mg Trolox eq./g DW), respectively. The lower DPPH radical scavenging activity was also found in heat-treated samples. These results are in agreement with Arabshahi-Delouee et al. (2007). Incubating moringa leaves extract at 100 ºC for 15 min resulted in a significant decrease in inhibition of lipid peroxidation by 17% using

thiobarbituric acid (TBA) assay. Heat processing may have resulted in degradation of antioxidants present in moringa leaves extract, thereby decreasing the activity.

FRAP of raw and heat-treated moringa peel, pulp, and seed extracts were shown in Table 2. FRAP of raw moringa peel (3.62 mg ferrous eq./g DW), seed (3.32 mg ferrous eq./g DW), and pulp extracts (3.28 mg ferrous eq./g DW) was comparable ($P>0.05$) and showed higher FRAP than all heat-treated samples ($P<0.05$). This method is based on the ability of a compound to donate one electron to Fe^{3+} to reduce it to $Fe^{2+,}$ whereas DPPH radical scavenging assay is involved hydrogen atom transfer.

Table 2 Ferric reducing antioxidant power and metal chelating activity of raw and heat-treated *Moringa oleifera* peel, pulp, and seed extracts

Sample	Ferric reducing antioxidant power (mg ferrous eq./g DW)	Metal chelating activity (mg EDTA eq./g DW)
Raw moringa peel	3.62^{a} ±0.08	0.41^{a} ±0.04
Raw moringa pulp	3.28^{ab}±0.25	0.42^{a} ±0.06
Raw moringa seed	3.32^{ab}±0.09	0.19^{b} ±0.01
Heat-treated moringa peel	2.13^{c} ±0.28	0.15^{b} ±0.01
Heat-treated moringa pulp	2.87^{b} ±0.30	0.13^{b} ±0.01
Heat-treated moringa seed	2.05^{c} ±0.01	0.01^{c} ±0.00

Means of the same column with different superscripts indicate significant difference ($P<0.05$).

Metal chelating activity of raw moringa pulp (0.42 mg EDTA eq./g DW) showed the highest content and was comparable with the raw moringa peel (0.41 mg EDTA eq./g DW) ($P\geq0.05$). Heating proceed resulted in a significant decrease in metal chelating activity of all samples ($P<0.05$). Transition metals act as catalysts that promote the generation of the first few radicals, which initiate the oxidative chain reaction. Thus, the chelating of transition metal ions by these extracts would reduce available transition metals, rendering the inhibition of the radical-mediated oxidative chain reactions (Zaid et al., 2012).

The decrease in FRAP and metal chelating activity of all samples might be due to the loss of naturally occurring antioxidants present in the extract or formation of novel compounds having prooxidant activity upon heat processing at 100 ºC for 15 min. These results demonstrated that antioxidant activities including DPPH radical scavenging activity, FRAP, and metal chelating activity of moringa peel, pulp, and seed samples depends on heat treatment.

CONCLUSION

Crude extract obtained from raw moringa peel showed the highest total phenolic content and DPPH radical scavenging activity followed by pulp and seed, respectively. The highest FRAP were obtained from the raw moringa peel extract, whereas the highest metal chelating activity was found in raw moringa pulp extract. Moringa peel, pulp and seed samples with heat treatment at 100 °C for 15 min showed lower total phenolic content and antioxidant properties than crude extract from raw moringa samples.

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