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THERAPEUTIC ANTIOXIDANT ACTIVITY OF *MONASCUS*-FERMENTED DURIAN SEED: A POTENTIAL FUNCTIONAL FOOD INGREDIENT

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ABSTRACT

Background: the phytochemical characteristic of *Monascus*-Fermented Products (MFPs) is well documented. During fermentation, *Monascus* produce various bioactivities metabolites that have an antioxidant, anti-hypertension, anti-cholesterol, anti-cancer, anti-inflamatory and anti-diabetics effects.

Purpose: to evaluate the antioxidant activity of *Monascus*-fermented durian seed extracts.

Methods: 50 gm of small cut durian seed was inoculated with *Monascus* sp. KJR2, which was employed as starter culture, to produce the MFPs. Dried MFDS was extracted at serial ethanol concentrations (0, 20, 40, 60, 70 and 80%) and tested against DPPH radical scavenging activity, phosphomolybdenum reduction and Ferric Reduction Activity Power (FRAP). Additionally, total phenolic and pigments contents were determined.

Results: the MFDS possesses antioxidant activity through DPPH radical scavenging, phosphomolybdenum reduction and FRAP. The highest DPPH radical scavenging of the MFDS extract is at ethanol concentration of 40%, FRAP at 60%, whereas the water extract possesses the highest reducing power of phosphomolybdenum assay. Total phenol and Monascus pigments contribute to the phosphomolybdenum reduction, but not to DPPH radical scavenging and FRAP.

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Conclusion: the phytochemical benefit of fermented durian seed extract has been ascertained as potential antioxidant food ingredient and reinstates its promising position in the region as effective indigenous traditional medicine.

Keywords: antioxidant activity; *Monascus*; durian seed; total phenolic; pigment.

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1. INTRODUCTION

For hundreds of years, *Monascus*-Fermented Products (MFPs) have been consumed traditionally in Asian countries, as food ingredients and traditional medicine. During fermentation, *Monascus* produces various metabolites which have biological activities that is, antioxidant, anti-hypertension, anti-cholesterol, anti-cancer, anti-inflamatory and anti-diabetic activities (Higashikawa et al., 2012; Lee et al., 2006; Li et al., 1998; Wei et al., 2003; Yang and Mousa, 2012). Oxidative stress is a main cause of the lack of normal body cell function, related to aging and various degenerative diseases such as atherioscle-rosis, cancer, diabetes, Alzheimer and Parkinson (Shi et al., 2012).

Studies on antioxidant activities of MFPs initiated by Aniya et al. (1999) through screening of 40 fungi species. They reported that 13 species of *Monascus* showed high DPPH scavenging activity (>40%) with *Monascus* anka had the highest antioxidant activity. Consequently, studies have been carried out on *Monascus* fungi to produce *Monascus*-fermented rice with antioxidant activity (Aniya et al., 2000; Dhale et al., 2007; Lee et al., 2008, 2009; Mohan-Kumari et al., 2011; Taira et al., 2002; Yang et al., 2006).

Some researchers had developed non-rice MFPs with antioxidant activity that is, *Monascus*-fermented adlay, *Monascus*-fermented soybean and *Monascus*-fermented dioscorea (Lee et al., 2008; Li et al., 2013; Shi et al., 2012; Tseng et al., 2006, 2012). Our recent study showed that *Monascus* fungi could grow on durian seed medium and produce various metabolites (Srianta et al., 2012, 2014), however, the evaluation of the antioxidant activity has not been fulfilled to date. Therefore, the specific objective of this study was to study the antioxidant activity of *Monascus*-fermented durian seed extracts.

2. MATERIALS AND METHODS

2.1 Culture

Monascus sp. KJR2 was maintained on Saboraud's Dextrose Agar (SDA) slant, preserved at 4°C and subcultured monthly. Following the growth of *Monascus* sp. KJR2 on SDA slants (at room temperature 30°C) under static conditions for 14 days, 10 mL of sterile distilled water was added and the spores were scraped under aseptic conditions. 0.1 mL of the spore suspension was inoculated into Saboraud's Dextrose Broth (SDB) and then was incubated at room temperature (30°C) for 10 days. It was used as starter culture to produce *Monascus*-fermented durian seed.

2.2 Production of MFDS

Durian seeds were obtained from local durian seller. Durian seeds were stored in a freezer (−4°C) until used. Durian seeds were boiled in a $CaCO_3$ solution of 5% w/v for 10 min. After the seed coat were peeled off, the seeds were cut into small size of 1 cm×1 cm×1 cm. A 50 g of small cut durian seed were transferred into 300 mL flask, mixed thoroughly, autoclaved at 121°C for 15 min, then left to cool to room temperature, inoculated with the spore suspension of *Monascus* sp. KJR2 and incubated at room temperature (30°C) for 14 days in static conditions (with manual shaking daily). *Monascus*-fermented durian seed were dried in an air drying oven at temperatures 45°C for 24 hr.

2.3 MFDS extraction

The dried MFDS was extracted at various ethanol concentration that is, 0; 20; 40; 60; 70 and 80%. The angkak powder was mixed with distilled water/ethanol at ratio 1:5, shaked in waterbath at 200 rpm for 1 hr, then centrifuged at 5000 g, 27°C for 30 min. The supernatant was filtered using Whatman no. 1. The filtrate was analysed

for the antioxidant activity through DPPH radical scavenging activity, phosphomolybdenum and Ferric reducing ability power, total phenolic content and pigments content.

2.4 DPPH scavenging activity assay

DPPH scavenging activity assay was carried out according to Tseng et al. (2006). A 3.8 mL sample extract was added to 0.2 mL DPPH (50 mg in 100 mL metanol) in a wrapped glass tube. The tube was withstand in a dark room for 30 min. The absorbance was measured at λ 517 nm. The percentage (%) inhibition was calculated with the control basis of DPPH solution absorbance value.

2.5 Ferric Reduction Activity Power (FRAP) assay

FRAP assay was carried out according to Kraboun et al. (2013). A 3.8 mL of FRAP reagent of mixed solution (Buffer asetat: TPTZ: $FeCl_3.6H_2O$=10:1:1) was transferred to a glass tube, added with 0.2 mL sample extract, then homogenised. The tube was withstand in a dark room for 30 min, then the absorbance was measured at λ 593 nm. Gallic acid was used as a standard.

2.6 Phosphomolybdenum assay

Phosphomolybdenum assay was carried out according to Chairote et al. (2009). The extracts were mixed with the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a glass tube. The tubes were incubated at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 725 nm. The total antioxidant capacity was expressed based on gallic acid equivalents.

2.7 Total phenolic content analysis

Total phenolic content of the angkak extract was determined with Folin-Ciocalteu reagent according to the method Lee et al. (2008) with slight modification, using Gallic Acid as a standard. A 0.1 mL of extract was placed into a 10 mL measuring flask and 0.5 mL of Folin-Ciocalteau reagent was added to the extract. The measuring flask was allowed to stand at room temperature for 5 min. Then, 1.5 mL of 20% (w/v) Na_2CO_3 was added to the mixture. The mixture was adjusted until the volume reaches 10 mL with distilled water. After 30 min at room temperature, absorbance was measured at 765 nm versus a blank by using a spectrophotometer (Shimadzu UV 1800, Japan). Total phenol value was expressed as mg Gallic Acid Equivalent/g.

2.8 Pigments content analysis

The *Monascus* pigments content was analysed according to Babitha et al. (2006) with slight modification. Absorbance of the angkak extract was measured using spectrophotometer (Shimadzu, UV 1601) at 392 nm, 470 nm and 501 nm for yellow, orange and red pigments, respectively. Pigment content was expressed as Absorbance (nm) at the wavelength per gram of dry substrate (AU/g).

2.9 Data analysis

The data were analysed using Analysis of Variance (ANOVA) at α=5%. If the ANOVA test results indicate a significant effect, this was followed by Duncan's Multiple Range Test (DMRT) at α=5% to determine the level of treatment that gives a significant difference.

3 RESULTS AND DISCUSSION

3.1 Antioxidant activity

Table 1 shows the antioxidant activity of *Monascus*-fermented durian seed extracts at various ethanol concentration (%).

Those extracts have antioxidant activity through DPPH scavenging, phosphomolybdenum reduction and Ferric reduction. There are no trend antioxidant activity through DPPH scavenging and Ferric reduction at increasing ethanol concentration, but phosphomolybdenum reduction activity decrease with increasing ethanol concentration.

MFDS extracts shows DPPH radical scavenging activity. Other studies also reported that *Monascus* fermented rice, soybean and adlay posses DPPH radical scavenging activity (Lee et al., 2008, 2009; Tseng et al., 2006; Yang et al., 2006). The DPPH radical scavenging of MFDS water extract at 0.2 g/mL was 35.26%, whereas MFDS ethanolic extracts at the same concentration was higher in a range of 43.19 and 56.26%. The similar findings also reported for water and ethanolic extracts of *Monascus* fermented soybean. At 5 mg/ml, scavenging abilities of the cold water

Table 1 Antioxidant activity of *Monascus*-fermented durian seed extracts at various ethanol concentration (%)

Ethanol concentration (%)	*DPPH radical scavenging activity (% inhibition)*	*Phosphomolybdenum (mg GAE/g)*	*FRAP (mg GAE/g)*
0	35.26[a]	256.99[e]	65.32[b]
20	43.19[b]	239.01[d]	91.68[d]
40	56.26[e]	229.01[d]	72.98[c]
60	51.37[c]	222.99[c]	93. 45[e]
70	54.03[d]	204.75[b]	59.92[a]
80	49.45[c]	152.60[a]	90.29[d]

Note: The different notation in the same column indicate the significant difference at α=5%.

extracts from *Monascus* fermented soybean with two *Monascus* different strains on DPPH radicals were 36.5% and 56.4%, whereas that of the ethanolic extracts were higher of 50.3 and 88.3% (Lee et al., 2008, 2009).

This indicated that DPPH radical scavenger components of MFDS and Monascus fermented soybean were more soluble in ethanol than in water. The highest DPPH radical scavenging activity of the MFDS extract is at ethanol concentration of 40%. Whereas, ferric reduction activity of MFDS extract of 0.2 g/mL at various ethanol concentration is in a range of 59.92 and 93.45 mg GAE/g. The highest FRAP is at ethanol concentration of 60%.

The antioxidant capacity of MFDS through phosphomolybdenum reduction assay is in a range of 152.60 and 256.99 mg GAE/g, equivalent to 31.32 and 51.39 mg GAE/mL. Those values are higher than those of *Monascus* fermented rice that in a range of 0.15 and 0.53 mg GAE/mL (Chairote et al., 2009). These results also showed that water extract posses higher activity than those of all ethanolic extracts. It indicated that the components contribute to the activity is that of more soluble in water.

3.2 Antioxidant components

Study on the antioxidant components of MFDS, total phenol and pigments contents has been examined. Table 2 shows total phenol and pigments content of MFDS extracts at various ethanol concentrations, whereas Table 3 shows the correlation of those components to the antixidant

Table 2 Total phenolic and pigments content of *Monascus*-fermented durian seed extracts at various ethanol concentrations (%)

Ethanol concentration (%)	*Total phenolic content (mg GAE/g)*	*Pigment content (AU/g)*		
		Yellow	*Orange*	*Red*
0	3.58[e]	1.121[d]	0.604[d]	0.464[c]
20	3.61[e]	1.103[d]	0.601[d]	0.548[d]
40	2.90[d]	1.178[e]	0.620[e]	0.608[e]
60	2.36[c]	0.815[c]	0.426[c]	0.479[c]
70	1.93[b]	0.691[b]	0.380b	0.434[b]
80	1.15[a]	0.511[a]	0.287[a]	0.346[a]

Note: The different notation in the same column indicate the significant difference at α=5%.

Table 3 Correlation of antioxidant activity and total phenol or pigments

	DPPH radical scavenging activity	*Phosphomolybdenum assay*	*FRAP assay*
Total phenol	−0.5789	0.9431	−0.1329
Yellow pigment	−0.3413	0.8884	−0.1875
Orange pigment	−0.3787	0.8850	−0.1977
Red pigment	0.1297	0.7034	−0.0507

activity. The results indicated that total phenol and pigments content possitively contributes to the phosphomolybdenum reduction. However the total phenol and pigments contents showed negative correlation to the DPPH radical scavenging and FRAP. The negative correlations of *Monascus* fermented waxy corn pigment intensity to DPPH radical scavenging and FRAP were also reported by Kraboun et al. (2013), but the values are higher than of this study. This can be explained on the basis that other metabolites such as monacolin K, GABA, and or dihydromonacolin MV contributes to the DPPH radical scavenging and FRAP.

4 CONCLUSIONS

The MFDS possessed antioxidant activity through DPPH radical scavenging, phosphomolybdenum reduction and FRAP. The highest DPPH radical scavenging of the MFDS extract is at ethanol concentration of 40%, FRAP at 60%, whereas the water extract possesses the highest reducing power of phosphomolybdenum assay. Total phenol and Monascus pigments contribute to the phosphomolybdenum reduction, but not to DPPH radical scavenging and FRAP. The phytochemical benefit of fermented durian seed extract has been ascertained as potential antioxidant food ingredient and restores its promising position in the indigenous traditional medicine in the region.

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