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Antioxidant properties of methanolic extracts from monascal adlay

Yu-Hsiu Tseng [a], Joan-Hwa Yang [b], Hui-Ling Chang [c], Yu-Ling Lee [b], Jeng-Leun Mau [b,*]

[a] Department of Baking Technology and Management, National Kaohsiung Hospitality College, 1 Sung-Ho Road, Kaohsiung 812, Taiwan, ROC
[b] Department of Food and Nutrition, Shih Chien University, Taipei 104, Taiwan, ROC
[c] Department of Food Science, National Chung-Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan, ROC

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Abstract

Both the fungus *Monascus* sp. and adlay possess functional components effective in improving human health. The fungus was inoculated into adlay and a new product was produced after the colonization of fungal mycelia. Our objective was to evaluate the antioxidant properties of methanolic extracts from inoculated products [monascal polished adlay (MPA) and monascal dehulled adlay (MDA)] as compared to uninoculated products [polished adlay (PA) and dehulled adlay (DA)]. With regard to EC_{50} values (mg extract ml^{-1}) of methanolic extracts, antioxidant activities were excellent and in the descending order of MDA (0.05) $\gg$ MPA (0.75) > DA (0.83) $\gg$ PA (6.35). Effectiveness in reducing powers was in a descending order of MPA (0.78) > MDA (1.53) $\gg$ PA (13.24) $\sim$ DA (13.67 mg ml^{-1}). Scavenging abilities on 1,1-diphenyl-2-picrylhydrazyl radicals and chelating abilities on ferrous ions were in the descending order of MPA > MDA > DA > PA. Total phenols were the major naturally occurring antioxidant components found. Overall, monascal adlay products displayed higher antioxidant activity, reducing power, scavenging and chelating abilities and higher in total phenol content than uninoculated adlay products.

Keywords: Monascal adlay; Antioxidant activity; Reducing power; Scavenging ability; Chelating ability; Antioxidant components

1. Introduction

Fungus *Monascus* has been used to prepare red fermented rice (anka, red koji) as a food colorant and traditional medicine in oriental countries for centuries (Bau & Mo, 1975). Metabolic products from fermentation of *Monascus* species are commonly utilized as food pigments or as antimicrobial agents. The components isolated from the fungus exert several biological actions and exhibit hypocholesterolemic (Endo, 1979, 1980), liver-protective and antitumor effects (Aniya, Yokomakura, Yonamine, Nagamine, & Nakanishi, 1998; Yasukawa, Takahashi, Yamanouchi, & Takido, 1996). Currently, *Monascus*-fermented products are used as a functional dietary supplement to reduce the cholesterol level in the human body (Tobert et al., 1982).

Adlay (Chinese pearl barley, soft-shelled Job's tears, *Coix lachryma-jobi* L. var. *ma-yuen* Stapf) is a grass crop that has long been used in traditional Chinese medicine and as a nourishing food due to its high nutritional value and special biological and functional effects on the human body. Adlay is widely planted in Taiwan, China, and Japan, where it is considered a healthy food supplement. According to the ancient Chinese medical book Pen-Tsao-Kang-Mu (Li, 1596), the seed of adlay was used in China for the treatment of warts, chapped skin, rheumatism and neuralgia, and as an anti-inflammatory or anti-helmintic agent. Coixenolide isolated from the adlay seed exhibited antitumor activity towards Ehrlich ascites sarcoma in mice (Tanimura, 1961; Ukita & Tanimura,

* Corresponding author. Tel.: +886 4 2285 4313; fax: +886 4 2287 6211.
E-mail address: jlmau@dragon.nchu.edu.tw (J.-L. Mau).

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1961). Numerous recent reports have also indicated that the consumption of adlay seed is beneficial to the human body (Chiang, Cheng, Chiang, & Chung, 2000; Kondo, Nakajima, Nozoe, & Suzuki, 1998; Kuo, Shih, Kuo, & Chiang, 2000; Otsuka, Hirai, Nagao, & Yamaski, 1988; Tsai, Yang, & Hsu, 1999; Yang & Tsai, 1998).

Both the fungus *Monascus* species and adlay possess functional components effective in maintaining human health. The fungus was inoculated into cooked adlay and a new monascal product was then produced after the colonization of fungal mycelia. The functional components of adlay are still present in the fermented adlay products along with those produced by the fungus (Chang, 2001). The taste quality of monascal adlay and its storage stability have been evaluated (Tseng, Yang, Chang, & Mau, 2004; Yang, Tseng, Chang, Lee, & Mau, 2005). However, the antioxidant properties of monascal adlay are not available. Accordingly, our objective was to study and compare the antioxidant properties of methanolic extracts from various adlay products, including inoculated adlay products [monascal polished adlay (MPA) and monascal dehulled adlay (MDA)] and uninoculated adlay products [polished adlay (PA) and dehulled adlay (DA)]. Antioxidant properties were assayed in terms of antioxidant activity by the conjugated diene method, reducing power, scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and chelating ability on ferrous ions. The contents of potential antioxidant components of methanolic extracts were also determined.

2. Materials and methods

2.1. Adlay products

Polished adlay [Taichung selected no. 4 (TCS-4)] (PA) was purchased at a local market in Taichung City, Taiwan. Dehulled adlay (DA) was obtained from a farm at Erhlin, Changhua County, Taiwan. *Monascus purpureus* Went (CCRC 31498) was obtained from the Culture Collection and Research Center, Food Industry Research and Development Institute, Hsinchu City, Taiwan. The fungus was inoculated onto malt extract agar (Difco) and incubated at 25 °C for 72 h. After pure culture was obtained, the mycelium was re-inoculated into potato dextrose broth (Difco) and incubated at 25 °C for 7 days. The culture was then homogenized in a Waring blender and inoculated into two kinds of autoclaved adlay, PA and DA, respectively, at an inoculation rate of 5%. New corresponding products, MPA and MDA, respectively, were then produced after the colonization of fungal mycelia for 7 days at 25 °C. Two *Monascus*-colonized adlay products as well as two uninoculated adlay products that were also autoclaved and used as controls, were air-dried in an oven at 40 °C.

For each product, three dried samples (~50 g each) were randomly selected and prepared for analyses. After a fine powder (20 mesh) was obtained using a mill (Retsch ultra centrifugal mill and sieving machine, Haan, Germany), a subsample (10 g) was extracted by stirring with 100 ml of methanol at 25 °C at 20*g* for 24 h and filtering through Whatman No. 4 filter paper. The residue was then extracted with two additional 100-ml portions of methanol as described above. The combined methanolic extracts were then rotary evaporated at 40 °C to dryness. The dried extract was used directly for analyses of antioxidant components or redissolved in methanol to a concentration of 50 mg ml^{-1} and stored at 4 °C for further uses.

2.2. Antioxidant activity

The antioxidant activity was determined by the conjugated diene method (Lingnert, Vallentin, & Eriksson, 1979). Each extract (1–20 mg ml^{-1}) in methanol (100 μl) was mixed with 2 ml of 10 mM linoleic acid emulsion (pH 6.5) in test tubes and placed in darkness at 37 °C to accelerate oxidation. After incubation for 15 h, 6 ml of 60% methanol (Mallinckrodt Baker, Paris, KY) in deionised water was added and the absorbance of the mixture was measured at 234 nm against a blank in a Hitachi U-2001 spectrophotometer. The antioxidant activity (AOA) was calculated as follows: AOA (%) = $[(\Delta A_{234}$ of control $- \Delta A_{234}$ of sample)/ΔA_{234} of control$] \times 100$. A control consisted of methanol and the reagent solution without methanolic extracts added and the procedure was carried out as described above. An AOA value of 100% indicates the strongest antioxidant activity. An AOA value of 100% indicates the strongest antioxidant activity. EC_{50} value (mg extract ml^{-1}) is the effective concentration at which the antioxidant activity was 50% and was obtained by interpolation from linear regression analysis. Vitamin C (ascorbic acid, Sigma Chemical Co., St. Louis, MO), butylated hydroxyanisole (BHA, Sigma) and vitamin E (α-tocopherol, Sigma) were used for comparison.

2.3. Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each extract (1–20 mg ml^{-1}) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6, Wako Pure Chemical Co., Osaka, Japan) and 2.5 ml of 1% potassium ferricyanide (Sigma), and the mixture was incubated at 50 °C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v, Wako) was added, the mixture was centrifuged at 200*g* for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride (Wako), and the absorbance was measured at 700 nm

against a blank in a Hitachi U-2001 spectrophotometer. A higher absorbance indicates a higher reducing power. EC_{50} value (mg extract ml^{-1}) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. Vitamins C and E and BHA were used for comparison.

2.4. Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals

Each extract (1–20 mg ml^{-1}) in methanol (4 ml) was mixed with 1 ml of methanolic solution containing DPPH (Sigma) radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank (Shimada, Fujikawa, Yahara, & Nakamura, 1992). The scavenging ability was calculated as follows: Scavenging ability (%) = $[(\Delta A_{517}$ of control $- \Delta A_{517}$ of sample$)/\Delta A_{517}$ of control$] \times 100$. EC_{50} value (mg extract ml^{-1}) is the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis. Vitamins C and E and BHA were used for comparison.

2.5. Chelating ability on ferrous ions

Chelating ability was determined according to the method of Shimada et al. (1992). To 2 ml of the mixture consisting of 30 mM hexamine (Wako), 30 mM potassium chloride (Sigma) and 9 mM ferrous sulphate (Union Chemical Works, Hsinchu, Taiwan) were added each extract (1–20 mg ml^{-1}) in methanol (2 ml) and 200 μl of 1 mM tetramethyl murexide (TMM, Sigma). After 3 min at room temperature, the absorbance of the mixture was determined at 485 nm against a blank. A lower absorbance indicates a higher chelating power. EC_{50} value (mg extract ml^{-1}) is the effective concentration at which ferrous ions were chelated by 50% and was obtained by interpolation from linear regression analysis. Ethylenediaminetetraacetic acid (EDTA, Sigma) was used for comparison.

2.6. Determination of antioxidant components

Ascorbic acid was determined according to the method of Klein and Perry (1982). Each methanolic extract (20 mg) was extracted with 10 ml of 1% metaphosphoric acid (Union) for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 9 ml of 2,6-dichloroindophenol (Sigma) and the absorbance was measured within 15 s at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (Sigma).

β-Carotene was extracted and analysed as described by Rundhaug, Pung, Read, and Bertram (1988). Each methanolic extract (20 mg) was extracted with a solution of 1% pyrogallol (Wako) in 10 ml of methanol/dichloromethane (1:1, v/v) for 45 min at room temperature, filtered through Whatman No. 4 filter paper and adjusted the volume to 10 ml using the same solution. The filtrate was then passed through a filter unit (13 mm, Lida Corp., Kenosha, WI) and filtered using a 0.45-μm CA filter paper prior to injection onto a high-performance liquid chromatograph (HPLC).

The HPLC system consisted of a Hitachi D-6200 pump, a Hitachi L-5000 LC controller, a Rheodyne 7161 injector, a 20-μl sample loop, a Hitachi D-2500 chromato-integrator, a Hitachi L-4000 UV detector, and a Prodigy 5 ODS-2 column (4.6×250 mm, 5 μm, Phenomenex Inc., Torrance, CA). The mobile phase was acetone/methanol/acetonitrile, 1:2:2 (v/v/v), at a flow rate of 0.7 ml min^{-1} and UV detection was at 470 nm. Content of β-carotene was calculated on the basis of the calibration curve of authentic β-carotene (Sigma).

Tocopherols were extracted and analysed according to the method of Carpenter (1979). Each methanolic extract (50 mg) was suspended in 6 ml of pyrogallol (6% in 95% ethanol) and 4 ml of 60% aqueous potassium hydroxide solution, and the resulting mixture was saponified at 70 °C for 20 min. Deionised water (15 ml) was added and the mixture was extracted with 15 ml of *n*-hexane. The organic layer was washed with deionised water to neutral, dried over anhydrous sodium sulphate, and rotary evaporated to dryness. The residue was redissolved in 5 ml of *n*-hexane and filtered prior to HPLC injection in the same manner as in the β-carotene assay.

The HPLC system was the same as for the β-carotene assay. The mobile phase was acetonitrile/methanol, 85:15 (v/v), at a flow rate of 1.0 ml min^{-1} and UV detection was at 295 nm. Content of each tocopherol was calculated on the basis of the calibration curve of each authentic tocopherol (Sigma).

Total phenols were determined according to the method of Taga, Miller, and Pratt (1984). Each methanolic extract (20 mg) was dissolved in a solution of 5 ml of 1.3% HCl in methanol/deionised water (60:40, v/v) and the resulting mixture (100 μl) was added to 2 ml of 2% aqueous sodium carbonate solution. After 3 min, 100 μl of 50% Folin–Ciocalteau's phenol reagent (Sigma) was added to the mixture. After 30 min standing, the absorbance was measured at 750 nm against a blank. The content of total phenols was calculated on the basis of the calibration curve of gallic acid (Sigma).

2.7. Statistical analysis

For each methanolic extract from adlay products, three samples were prepared for assays of every

antioxidant attribute and component. The experimental data were subjected to an analysis of variance for a completely random design to determine the least significant difference at the level of 0.05.

3. Results and discussion

3.1. Extraction yield

Following the extraction with methanol, the yields were in a descending order of MDA > MPA ≫ DA ~ PA (Table 1). The higher yields of MDA and MPA were mainly due to the fact that after the colonization of fungal growth, MDA and MPA were degraded and contained more small and water soluble substances. The yields of MDA were similar to those of monascal polished rice (MPR, 33.03%) and monascal dehulled rice (MDR, 30.66%), whereas the yield of DA was similar to that of dehulled rice (DR, 6.27%) (Tseng, Lee, Yang, & Mau, 2003).

3.2. Antioxidant activity

Using the conjugated diene method, at 0.1 mg ml^{-1}, the methanolic extract from MDA showed a moderate antioxidant activity of 52.7%, whereas methanolic extracts from PA, DA and MPA exhibited low antioxidant activities of 7.8%, 8.2% and 12.7%, respectively (Fig. 1). At 1 mg ml^{-1}, only the methanolic extract from PA showed a low antioxidant activity of 15.8%, whereas antioxidant activities of methanolic extracts from DA, MPA and MDA were in the middle range of 59.6–76.5%. At 10–20 mg ml^{-1}, antioxidant activities of four methanolic extracts increased with the increased concentrations to about 94%. With regard to antioxidant activity, monascal adlay was slightly better than uninoculated adlay. However, antioxidant activities were 96.7% and 91.2% at 0.01 mg ml^{-1} for BHA and vitamin E, respectively, and 84.2% at 10 mg ml^{-1} for vitamin C.

Similarly, using the same method, at 0.1 mg ml^{-1}, methanolic extracts from polished rice (PR) and DR showed moderate antioxidant activities of 43.9% and 38.0%, respectively, whereas no activity was observed for methanolic extracts from MPR and MDR (Tseng et al., 2003). However, at 10 mg ml^{-1}, methanolic extracts from PR, DR, MPR and MDR exhibited high antioxidant activities of 98.0%, 97.1%, 81.1% and 90.0%, respectively (Tseng et al., 2003). With regard to antioxidant activity, it seems that various rice products were better than corresponding adlay products shown in Fig. 1, except for MDA, which was the most effective.

Table 1
Extraction yield of methanolic extracts from various adlay products

	Extraction %[a] (w/w)
Polished adlay (PA)	4.08 ± 0.22 C[b]
Dehulled adlay (DA)	6.09 ± 0.55 C
Monascal polished adlay (MPA)	23.37 ± 0.72 B
Monascal dehulled adlay (MDA)	29.68 ± 1.69 A

[a] Extracted from dried materials (10.00 g). Each value is expressed as mean ± standard deviation (n = 3).

[b] Means with different letters within a column are significantly different (p < 0.05).

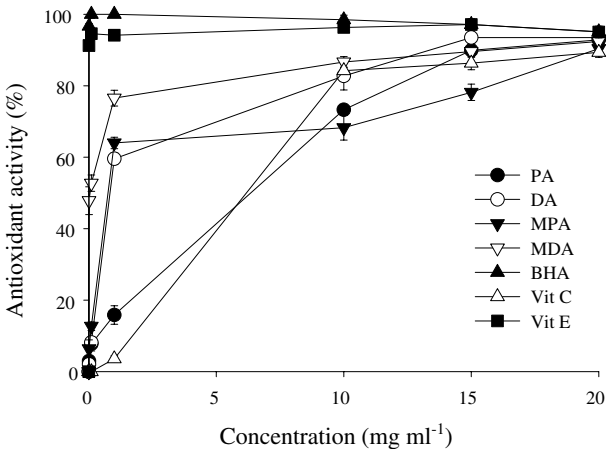

Fig. 1. Antioxidant activity of methanolic extracts from various adlay products (conjugated diene method). Each value is expressed as mean ± standard deviation (n = 3). PA, polished adlay; DA, dehulled adlay; MPA, monascal polished adlay; MDA, monascal dehulled adlay.

3.3. Reducing power

Reducing powers of the methanolic extracts increased in two patterns with increased concentrations, i.e., a fast increase for monascal adlay products (MPA and MDA) and a slow increase for uninoculated adlay products (PA and DA) (Fig. 2). At 1 mg ml^{-1}, reducing powers of

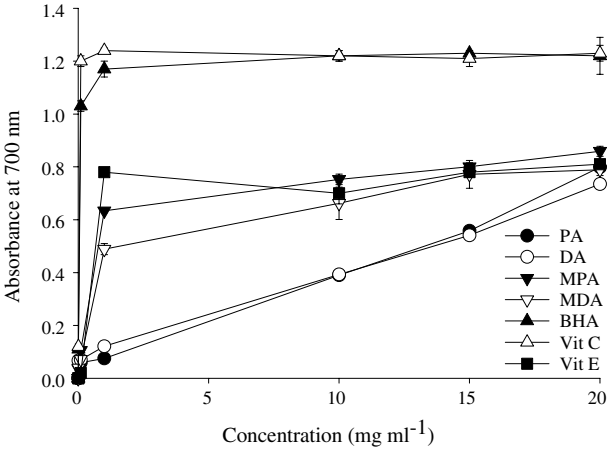

Fig. 2. Reducing power of methanolic extracts from various adlay products. Each value is expressed as mean ± standard deviation (n = 3). PA, polished adlay; DA, dehulled adlay; MPA, monascal polished adlay; MDA, monascal dehulled adlay.

methanolic extracts were 0.08, 0.12, 0.63 and 0.49 for PA, DA, MPA and MDA, respectively. At 20 mg ml^{-1}, reducing powers of methanolic extracts were in the range of 0.73–0.86. However, reducing powers of BHA and vitamin C were 1.03 and 1.20 at 0.1 mg ml^{-1}, respectively. At 1 mg ml^{-1}, vitamin E showed a reducing power of 0.78 and as the concentrations increased it exhibited the same pattern as methanolic extracts from MPA and MDA.

Tseng et al. (2003) found that at 20 mg ml^{-1}, methanolic extracts from PR and DR showed reducing powers of 0.38 and 0.45 whereas at 1 mg ml^{-1}, reducing powers of those from MPR and MDR were 0.65 and 0.22, respectively. Obviously, uninoculated rice products were less effective in reducing power than uninoculated adlay products. However, with regard to reducing power, monascal adlay products were comparable to monascal rice products whereas monascal adlay products were better than uninoculated adlay products.

3.4. Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals

At 1 mg ml^{-1}, scavenging abilities on DPPH radicals were 6.2%, 16.0%, 41.7% and 20.9% for methanolic extracts from PA, DA, MPA and MDA, respectively (Fig. 3). At 1–10 mg ml^{-1}, the scavenging abilities increased to 81.1–94.1%. Generally, four methanolic extracts exhibited the same pattern. However, scavenging abilities of BHA, vitamins C and E were in the range of 90.0–96.4% at 0.1 mg ml^{-1}.

At 1 mg ml^{-1}, MPR scavenged 88.6% of DPPH radicals whereas scavenging abilities of PR, DR and MDR were 10.5, 9.2 and 24.3%, respectively (Tseng et al., 2003). Similarly, at 1–10 mg ml^{-1}, scavenging abilities increased to 92.7–96.7% for four methanolic extracts from various rice products (Tseng et al., 2003). With regard to scavenging ability on DPPH radicals, monascal adlay products were comparable to monascal rice products and monascal adlay products were similar to uninoculated adlay products.

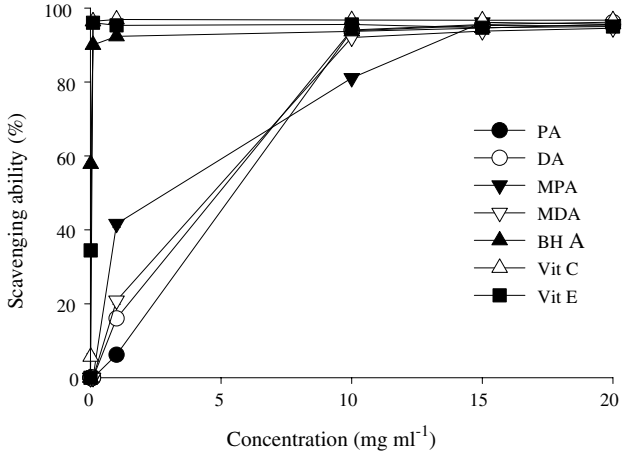

Fig. 3. Scavenging ability of methanolic extracts from various adlay products on 1,1-diphenyl-2-picrylhydrazyl radicals. Each value is expressed as mean ± standard deviation (n = 3). PA, polished adlay; DA, dehulled adlay; MPA, monascal polished adlay; MDA, monascal dehulled adlay.

3.5. Chelating ability on ferrous ions

At 1 mg ml^{-1}, chelating abilities of methanolic extracts from PA, DA, MPA and MDA on ferrous ions were 7.0%, 17.8%, 45.0% and 26.5%, respectively (Fig. 4). At 1–20 mg ml^{-1}, chelating abilities of methanolic extracts from DA, MPA and MDA increased steadily to 96.1–97.5%, whereas the methanolic extract from PA chelated 77.4% of ferrous ions at 20 mg ml^{-1}. However, EDTA showed an excellent chelating ability of 92.0% at 0.1 mg ml^{-1}.

At 1 mg ml^{-1}, chelating abilities of methanolic extracts from PR, DR, MPR and MDR on ferrous ions were 13.5%, 9.6%, 34.0% and 31.5%, respectively (Tseng et al., 2003). At 10 mg ml^{-1}, chelating abilities of methanolic extracts from PR, DR, MPR and MDR were in the range of 69.3–91.6% (Tseng et al., 2003). It seemed that chelating abilities of various rice products were better than those of various monascal products. However, these monascal products showed higher chelation of ferrous ions. Since ferrous ions are the most effective prooxidants in the food system (Yamaguchi, Tatsumi, Kato, & Yoshimitsu, 1988), high chelating abilities of methanolic extracts from various monascal products would be beneficial.

3.6. EC_{50} values in antioxidant properties

The antioxidant properties assayed herein are summarized in Table 2 and the results are normalized and expressed as EC_{50} values (mg methanolic extract per

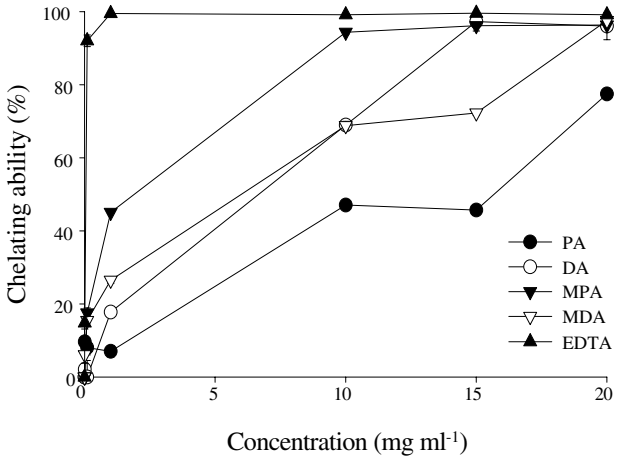

Fig. 4. Chelating ability of methanolic extracts from various adlay products on ferrous ions. Each value is expressed as mean ± standard deviation (n = 3). PA, polished adlay; DA, dehulled adlay; MPA, monascal polished adlay; MDA, monascal dehulled adlay.

Table 2
EC_{50} values of methanolic extracts from various adlay products in antioxidant properties

	EC_{50} value[a] (mg extract ml^{-1})			
	PA[b]	DA[b]	MPA[b]	MDA[b]
Antioxidant activity (conjugated diene method)	6.35 ± 0.05 A[c]	0.83 ± 0.01 B	0.75 ± 0.02 C	0.05 ± 0.01 D
Reducing power	13.24 ± 0.09 B	13.67 ± 0.10 A	0.78 ± 0.02 D	1.53 ± 0.11 C
Scavenging ability on DPPH radicals	5.49 ± 0.12 A	4.92 ± 0.06 B	2.91 ± 0.08 C	4.68 ± 0.03 B
Chelating ability on ferrous ions	15.68 ± 0.19 A	6.67 ± 0.14 B	1.91 ± 0.06 D	6.01 ± 0.05 C

[a] EC_{50} value: the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals were scavenged by 50%; and ferrous ions were chelated by 50%, respectively. EC_{50} value was obtained by interpolation from linear regression analysis.
[b] PA, polished adlay; DA, dehulled adlay; MPA, monascal polished adlay; MDA, monascal dehulled adlay.
[c] Each value is expressed as mean ± standard deviation ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$).

ml) for comparison. With regard to EC_{50} values of methanolic extracts, antioxidant activities by the conjugated diene method were excellent and in the descending order of MDA ≫ MPA > DA ≫ PA. Effectiveness in reducing powers inversely correlated with EC_{50} value and was in a descending order of MPA > MDA ≫ PA ∼ DA. Scavenging abilities on DPPH radicals were in the descending order of MPA > MDA > DA > PA. Chelating abilities on ferrous ions were in a descending order of MPA > MDA > DA > PA. All EC_{50} values of methanolic extracts from monascal products were below 10 mg ml^{-1}, indicating that monascal products exhibited higher antioxidant properties. All EC_{50} values of methanolic extracts from uninoculated products were below 20 mg ml^{-1}, indicating that uninoculated products exhibit high antioxidant properties.

For four antioxidant properties assayed, methanolic extracts from monascal products were better than those from uninoculated products. It seemed that adlay prior to monascal colonization exhibited high antioxidant properties. However, after fungal fermentation, the monascal adlay showed higher antioxidant properties. For two monascal adlay products, MDA exhibited higher antioxidant activity whereas MPA showed higher reducing power, scavenging ability on DPPH radicals and chelating ability on ferrous ions. For two uninoculated adlay products, DA exhibited higher antioxidant activity, scavenging ability on DPPH radicals and chelating ability on ferrous ions whereas PA showed higher reducing power.

When the extraction yields were taken into consideration, EC_{50} values (mg dried sample per ml) in antioxidant activity were 0.17, 3.21, 13.63 and 155.6 mg ml^{-1} for MDA, MPA, DA and PA, respectively. EC_{50} values in reducing power were 3.34, 5.15, 224.5 and 324.5 mg ml^{-1} for MPA, MDA, DA and PA, respectively. EC_{50} values for scavenging ability on DPPH radicals were 12.45, 15.77, 80.79 and 134.6 mg ml^{-1} for MPA, MDA, DA and PA, respectively. EC_{50} values in chelating ability on ferrous ions were 8.17, 20.25, 169.5 and 384.3 mg ml^{-1} for MPA, MDA, DA and PA, respectively.

Although BHA, vitamins C and/or E exhibited high antioxidant activity, reducing power and scavenging ability on DPPH radicals and EDTA showed high chelating ability on ferrous ions, they are additives and used or present in mg levels in foods. Various adlay products especially monascal adlay products could be used in g or hundreds of g levels as food or a food ingredient.

3.7. Antioxidant components

Naturally occurring antioxidant components, including ascorbic acid, tocopherols, and total phenols, were found in methanolic extracts from various adlay products (Table 3). β-Carotene was not found, whereas the contents of ascorbic acid and tocopherols were in the range of 0.04–0.15 mg g^{-1}. However, total phenols were the major naturally occurring antioxidant components found and methanolic extracts from monascal adlay

Table 3
Contents of ascorbic acid, β-carotene, tocopherols and total phenols of methanolic extracts from various adlay products

Compound	Content (mg g^{-1})			
	PA[a]	DA[a]	MPA[a]	MDA[a]
Ascorbic acid	0.12 ± 0.05 A[b]	0.04 ± 0.04 B	0.11 ± 0.02 A	0.15 ± 0.05 A
β-Carotene	nd[c]	nd	nd	nd
α-Tocopherol	0.02 ± 0.01 C	0.02 ± 0.01 C	0.14 ± 0.01 A	0.06 ± 0.01 B
γ-Tocopherol	0.02 ± 0.01 B	0.06 ± 0.01 A	0.03 ± 0.01 B	0.03 ± 0.01 B
δ-Tocopherol	0.04 ± 0.01 A	0.01 ± 0.01 B	0.02 ± 0.01 B	0.02 ± 0.01 B
Total phenols	3.05 ± 0.05 D	9.73 ± 0.07 C	25.45 ± 0.07 B	29.75 ± 0.25 A

[a] PA, polished adlay; DA, dehulled adlay; MPA, monascal polished adlay; MDA, monascal dehulled adlay.
[b] Each value is expressed as mean ± standard deviation ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$).
[c] Not detected.

products contained more total phenols than those from uninoculated adlay products. Total antioxidant components varied among methanolic extracts and were 3.25, 9.86, 25.75 and 30.01 mg g^{-1} for PA, DA, MPA and MDA, respectively.

Phenols such as BHT and gallate were known to be effective antioxidants (Madhavi, Singhal, & Kulkarni, 1996). Yen, Duh, and Tsai (1993) found that the antioxidant activity of the methanolic extract from peanut hulls correlated with its content of total phenols. Therefore, the high content of total phenols in all methanolic extracts might explain high antioxidant properties in various adlay products.

Overall, monascal adlay products, MPA and MDA, exhibited higher antioxidant activity, reducing power, scavenging and chelating abilities and higher in total phenol content than uninoculated adlay products, PA and DA. To study the antioxidant mechanisms by some specific phenolic components or secondary metabolites produced by *Monascus* species during fungal growth, the fractionation of the methanolic extract and further identification are in progress.

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