



Research Article

Antioxidant Activity of Ethanol Extract of Red Yeast Rice and its Fractionation Products

¹Hasim, ¹Dimas Andrianto, ¹Wira Islamiati, ¹Annisa Farhiah Wahdah Hamid and ²Didah Nur Faridah

¹Department of Biochemistry, Faculty of Mathematic and Natural Sciences, IPB Darmaga Campus, Bogor Agricultural University, 16680, Bogor, Indonesia

²Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, IPB Darmaga Campus, Bogor Agricultural University, 16680, Bogor, Indonesia

Abstract

Background and Objective: Red yeast rice (angkak) can be used as therapeutic agents for controlling cholesterol and blood sugar, given the bioactive compounds contained. The research was aimed to study antioxidant activity, total phenolic and flavonoid contents of crude ethanol extract of red yeast rice and its fractions using liquid-liquid partition. **Materials and Methods:** The crude ethanol extract of red yeast rice was fractionated using organic solvents with different polarity: n-hexane, dichloromethane, ethyl acetate and water. Antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). **Results:** The highest antioxidant activity was from ethyl acetate fraction with IC_{50} value of 12.82 mg L^{-1} and $190.84 \text{ mg TEAC g}^{-1}$ (FRAP method). Total phenolic and flavonoid content were also the highest in ethyl acetate fraction, at $66.23 \text{ mg GAE g}^{-1}$ and $22.09 \text{ mg QE g}^{-1}$, respectively. **Conclusion:** Fractionation of ethanol extract of red yeast rice showed that ethyl acetate fraction had the highest total phenols, flavonoids and antioxidant activity compared to the crude ethanol extract, n-hexane, dichloromethane and water fraction of red yeast rice.

Key words: Red yeast rice, antioxidant activity, total phenolic, total flavonoid, fractionation

Citation: Hasim, Dimas Andrianto, Wira Islamiati, Annisa Farhiah Wahdah Hamid and Didah Nur Faridah, 2018. Antioxidant activity of ethanol extract of red yeast rice and its fractionation products. *Res. J. Phytochem.*, 12: 52-59.

Corresponding Author: Hasim, Department of Biochemistry, Faculty of Mathematic and Natural Sciences, IPB Darmaga Campus, Bogor Agricultural University, 16680, Bogor, Indonesia Tel: +62813 1920 9692 Fax: (0251) 8423267

Copyright: © 2018 Hasim *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Degenerative diseases become the main cause of death in Indonesia. Generally, degenerative diseases are indicated by a continuous deterioration of function or structure of cells which can accumulate over time and lead to damaged tissues or even organs. One of the main causes of the diseases is the activity of free radicals in the body. Free radical is defined as a molecule, atom or some groups of atoms that has one or more unpaired electrons in their outer orbital, causing them to be unstable free and highly reactive¹. Free radicals can react with macromolecules such as proteins, lipids, carbohydrates and even nucleic acids and cause oxidative damage of those macro-molecules. Inability in destroying excessive free radicals will lead to oxidative stress. This condition initiates the development of degenerative diseases^{2,3}.

Free radicals are generally involved in a chain reaction, a series of reactions that regenerate new radicals that can start a new reaction and results in a continuous formation of radicals⁴. Our body elaborates defensive mechanisms against free radical oxidation to stop the chain reaction. The defense includes removing free radical catalysts, binding protein with redox active metal ions, protecting against macromolecule damage and reducing free radicals by donating electrons². These mechanisms are accounted by the presence of antioxidants, any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate¹.

Human body produces several types of endogenous antioxidants. However, the defensive mechanisms will be disturbed when excessive free radicals are formed, leading to sickness. The production of endogenous antioxidants declines as people age. Meanwhile, modern lifestyle is often considered unhealthy and promotes the formation of free radicals in the body. Therefore, exogenous antioxidants are important to suffice the needs for anti-oxidants².

Red yeast rice can be a source for exogenous antioxidant. Red yeast rice is rice fermented by *Monascus purpureus* yeast⁵. It has been long used as red colorant for food, meat and fish preservative⁶, an anti-hypertensive, hypo-cholesterolaemic, anti-microbial, anti-oxidative and anti carcinogenetic agent⁷.

Rajasekaran and Kalaivani⁸ reported that red yeast rice is potential as antioxidant with a high *in vitro* activity of $13921 \pm 23 \mu\text{mol trolox g}^{-1}$. Tisnadaja and Bustanussalam⁹ found that ethanol extract of red yeast rice had antioxidant activity with IC_{50} value of 111.10 mg L^{-1} .

Aniya *et al.*¹⁰ performed screening on 40 different species of yeast, including *Monascus* (31), *Aspergillus* (8) and *Rhizopus* (1). They revealed that 13 species of *Monascus* and 3 species of *Aspergillus* had inhibitory activity against DPPH radicals by >40% with *Monascus anka* or *Monascus purpureus* showing the highest activity¹⁰. The compounds in red yeast rice that can act as antioxidant are dimerumic acid¹¹, dihydromonacolin-MV, γ -aminobutyric acid (GABA), ankaflavin and monascin¹².

Fermentation of soy by *Monascus* increased the antioxidant activity (Fe ion reduction, DPPH radical scavenging, Fe ion chelating) which was correlated with the total phenolic content^{13,14}. Meanwhile, Hasim *et al.*¹⁵ reported that after fermentation by *Bacillus subtilis natto*, both genistein and daidzein concentration in black soybean increased up to 8 times as much as those in the raw one. The study proved that fermentation by *Bacillus subtilis natto* improves genistein and daidzein content in black soybean varieties detam 2. This occurred as fermentation transforms isoflavones in their glycosides form to aglycones.

Several studies on antioxidant compounds of red yeast rice extract have been done. However the antioxidant activity of its fractionation products remain unknown. Fractionation can be used to separate bioactive compounds in an extract based on their polarity to obtain purer compounds¹⁶. This study aimed to determine the antioxidant activity as well as total phenolic and flavonoid content of red yeast extract and its fractionation products.

MATERIALS AND METHODS

Place and time: This study was conducted in Biopharmaca Research Center and Research Laboratory of Biochemistry Department, Bogor Agricultural University. This research was implemented gradually from January-August, 2016.

Preparation of red yeast rice samples: Sample was oven dried, 100 g of red yeast rice (Bogor, Indonesia) was put in an oven (NDO-700, Eyela, Japan) at 50°C for 6 h, followed by grinding and sieving with 40 mesh sieve^{17,18}.

Extraction of red yeast rice: The extraction was done by maceration. Red yeast rice (20 g) was mixed with ethanol 95% (Merck, Germany) (400 mL). The mixture was macerated at 110 rpm for 2 h and filtered with filter paper (Whatman, Merck, Germany). The extraction was repeated on the residue at the same speed and duration. The filtrate was concentrated with rotary evaporator (N-1300S-W, Eyela, Japan) to obtain crude extract of red yeast rice¹⁹.

Fractionation of ethanol extract by liquid-liquid partition:

The crude ethanol extract of red yeast rice obtained from the previous step was fractionated based on their polarity. The fractionation was performed by liquid-liquid extraction with n-hexane (T and T chemical, Indonesia), dichloromethane (Jinan Shijitongda Chemical Co, Ltd., China), ethyl acetate (Dwi Prima Jaya Ltd., Indonesia) and water as solvent. The first fractionation was done by dissolving ethanol extract of the red yeast rice (0.5 g) in n-hexane:water (1:1). The mixture was let to separate in a separating funnel for 30 min until two distinct layers were formed, namely n-hexane and water layer. The n-hexane layer was separated from water layer and stored as n-hexane fraction. The water layer was again fractionated by adding dichloromethane to obtain dichloromethane and water layer. Dichloromethane layer was collected and stored as dichloromethane fraction. Further fractionation was performed to the water layer with ethyl acetate to obtain ethyl acetate and water fraction. The fractionation was performed three times for each solvent. The n-hexane, dichloromethane, ethyl acetate and water fraction were concentrated with rotary evaporator at 50°C and stored²⁰ at 4°C.

Determination of antioxidant activity with DPPH method:

Each red yeast rice extract (750 µL) from the fractionation process was mixed with DPPH solution 152 µM in methanol (750 µL) and incubated at 37°C for 20 min. The absorbance was measured with spectrophotometer UV-Vis (Genesys 10UV, Thermo scientific-USA) at 517 nm. The analysis was done in triplicate. The percentage of inhibition was determined as follow²¹:

$$\text{Inhibition (\%)} = \frac{[A_{\text{blank}} - A_{\text{sample}}]}{A_{\text{blank}}} \times 100$$

where, A_{blank} was absorbance of control (containing all reagents without sample) and A_{sample} was absorbance of samples. The IC_{50} (concentration of 50% inhibition) was determined from linear regression of the inhibition (%).

Determination of antioxidant activity with ferric reducing antioxidant power method^{22,23}:

Ferric reducing antioxidant power (FRAP) reagent consisted of acetate buffer (Sigma-Aldrich, St.Louis, MO, USA) solution 300 mM (pH 3.6), 2,4,6-tripyridyl-s-triazine (Sigma-Aldrich, St.Louis, MO, USA) (TPTZ) solution 10 mM in HCl, 40 mM and $FeCl_3 \cdot 6H_2O$ 20 mM. FRAP reagent was prepared by mixing acetate buffer (25 mL), TPTZ solution (2.5 mL) and $FeCl_3 \cdot 6H_2O$ solution (2.5 mL). The

solutions were mixed shortly before the analysis and incubated at 37°C for 30 min. Trolox was used as standard solution. Sample (10 µL) was then added with FRAP reagent (990 µL), homogenized and incubated at room temperature for 4 min. The absorbance was measured at 593 nm with spectrophotometer UV-Vis. The concentration value of sample was calculated by putting the absorbance value into the line equation at trolox standard curve.

Determination of total phenolic content: Total phenolic content was determined with Folin Ciocalteu method referring to study by Vongsak *et al.*²¹. Sample at 1000 µg mL⁻¹ (200 µL) was mixed with Folin Ciocalteu (Merck, Germany) 10% reagent (500 µL) and $NaHCO_3$ (Merck, Germany) 7.5% (800 µL) and incubated at room temperature for 30 min. The absorbance was measured at 765 nm with spectrophotometer UV-Vis. Gallic acid (JT Baker Chemical Co, USA) was used as standard to make standard curve. Total phenolic content of the sample was expressed as mg gallic acid/g extract (mg GAE g⁻¹ extract).

Determination of total flavonoid content: Total flavonoid content was determined according to Vongsak *et al.*²¹. Sample at 1000 µg mL⁻¹ (500 µL) was mixed with $AlCl_3$ (Merck, Germany) 2% (500 µL) and incubated at room temperature for 30 min. The absorbance was measured at 415 nm with spectrophotometer UV-Vis. Blank solution containing sample without the addition of $AlCl_3$ 2% was treated the same as samples. Quercetin (Sigma-Aldrich, USA) was used as standard for calibration curve. Total flavonoid content was expressed as mg QE g⁻¹ extract.

Statistical analysis: All measurements were conducted 3 times. Data were presented as means with their standard deviations (SD). Data analysis was done using mean value comparison analysis. Comparative analysis of mean values was performed by one-way ANOVA followed by least significant difference (LSD) test using the Statistical Product and Service Solutions (SPSS) software version 21 (SPSS Ltd., USA). A p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Total phenolic content: The phytochemical screening performed by Ismail *et al.*²⁴ showed that crude extract of red yeast rice positively contained several active compound such as flavonoid, saponin and alkaloid, potentially as antioxidant. Flavonoid and saponin belong to phenolic group which were capable of scavenging free radicals.

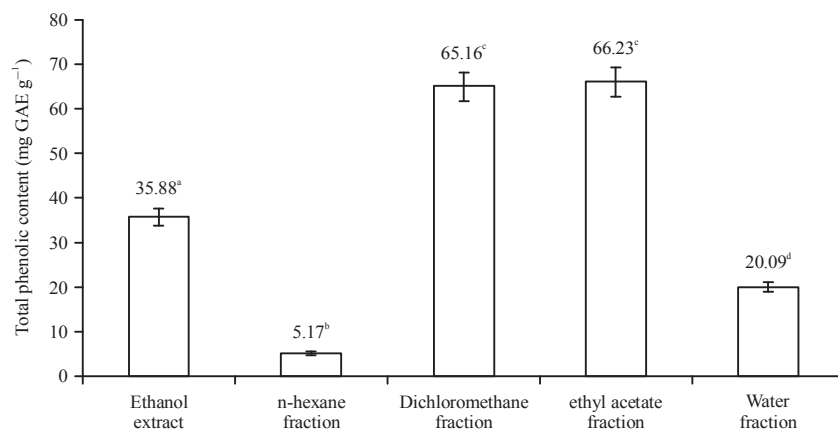


Fig. 1: Total phenolic content of red yeast rice extract and its fractions

Values are means with their standard deviations represented by vertical bars. The values followed by different superscript letters show significant differences ($p < 0.05$)

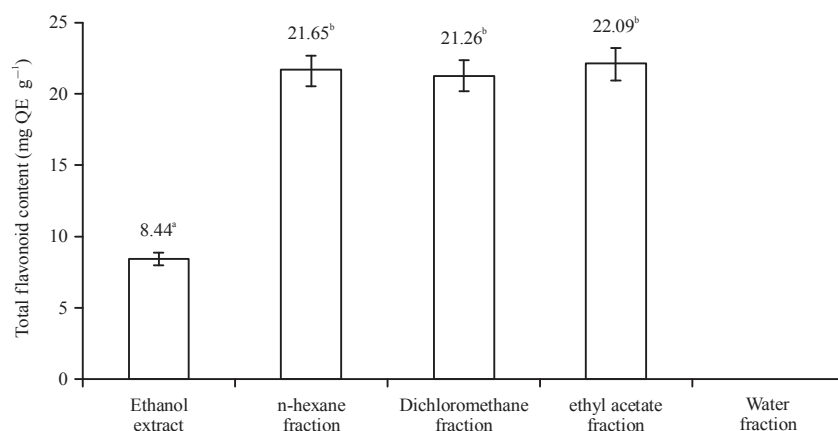


Fig. 2: Total flavonoid content of red yeast rice extract and its fractions

Values are means with their standard deviations represented by vertical bars. The values followed by different superscript letters show significant differences ($p < 0.05$)

Total phenolic contents of each extract and fraction of red yeast rice were presented on Fig. 1. The value was expressed as mg of gallic acid equivalent (GAE)/g (mg GAE g^{-1}). The highest phenolic content was found in ethyl acetate fraction and dichloromethane fraction, while the lowest was in n-hexane fraction.

Total phenolic content in red yeast rice was the highest in ethyl acetate, followed by in dichloromethane, ethanol extract, water and n-hexane fraction respectively. It indicated that phenolic compound in red yeast rice is more soluble in semi polar solvents such as ethyl acetate and dichloromethane. Aniya *et al.*¹¹ stated that the phenolic compound in red yeast rice is dimeric acid which is polar and easily dissolves in polar and semi polar solvents, but not in the nonpolar ones. This also explains why n-hexane fraction had the lowest total phenolic content.

The total phenolic content of red yeast rice ranged from 5.17-66.23 mg GAE g^{-1} . This range was higher than that found by Yeap *et al.*²⁵, which was 0.81-16.20 mg GAE g^{-1} . The difference in total phenolic content between the two studies was accounted to the type of solvent used during extraction. Additionally, fractionation process in present study could increase the purity and concentration of phenolic compound of the samples.

Total flavonoid content: Total flavonoid content was expressed as mg of quercetin equivalent in gram (mg QE g^{-1}). Total flavonoid of the extract and fractions of red yeast rice were presented on Fig. 2. The highest total flavonoid was found in dichloromethane fraction while the lowest was in the water fraction. Flavonoid compound was not even detected in the water fraction of red yeast rice.

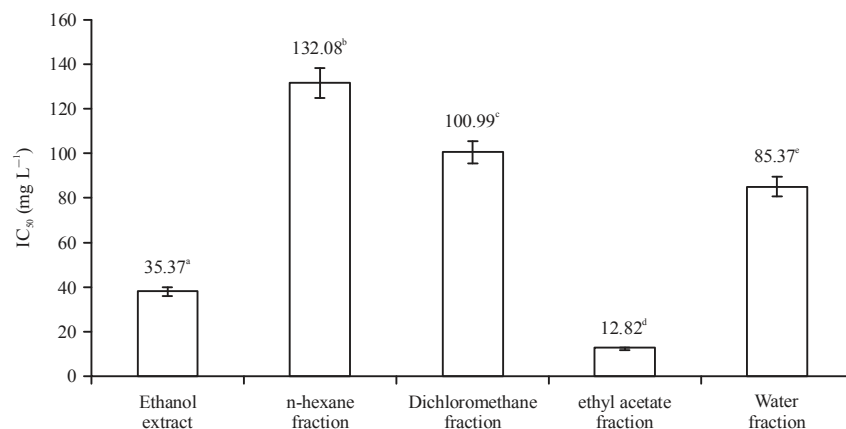


Fig. 3: IC₅₀ of red yeast rice extract and its fractions

Values are means with their standard deviations represented by vertical bars. The values followed by different superscript letters show significant differences ($p < 0.05$)

The total flavonoid in red yeast rice was the highest in ethyl acetate fraction followed by that in dichloromethane, n-hexane, ethanol extract and water fraction, respectively. Pigments in red yeast rice are yellow (monascin and ankaflavin), pink (monascurbin and rubropunctatin) and red pigment (monascorubrin dan rubropunctamine)²⁶. The pigments contain anthocyanin of flavonoid group that makes red yeast rice potential as antioxidant source. Anthocyanin has a polar structure that helps them dissolve easily in polar and semi polar solvents²⁷.

Total flavonoid in each fraction of red yeast rice was within the range of 0-22.09 mg QE g⁻¹. Meanwhile, that of other fermentation products, such as cheese was around 1.8-7.7 mg QE g⁻¹, fermented soy products around 1.9 mg QE g⁻¹, fermented macroalgae around 11.55-21.48 mg QE g⁻¹ ²⁸⁻³⁰. This result showed how red yeast rice contains higher total flavonoid than those three fermentation products.

Antioxidant activity of red yeast rice: The antioxidant activity was expressed in IC₅₀. Ethyl acetate fraction showed the highest antioxidant activity with IC₅₀ value of 12.82 mg L⁻¹, while n-hexane fraction had the lowest one with IC₅₀ value of 132.08 mg L⁻¹ (Fig. 3).

The IC₅₀ value of ethyl acetate fraction (12.82 mg L⁻¹) was higher than that of vitamin C (5.47 mg L⁻¹). According to Jun *et al.*³¹, antioxidant activity was considered as strong when the IC₅₀ value is <50 ppm, active when it is 50-100 ppm, moderate when it is 101-250 ppm, weak when it is 250-500 ppm and not active when it is >500 ppm. Ethyl acetate fraction had strong activity as its IC₅₀ values was less than 50 ppm. Kwon³² reported that ethyl acetate extract of red yeast rice

showed a high activity with IC₅₀ (0.13 mg L⁻¹). Therefore, water and dichloromethane fraction demonstrated an active antioxidant activity, while n-hexane fraction, with the lowest antioxidant activity showed a moderate one.

Crude ethanol extract of red yeast rice with IC₅₀ value of 35.37 mg L⁻¹ was also considered having strong antioxidant activity. Tisnadaja and Bustanussalam⁹ found that ethanol extract of red yeast rice had moderate antioxidant activity with IC₅₀ value of 111.10 mg L⁻¹. The extraction was performed by reflux with ethanol without any fractionation process. In this study, process extraction used maceration method.

Antioxidant activity of red yeast rice extract and fractions with FRAP method were presented in Fig. 4. Ethyl acetate fraction had the highest antioxidant activity while water fraction had the weakest antioxidant activity. Ethyl acetate fractionation had the highest antioxidant activity in both FRAP and DPPH methods. The main red pigments in red yeast rice were anthocyanin monascurbin and monascoflavin³³. Anthocyanins belong to flavonoid group and act as antioxidant³⁴.

It was because most of secondary metabolites, including antioxidants, are semi-polar that they are more readily soluble in semi-polar solvents, such as ethyl acetate and dichloromethane. Additionally, Tanaya *et al.*³⁵ stated that ethyl acetate as solvent could dissolve compounds from flavonoid, triterpenoid and tannin group. Meanwhile, steroids, quinone and terpenoids are more commonly found in dichloromethane³⁶. Each fraction of red yeast rice had antioxidant activity of 31.63-190.84 mg TE g⁻¹. Ethyl acetate fraction showed the highest ability in reducing Fe ion and scavenging DPPH radicals of all fractions. This fraction was also the highest in total phenolic and flavonoid content. Studies

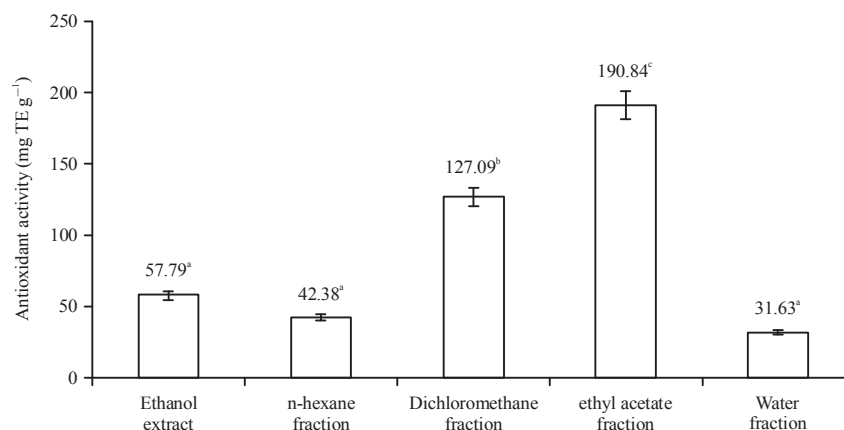


Fig. 4: Antioxidant activity of red yeast rice extract and its fractions with FRAP method

Values are means with their standard deviations represented by vertical bars. The values followed by different superscript letters show significant differences ($p < 0.05$)

demonstrated positive correlation between antioxidant activity and total phenol and flavonoids. Strong correlation between them was found in broccoli³⁷, chili, mung bean, snaps, spinach³⁸ and sweet potato³⁹. The increasing antioxidant activity proportional to total phenolic and flavonoid content suggests that phenolic compounds are responsible for the antioxidant activity of food.

CONCLUSION

Fractionation of ethanol extract of red yeast rice showed that ethyl acetate fraction had the highest total phenols, flavonoids and antioxidant activity compared to the crude ethanol extract, n-hexane, dichloromethane and water fraction of red yeast rice. There is a correlation between antioxidant activity and total phenol and flavonoids.

SIGNIFICANCE STATEMENT

The study was conducted to evaluate the antioxidant activity as well as total phenolic and flavonoid contents of each fraction of crude ethanol extract of red yeast rice. It was found that the highest antioxidant activity was significantly demonstrated by the ethyl acetate fraction of red yeast rice extract. In line with its antioxidant activity, that fraction also gave the highest levels of total phenolic and flavonoids compared to other fractions. The study result provides information about the best solvent for red yeast rice extraction related to antioxidant studies, that is semi-polar solvents especially ethyl acetate. This information will assist the researchers on performing the isolation and purification of certain antioxidant compound in red yeast rice and also the antioxidant activity test *in vivo*.

ACKNOWLEDGMENTS

The authors would like to acknowledge Ministry of Research, Technology and Higher Education of Indonesia for financial support through Program Penelitian Strategis Aplikatif 2016. The grant number is 079/SP2H/LT/DRPM/ii/2016.

REFERENCES

- Halliwell, B. and J.M. Gutteridge, 1989. Free Radicals in Biology and Medicine. Clarendon Press, Oxford, UK.
- Deshpande, S.S., U.S. Deshpande and D.K. Salunkhe, 1995. Nutritional and Health Aspects of Food Antioxidants. In: Food Antioxidants. Technological, Toxicological and Health Perspectives, Madhavi, D.L., S.S. Deshpande and D.K. Salunkhe (Eds.). Marcel Dekker, New York, ISBN: 9780824793517, pp: 361-470.
- Shinde, A., J. Ganu and P. Naik, 2012. Effect of free radicals and antioxidants on oxidative stress: A review. J. Dental Allied Sci., 1: 63-66.
- Sen, S., R. Chakraborty, C. Sridhar, Y.S.R. Reddy and B. De, 2010. Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. Int. J. Pharm. Sci. Rev. Res., 3: 91-100.
- Steinkraus, K.H., 1983. Handbook of Indigenous Fermented Foods. Marcel Dekker, Inc., New York, USA.
- Ma, J., Y. Li, Q. Ye, J. Li and Y. Hua *et al.*, 2000. Constituents of red yeast rice, a traditional Chinese food and medicine. J. Agric. Food Chem., 48: 5220-5225.
- Wang, T.H. and T.F. Lin, 2007. *Monascus* rice products. Adv. Food Nut. Res., 53: 123-159.

8. Rajasekaran, A. and M. Kalaivani, 2011. Hypolipidemic and antioxidant activity of aqueous extract of *Monascus purpureus* fermented Indian rice in high cholesterol diet fed rats Turk. J. Med. Sci., 41: 25-32.
9. Tisnadjaja, D. and Bustanussalam, 2012. Hubungan profil kromatografi dari ekstrak angkak dengan aktivitas antioksidannya. Prosidings of Seminar Nasional XXI Kimia dalam Industri dan Lingkungan, Desember 6, 2012, Yogyakarta, Indonesia.
10. Aniya, Y., T. Yokomakura, M. Yonamine, K. Shimada, T. Nagamine, M. Shimabukuro and H. Gibo, 1999. Screening of antioxidant action of various molds and protection of *Monascus anka* against experimentally induced liver injuries of rats. Gen. Pharmacol.: Vasc. Syst., 32: 225-231.
11. Aniya, Y., I.I. Ohtani, T. Higa, C. Miyagi and H. Gibo *et al.*, 2000. Dimerumic acid as an antioxidant of the mold, *Monascus anka*. Free Radical Biol. Med., 28: 999-1004.
12. Dhale, M.A., S. Divakar, S.U. Kumar and G. Vijayalakshmi, 2007. Isolation and characterization of dihydromonacolin-MV from *Monascus purpureus* for antioxidant properties. Applied Microbiol. Biotechnol., 73: 1197-1202.
13. Lee, Y.L., J.H. Yang and J.L. Mau, 2008. Antioxidant properties of water extracts from *Monascus* fermented soybeans. Food Chem., 106: 1128-1137.
14. Lee, Y.L., J.H. Yang and J.L. Mau, 2009. Antioxidant properties of ethanolic and methanolic extracts from *Monascus*-fermented soybeans. J. Food Biochem., 33: 707-727.
15. Hasim, P. Astuti, S. Falah and D.N. Faridah, 2015. Bacillus subtilis natto fermentation to improve aglycone isoflavones content of black soybean varieties detam 2. Int. Food Res. J., 22: 2558-2564.
16. Rocha-Santos, T. and A. Duarte, 2014. Analysis of Marine Samples in Search of Bioactive Compounds. Elsevier, Oxford, UK., ISBN: 9780444633590, Pages: 312.
17. Zubaidah, E. and D.P. Sari, 2015. [The effect of mung bean addition on rice media IR36 toward pigment and lovastatin of Angkak]. J. Pang. Agroindus., 3: 962-971.
18. Damayanthi, E., 2002. [Characteristics of stabilized rice (*Oryza sativa*) brand and *in vitro* antioksidant and cuncer cell line antiproliferation activities of rice bran oil and its fractions]. Ph.D. Thesis, Bogor Agricultural University, Bogor, Indonesia.
19. Singgih, M., V. Saraswaty, D. Ratnaningrum, S. Priatni and S. Damayanti, 2014. The influence of temperature and ethanol concentration in monacolin K extraction from *Monascus* fermented rice. Proc. Chem., 9: 242-247.
20. Andrianto, D., T. Katayana and T. Suzuki, 2015. Screening of antioxidant and antihyperlipidemic potencies of indonesian underutilized fruits. J. For. Biomass Utilization Soc., 10: 19-25.
21. Vongsak, B., P. Sithisarn, S. Mangmool, S. Thongpraditchote, Y. Wongkrajang and W. Gritsanapan, 2013. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Ind. Crop Prod., 44: 566-571.
22. Benzie, I.F.F. and J.J. Strain, 1996. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Biochem., 239: 70-76.
23. Tananuwong, K. and W. Tewaruth, 2010. Extraction and application of antioxidants from black glutinous rice. LWT-Food Sci. Technol., 43: 476-481.
24. Ismail, A.I., Hasim, S. Falah and D.N. Faridah, 2016. α -Glucosidase inhibition by red yeast rice extract and fractions as *in vitro* antidiabetes. Der Pharm. Chem., 8: 46-49.
25. Yeap, S.W., B.K. Beh, J. Kong, W.Y. Ho and H.M. Yusof *et al.*, 2014. *In vivo* hypocholesterolemic effect of MARDI fermented red yeast rice water extract in high cholesterol diet fed mice. Evid.-Based Complement. Altern. Med., Vol. 2014. 10.1155/2014/707829.
26. Pattanagul, P., R. Pinthong, A. Phianmongkhon and N. Leksawasdi, 2007. Review of Angkak production (*Monascus purpureus*). Chiang Mai J., 34: 319-328.
27. Wallace, T.C. and M.M. Giusti, 2014. Anthocyanins in Health and Disease. CRC Press, Boca Raton, UK., ISBN: 9781439894767, Pages: 368.
28. Katawal, I., G.P. Kharel and B.H. Poudel, 2016. Antioxidant activity and cytotoxicity of Nepalese nak cheese. Biol. Chem. Res., 3: 234-245.
29. Yang, Y.C., H.Y. Lin, K.Y. Su, C.H. Chen and Y.L. Yu *et al.*, 2012. Rutin, a flavonoid that is a main component of *Saussurea involucrate*, attenuates the senescence effect in d-galactose aging mouse model. Evid.-Based Complement. Altern. Med., Vol. 2012. 10.1155/2012/980276.
30. Sakulpong, A., A. Wongklom and P. Moonsin, 2015. Total phenolics, flavonoids and antioxidant activity of macroalgae fermented with lactic acid bacteria. J. Food Sci. Agric. Technol., 1: 177-181.
31. Jun, M., H.Y. Fu, J. Hong, X. Wan, C.S. Yang and C.T. Ho, 2003. Comparison of antioxidant activities of isoflavones from kudzu root (*Pueraria lobata* Ohwi). J. Food Sci., 68: 2117-2122.
32. Kwon, S.C., 2012. Antioxidant properties of red yeast rice (*Monascus purpureus*) extracts. J. Korean Soc. Food Sci. Nutr., 41: 437-442.
33. Loret, M.O. and S. Morel, 2010. Isolation and structural characterization of two new metabolites from *Monascus*. J. Agric. Food Chem., 5: 1800-1803.
34. Taira, J., C. Miyagi and Y. Aniya, 2002. Dimerumic acid as an antioxidant from the mold, *Monascus anka*. The inhibition mechanisms against lipid peroxidation and heme protein-mediated oxidation. Biochem. Pharmacol., 63: 1019-1026.

35. Tanaya, V., R. Retnowati and Suratmo, 2015. Fraksi semi polar dari daun mangga kasturi (*Mangifera casturi* Kosterm). *Kimia Student J.*, 1: 778-784.
36. Widiyantoro, A., 2014. Metabolit sekunder prospektif dari famili simaroubaceae. *J. Penelitian Saintek*, 19: 14-22.
37. Gawlik-Dziki, U., 2008. Effect of hydrothermal treatment on the antioxidant properties of broccoli (*Brassica oleracea* var. *Botrytis italica*) florets. *Food Chem.*, 109: 393-401.
38. Turkmen, O., S. Sensoy, A. Dursun and M. Turan, 2004. Sewage sludge as a substitute for mineral fertilization of Spinach (*Spinacia oleracea* L.) at two growing periods. *Acta Agric. Scand. Sec. B: Soil Plant Sci.*, 54: 102-107.
39. Bellail, A.A., O.E. Shaltout, M.M. Youssef and A.M.A. El Gamal, 2012. Effect of home-cooking methods on phenolic composition and antioxidant activity of sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars grown in Egypt. *Food Nutr. Sci.*, 3: 490-499.