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PHARMACY AND ADVANCED PHARMACEUTICAL SCIENCES

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Pharmaceutical Science*

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METAL CHELATING ACTIVITY OF RICE BRAN AND RICE HUSK

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ABSTRACT

Free radical-induced oxidative stress is the root cause for many human diseases. Naturally occurring antioxidant supplements from plants are vital to counter the oxidative damage in cells. The main objective of the present study was to explore and compare the antioxidant activity of rice bran and rice husk extracted from rice milling waste. N-hexane extracts of rice bran and rice husk were used to study their in vitro antioxidant activities using metal chelating activity (iron (II)-phenanthroline complex). Vitamin E was used as standard material. The ability of the sample to chelate metal ion (ferrous ion) was calculated relative to the control and expressed as % inhibition. % inhibition of two samples were analyzed with student test ($P=0.05$). The results have shown that at the same concentration (10 ppm), rice husk extract, rice bran extract and vitamin E have the different activity, i.e.: 0.51%, 2.27% and 5.55% in inhibition of chelat formation, respectively. In conclusion, metal chelating activity of rice husk extract is smaller than rice bran extract. Activity of rice bran extract is almost a half from vitamin E, so this extract is still potential to be developed as source of antioxidant compounds.

Key words: rice bran, rice husk, metal chelating activity, antioxidant

INTRODUCTION

Rice bran is a rich source of natural antioxidants which can be used as free radical scavengers. It is widely recognized that many of the today's diseases are due to the oxidative stress that result from an imbalance between formation and neutralization of pro-oxidants (Hazra et al., 2008; Braca et al., 2002). Cells have developed antioxidant mechanisms to quench the free radicals but when the generation of free radicals exceeds the scavenging capacity of the cell, the excess free radicals seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells resulting the induction of lipid peroxidation which leads to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases (Rao et al., 2010). The free radicals are known to be scavenged by synthetic antioxidants, but due to their adverse side effects leading to carcinogenicity; search for effective and natural antioxidants has become crucial (Choi et al., 2007; Adeolu et al., 2009).

Rice bran is a by-product of rice milling which contains a significant amount of natural phytochemicals including sterols, higher alcohols, gamma-oryzanol, tocopherols, tocotrienols and phenolic compounds (Nam et al., 2006; Isao et al., 2004; Devi & Arumughan, 2007). These bioactive molecules have known to reduce serum cholesterol, decrease the incidence of atherosclerosis and also have antitumor properties (Deepa et al., 2008; Simi & Abraham, 2008; Halliwell, 1992; Itani & Ogawa, 2004).

In addition to rice bran, a by-product of rice milling is rice husk. This process yields rice husk and rice bran between 15-20% and 8-12%, respectively. Husk is outer skin of rice, while bran is epidermis of rice. If the national dry paddy production is 49.8 million tons/year, therefore the products of husk and bran are 7.5-10 million tons/year and 4-6 million tons/year, respectively. Utilization of rice by-products are still limited, sometimes even pollute the environment. These materials actually have an economic value well if be handled correctly. They can increase the value added in agro-industry system of rice. Some alternatives include the use of rice husk as a growing medium for mushrooms and ornamental plants, fuel, ash scrub, a mixture of tiles and biodiesel makers (Rachmaniah et al., 2007).

Bran can be utilized in the manufacture of breakfast cereals and in the increasing of dietary fiber (Hermanianto et al., 1999; Widowati, 2001).

Antioxidant activity of rice bran has been studied previously using several methods, i.e.: reducing power, total antioxidant activity, nitric oxide scavenging capability and DPPH scavenging assay. The reducing power of the rice bran extracts increased with the increasing concentration and a significant change was observed at 0.1 to 0.5 mg/ml. Total antioxidant activity of the rice bran extracts also increased with the increasing concentration of the extracts and a significant change was observed at 0.02 to 0.1 mg/ml. On DPPH test, the extracts showed activity with an IC_{50} 30.85 μ g/ml (Rao et al., 2010).

Since rice husk is paddy skin to, hence it is supposed contain antioxidant compounds. At the other hand, there is no antioxidant activity test on rice husk recently. Based on the background mentioned, it is important to study the antioxidant activity of rice husk and compared it to rice bran and synthetic antioxidant.

There are many mechanisms of action of antioxidant compounds in counteracting the effects of oxidants. Antioxidant activity test was used in this study is metal chelating activity. This method is useful to investigate the function of antioxidants that can bind metals (Limantara et al., 2009). Solvent was used to extract the rice husk and rice bran is n-hexane. It was subjected to attracts non-polar compounds contained in both materials. Antioxidant activity of rice husk and rice bran were also compared to synthetic antioxidant vitamin E. The antioxidant capacity is determined from the IC_{50} (Inhibition Concentration 50), the concentration of test material that can inhibit 50% the formation of chelate (Lim et al., 2007).

METHODOLOGY

Materials and equipments

The samples of rice bran and rice husk were obtained from the rice milling unit, UD Eka Jaya located at Surabaya, East Java in september 2010. Standard α -tocopherol was purchased from Sigma-Aldrich Chemical Co. n-hexane and methanol were purchased from Mallinckrodt, while $FeSO_4$, 1,10-phenantroline and ethyl acetate were purchased from Merck. Demineralised water was used as reagent's solvent.

Equipments were used iclude rotary evaporator (IKA[®] WERKE RV06-ML), analitical balance (Sartorius), Spectrophotometer UV-Vis (Shimadzu), vortex (Branson 1200), Maxi-mix (Thermolyne tipe 1600), and glassware equipments.

Methods

Preparation of rice husk and rice bran extract

Rice husk was washed and then dried at ambient temperature. After that, 150 g of dried rice husk was extracted thrice with certain volume of n-hexane for 2 h in an electrical stirrer at room temperature. Next, the extracts were filtered and evaporated under vaccum using a rotary evaporator (50°C) until thick extracts were obtained. Rice bran extracts were prepared in same manner without washing on starting materials. Finally, rice bran and rice husk extracts were dissolved in methanol until certain concentration (called as mother extract). From this concentration, working extracts were prepared in several concentrations.

Metal chelating activity

The metal chelating activity of extracts were measured qualitatively by the decrease in the intensity color of the iron (II)-phenantroline complex. Rice husk and rice bran extracts were applied on TLC plate Si Gel 60F₂₅₄, eluted with n-hexane:ethyl acetate (17:3) and sprayed with mixture of 1.68% $FeSO_4$ and 0.32% 1,10-phenantroline. Metal chelating inhibition was shown by light spot color with orange background.

Metal chelating activity tests were performed quantitatively according to **Lim et al. (2007)** with modification, as follow: 2 ml of 0.056% $FeSO_4$, and 2.0 ml of 0.0108% phenantroline were mixed with 1.0 ml of sample (with different dilutions). The mixture was allowed to equilibrate for 10 min before

measuring the absorbance at 509 nm. Sample solutions with appropriate dilutions were used as blanks as the extracts may also absorb at this wavelength. The ability of the sample to chelate ferrous ion was calculated relative to the control (consisting of iron and phenantroline only) using the formula,

$$\text{Chelating effect (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of test sample.

Statistical analysis

The measurements of samples were replicated at four times and the standard at twice. The results were statistically analysed with Student Test. Statistical significance was accepted at a level of $P < 0.05$

RESULT AND DISCUSSION

In this study, the using of n-hexane as menstrum was subjected to extract the non polar compounds such as sterols, higher alcohols, gamma-oryzanol, tocopherols and tocotrienols. These compounds have been supposed to have antioxidant activity. From 150.0 g rice husk and rice bran were obtained 74.6 mg (0.05%) and 1.2 g (0.8%) dry extracts, respectively. The metal chelating activity of rice husk and rice bran extracts are illustrated qualitatively in fig. 1.

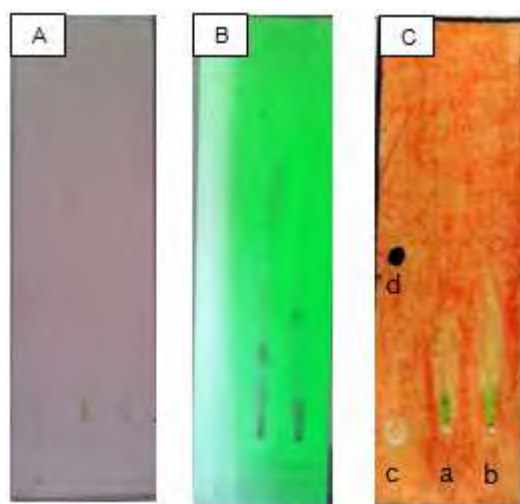


Fig. 1. TLC profile of rice husk extract (a), rice bran extract (b), vitamin E (c)* and catechin (d)* on Si gel 60 F254, eluted with n-hexane:ethyl acetate (17:3) under vis (A), uv 254 (B), and sprayed with mixture of 1.68% FeSO_4 and 0.32% 1.10-phenantroline (C)

*not eluted

From the fig. 1 (C), there are three white spots (a, b, c) and a black spot (d) with orange background. This indicated that both rice bran and rice husk extracts contain some compounds which inhibit the metal chelating reaction.

The chelating ability of the extract measures how effective the compounds in it can compete with phenantroline for ferrous ion. The iron-phenantroline complex has maximum absorbance at 509 nm and a large decrease in absorbance indicates strong chelating power. By forming a stable iron(II) chelate, an extract with high chelating power reduces the free ferrous ion concentration thus decreasing the extent of Fenton reaction which is implicated in many diseases (Halliwell & Gut-teridge, 1990).

Data of ferrous chelating activity of rice bran and rice husk are shown in table 1. Although there have been performed activity test with several concentrations of sample (both rice bran and rice husk extracts at 7-21 ppm), but only one concentration showed inhibition activity, i.e: 10 ppm. Therefore IC₅₀ of rice bran extract can not be calculated and neither can rice husk extract. From T test, there is found a significant difference between rice bran and rice husk extracts (t stat: 314.3911 > t critical two-tail: 2.447)

Table 1. Metal chelating activity of rice husk and rice bran extracts

Replication	Rice bran extract		Rice husk extract	
	Concentration (ppm)	Inhibition (%)	Concentration (ppm)	Inhibition (%)
1	10.62	2.28	10.044	0.51
2	10.53	2.26	10.026	0.50
3	10.62	2.26	10.044	0.51
4	10.35	2.26	10.044	0.52
Mean	10.53	2.27	10.04	0.51

It is found that rice brand and rice husk extract have low chelating power. For comparison, at approximately same concentration (10 ppm) vitamin E has inhibition 5.52%. This value is derived from linear correlation equation between concentrations of vitamin E versus % inhibition (table 2).

Table 2. Metal chelating activity of vitamin E

Replication	Concentration (ppm)	A _{sample}	A _{control}	Inhibition (%)	IC ₅₀ (ppm)
1	12.3	0.803	0.865	7.17	234.4 (y = 4.3098 + 0.1949x)
	24.6	0.778		10.06	
	49.2	0.77		10.98	
	61.5	0.719		16.88	
	73.8	0.703		18.73	
	86.1	0.675		21.97	
2	12.1	0.808	0.863	6.37	244.4 (y = 3.9635 + 0.1884x)
	24.2	0.772		10.54	
	48.4	0.766		11.24	
	60.5	0.749		13.21	
	72.6	0.72		16.57	
	84.7	0.666		22.82	

Although the metal chelating activity of rice bran and rice husk extract are low, but it can not be concluded that both have low antioxidant activity. This can be caused by many mechanisms of action of antioxidant. For comparison, guava has very potent primary antioxidant property but its function as secondary or preventive antioxidant is poor. At the other hand, langsung though acts as a weak primary antioxidant, can act as a moderate secondary antioxidant. Primary antioxidants scavenge radicals to inhibit chain initiation and break chain propagation. Secondary antioxidants suppress the formation of radicals and protect against oxidative damage such as bind to metal ions.

CONCLUSIONS

Rice bran extracts have shown metal chelating activity greater than rice husk extracts, respectively 2.26% and 0.51% at 10 ppm of extract. At 10 ppm, vitamin E has inhibition 5.52% and IC₅₀ equivalent to 239.4 ppm. Although inhibition activity of rice bran extracts are smaller than vitamin E, but there is a good possibility to use this waste material as antioxidant sources.

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