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Antioxidant Properties of *Garcinia mangostana* L (Mangosteen) Rind

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Abstract

Many diseases correlate with antioxidant deficiencies. *Garcinia mangostana* L rind (GMR) belong to waste product, contains xanthones which are antioxidant compounds. The aim of this study was to determine antioxidant properties of its ethanolic extract, hexane, ethylacetate, butanol, and water fractions in DPPH scavengingactivity, level of SOD and total antioxidant (TAS) compared against α-mangostin. Extract and all of these fractions had high DPPH trapping activity while α-mangostin had low activity. Level of SOD was highest in GMR water fraction while TAS level was highest in GMR ethylacetate fraction. It was concluded that GMR products had potential antioxidant properties

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Keywords: *Garcinia mangostana* L (mangosteen) rind, DPPH, SOD, TAS

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1. Introduction

Antioxidant deficiency correlates with many diseases. Lipid peroxidation is induced by oxidative stress and is a key process in many pathological events. The destruction of membrane lipid caused by unsaturated lipid oxidation producing malondialdehyde as breakdown products is mutagenic and carcinogenic[1]. *G.mangostana* rind, as waste product, contains a lot of water soluble antioxidant[2]. Various kinds of xanthones in *G.mangostana* rind had been proven to have strong antioxidant activity included alpha mangostin[3]. According to Zarena and Udaya Sankar (2009), antioxidant activity among these rind extracts was different each other deepending on the solvent: *G.mangostana* rind extract especially acetone and ethyl acetate extract was effective to inhibit lipid peroxidation induced by ferrous sulphate-ascorbate system in the linolenic acid medium as lipid phase model system[4]. Besides

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containing antioxidant activities, it has antitumoral, anti-inflamatory, antiallergy, antibacterial, antiviral , and antifungal activities[5,6]. According to Palakawong *et al*. in their study, antioxidant activity of *G.mangostana* rind extracted with 50 % ethanol, which IC_{50} was 5.94 µg/ml, was the strongest compared to its bark and leaves as well[7]. The aim of this study was to evaluate the antioxidant properties of *G. mangostana* L (GMR) rind 96 % and 70 % ethanolic extract and hexane, ethyl acetate, butanol, and water fraction by measuring DPPH scavenging activity, SOD activity, and total antioxidant status of these samples

2. Experiments

2.1. Material

Ripe *G.mangostana* L which had purple colour collected from Subang (West Java), destilated water, 96 % and 70 % ethanol, hexane, ethyl acetate, butanol, methanol solution, DPPH solution (Sigma Aldrich), Cayman's Superoxide Dismutase Assay Kit, and Superoxide Dismutase Assay Kit

2.2. Extraction and`fractionation

Mangosteen fruit rind was washed as clean as possible, dried at 37 C, pounded, and macerated using 96% and 70% ethanol, until the filtrate becoming no colour anymore. The filtrate was evaporated until becoming pasta like extract. Parts of the 96% ethanolic extracts was macerated again using hexane-water mixture in 1:1 ratio until this filtrate becoming colourless again and then this filtrate was evaporated also becoming hexane fraction. The last residu was treatedsuch as before consecutively using ethyl acetate-water mixture 1:1, butanol-water mixture 1:1, and water only to get ethyl acetate fraction, butanol fraction, and water fraction.

2.3. DPPH scavenging activity assay

The DPPH assay was carried out as described by Unlu *et al*.[8]. To obtain the IC_{50} value, 50 µL of each extract and fractions of GMR at a range of various final concentrations 100; 50; 25; 12.5; 6.25; 3.125; 1.563; 0.781; 0.391 and 0.195 µg/mL in methanol solution were pippeted and put into the microplate and then added with 200 µL of 0.077 mmol/L DPPH (Sigma Aldrich) in methanol.The reaction mixtures were shaken vigorously and kept in the dark for 30 minutes at room temperature, furthermore DPPH scavenging activity was determined by microplate reader at 517 nm.

The radical scavenging activity of each sample was determined by the ratio of DPPH absorption decrease against the absorption of DPPH solution in the absence of test sample (negative control) using the following equation.

$$Scaveninge(\%) = \frac{Ac - As}{AC} x100$$

A_S: absorbance of samples, A_C: negative control absorbance (without sample)

2.4. Superoxide Dismutase (SOD) activity assay

The SOD assay was carried out using Cayman's Superoxide Dismutase Assay Kit. 10 µL of three series of concentrations including 500; 125; 31.25 µg/mL of GMRextract and fractions were pippeted into sample wells, then 200 µL diluted radical detector was added into each wells. For SOD standard wells, 200 µL diluted radical detector and 10 µL standard was pippeted into the wells (tubes A-G). To intitiate the reactions, 20 µL diluted xanthine oxidase was added into each wells. This well plate was shaked for few seconds for mixing and incubated on shaker for 20 minutes at room temperature. Absorbance of each well was read at 450 nm curve and linearized rate (LR). The SOD level in U/ mL was determinate by the equation below.

$$SOD = \frac{(sampleLR - y.\text{int}ercept).x.0.23mL}{Slope} x.sample.dilution$$

2.5. Total Antioxidant Status (TAS) assay

The TAS assay was carried out using Cayman's Antioxidant Assay Kit. Into the each of the sample wells, 10 µL of three series of concentrations including 500; 125; 31.25 µg/mL of GMRextract and fractions, 10 µL metmyoglobin, and 150 µL chromogen were added. For Trolox standard wells, 10 µL trolox standard (tubes A-G), metmyoglobin, and 150 µL chromogen were put. To intitiate the reactions, 40 µL hydrogen peroxide was added into all wells. The plate was incubated on a shaker for 5 minutes at room temperature. Absorbance was read at 750 nm using Elisa Reader. The TAS was calculated using the linear regression equation of the standard curve written in the following equation[3].

$$TAS(mM) = \frac{absorbance - y\,\text{int}ercept}{slope} x.dilution \quad (3)$$

3. Results and Discussion

To assure the quality of this crude drugs, proximate analysis was done as shown in Table 1 below.

Table 1. Proximate Analysis of *G.mangostana*L Rind Crude Drug

No	Proximate Analysis	%
1.	Water content	10.31
2.	Ash	20.54
3.	Protein	3.43
4.	Rough fiber	25.53
5.	lipid	0.54
6.	Carbohydrate	49.96

Because of water content of this simplicia is only 10,31 %, this material is dry enough to protect from fungus contamination. DPPH scavenging activity of GMR extract and its various fractions and alpha mangostin is shown in the Figure1.

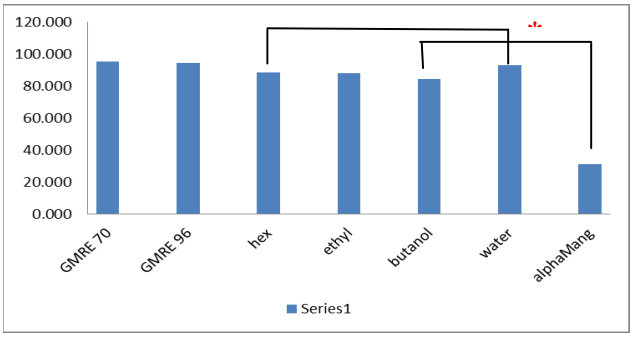

Figure 1. DPPH Scavenging Activity of GMR Extract, Fractions, and Alpha Mangostin at 500 µg/ mL * means significant difference between these groups

According to DPPH free radical scavenging activity, the IC_{50} of these materials is shown in the Table 2 below.

Table 2. IC_{50} of DPPH Scavenging Activity of GMR and Alpha Mangostin

Extract/ Fraction	IC_{50} (µg/mL)
96 % alcohol extract	7,48 ± 0,19
70 % alcohol extract	6.563 ± 0.311
Hexane fraction	3,62 ± 0,04
Ethyl acetate fraction	13,29 ± 0,12
Butanol fraction	12,23 ± 0,13
Water fraction	10,31 ± 0,04
Alpha mangostin	66,63 ± 34,65

IC_{50} of DPPH scavenging activity of GMR96% and 70 % ethanolic extracts were (7,48 ± 0,19)% and (6.563 ± 0.311)%. These IC_{50} is higher, that's meant less DPPH scavenging activity, than reported by Palakawong *et al*.[7] which was 5.94 µg/mL although they used 50% ethanol for the extraction. The different region of *G.mangostana* L collection and degree of the fruit ripeness might play a role to cause it.

Similar results were concluded about different antioxidant activity in using various extraction solution for sample extraction by Zarena and Udaya Sankar[4]. This difference could be caused by different extraction capacity of each solution to extract GMR polar active compund[4]. Phenolic compound content of GMR extract such as tannin, alpha mangostin, epicatechin was different if solvent extraction was different. Therefore DPPH and hydroxyl radical scavenging and also lipid peroxidation inhibition activity was different in several extraction solution.Water extract had stronger capacity than methanol extract whilemethanol extract had stronger capacitythan hexane extract[9]. Our study had the similar results with that ones.

Total antioxidant status/ total antioxidant capacity (mM Trolox) of these GMR extracts, fractions, and alpha mangostin is shown in Figure 2.

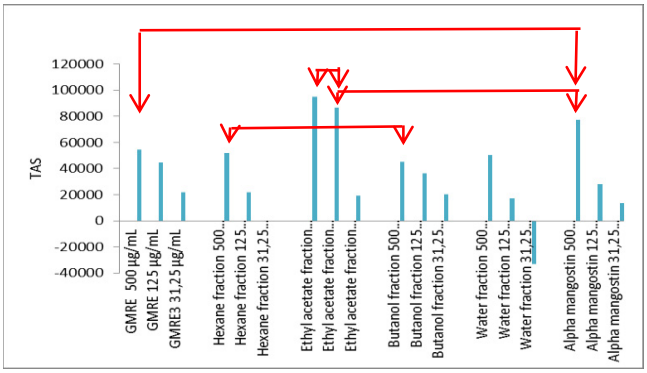

Fig. 2. Total Antioxidant Status (Total Antioxidant Capacity) of GMR (*G.mangostana* L rind 96 % ethanolic extract), Fractions, Alpha Mangostin ($p < 0,05$). GMRE =, *= means significant difference between these groups.

At the same concentration, 500 µg/ mL, TAS of ethyl acetate fraction was the highest among other materials, followed by alpha mangostin. GMR extract showed same TAS as hexane fraction and water fraction as well, while butanol fraction showed the lowest ($p < 0,05$).

SOD activity of GMR 96% ethanolic extract, hexane, ethyl acetate, butanol, and water fraction is shown in the following figure.

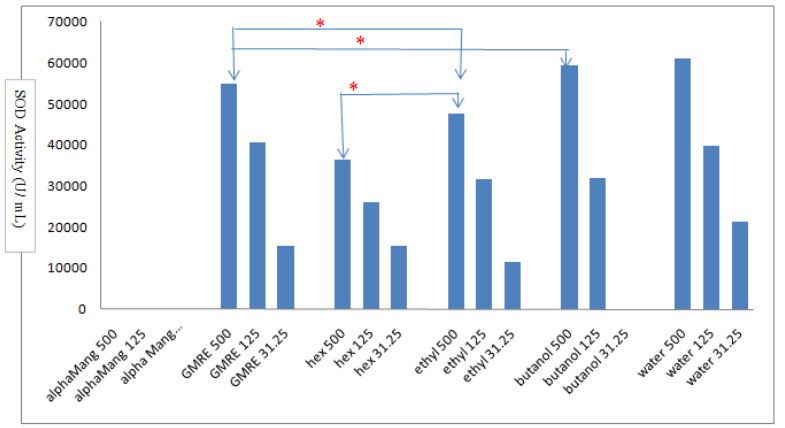

Fig. 3. SOD Activity of GMR 96% Ethanolic Extract, Hexane, Ethyl Acetate, Butanol, and Water Fraction * means significant difference between each group. ($p < 0,05$)

At concentration 500 µg/ mL,comparing to other materials, GMR extract and also water fraction had the strongest DPPH scavenging activity, in contrast against alpha mangostin which had the lowest DPPH scavenging activity. But alpha mangostin had higher TAS than GMR extract ($p < 0,05$), it may be caused by other antioxidant mechanism of alpha mangostin besides free radical scavenging such as other enzymatic mechanism besides SOD. GMR extract and water fraction had same TAS and DPPH scavenging activity although water fraction, also butanol fraction, had the most SOD activity ($p < 0,05$). Other antioxidants beside SOD activity could play more role in GMRextract. Although butanol fraction had the strongest SOD activity, lack of other antioxidant properties might cause its lowest TAS. Ethyl acetate fraction had the highest TAS even though in lower concentration i.e. 125 µg/mL comparing to other samples ($p < 0,05$).

4. Conclusion

In clonclusion,*Garcinia mangostana* L rind products had potential antioxidant properties.

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References

1. Miyake T, Shibamoto T. Antioxidant Activities of Natural Compounds Found in Plants. *J Agric Food Chem* 1997; **45**: 1819-22.
2. Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsapanan W. Effect of *Garcinia mangostana* on inflammation caused by *Propionibacterium acnes*. *Fitoter* 2007; **78**: 401-8.
3. Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD. 2006. Antioxidant Xanthones from The Pericarp of *Garcinia mangostana* (Mangosteen). *J Agric Food Chem* 2006; **54**: 2077-82.
4. Zarena A S, Udaya Sankar K.Screening of Xanthone from Mangosteen (*Garcinia mangostana* L.) Peels and Their Effect on Cytochrome C Reductase and Phosphomolybdenum Activity. *J Nat Prod* 2009; **2**: 23-30.
5. Suksamrarn S, Komutiban O, Ratananukul P, Chimnoi N, Lartpornmatulee N, Suksamrarn A. Cytotoxic Prenylated Xanthones from The Young Fruit of *Garcinia mangostana*. *Chem Pharm Bull* 2006; **54**: 301-5.
6. Pedraza-Chaverri J, Cardenas-Rodriguez N, Orozco- Ibarra M, Perez-Rojas J M. 2008. Medicinal Properties of Mangosteen (*Garcinia mangostana*). *Food Chem Toxicol* 2008; **46**: 3227-3239.
7. Palakawong C, Sophanodora P, Pisuchpen S, Phongpaichit S. Antioxidant and Antimicrobial Activities of Crude Extracts from Mangosteen (*Garcinia mangostana* L.) Parts and Some Essential Oils. *Int Food Res J* 2010; **17**: 583-9
8. Unlu GV, Candan F, Sokmen A, Dafefera D, Polissiou M, Sokmen E, et al. Antimicrobial and Antioxidant Activity of The Essential Oil and Methanol Extracts of *Thymus pectinatus* Fisch. Et Mey. Var. pectinatus (Lamiaceae). *J Agric Food Chem* 2003; **51**: 63-7.
9. Ngawhirunpat T, Opanasopi P, Sukma M, Sittisombut C, Kat A, Adachi I. 2010. Antioxidant, Free Radical-Scavenging Activity and

Cytotoxicity of Different Solvent Extracts and Their Phenolic Constituents from The Fruit Hull of Mangosteen (*Garcinia mangostana*). *Pharm Biol* 2010; **48**: 55-62.