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Perbandingan Aktivitas Antioksidan pada Ekstrak Murni, Granul dan Tablet *Effervescent* Ekstrak Kulit Manggis (*Garcinia mangostana* L.)

Comparison of Antioxidant Activity of Pure Extract, Granule, and Effervescent Tablet of Mangosteen rind Extract (*Garcinia Mangostana* L.)

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ABSTRACT

Kulit manggis (*Garcinia mangostana L.)* mengandung senyawa alkaloid, flavonoid, triterpenoid, tanin dan polifenol. Senyawa metabolit sekunder flavonoid yang terdapat antioksidan di dalamnya. Penelitian pengujian aktivitas antioksidan dengan sampel ekstrak murni, granul *effervescent* dan tablet *effervescent* dari ekstrak kulit manggis ini dilakukan untuk mengetahui berapa kadar antioksidan yang terdapat pada masing-masing sediaan. Metode ekstraksi yang digunakan dalam penelitian ini yaitu maserasi dengan pelarut etanol 96% kemudian *di rotary evaporator* dan di water bath pada suhu 40°C untuk mendapatkan ekstrak kental. Diperoleh rendemen ekstrak sebesar 23,4% yang memenuhi persyaratan sebagai ekstrak yang baik. Ekstrak kental kemudian di oven pada suhu 40°C untuk mendapatkan ekstrak kering. Kemudian ekstrak kulit manggis dilakukan skrining fitokimia untuk mengetahui senyawa metabolit sekunder apa saja yang terdapat di dalamnya. Kemudian dilakukan uji aktivitas antioksidan dengan menggunakan metode DPPH menggunakan metanol sebagai pelarut dan kuersetin sebagai kontrol positif. Hasil IC_{50} yang paling kuat terdapat pada ekstrak murni kulit manggis dengan nilai 0,037 ppm, granul *effervescent* dengan nilai IC_{50} 0,607 ppm dan tablet *effervescent* dengan nilai IC_{50} 2,517 ppm yang berarti semakin kecil nilai IC_{50} maka semakin tinggi kemampuan antioksidan dalam menangkal radikal bebas.

ABSTRACT

The mangosteen rind (*Garcinia mangostana* L.) contains alkaloids, flavonoids, triterpenoids, tannins, and polyphenols. Flavonoids are secondary metabolites found in it which act as antioxidants. Research on antioxidant activity testing with samples of pure extract, effervescent granules, and effervescent tablets from mangosteen rind extract was conducted to determine the antioxidant content in each formulation. The extraction method used in the study was maceration with 96% ethanol solvent followed by rotary evaporation and a water bath at 40°C to obtain a concentrated extract. The yield of the extract was 23.4%, meeting the criteria for a good extract. The concentrated extract was then dried in an oven at 40°C to obtain dry extract. Subsequently, phytochemical screening of the mangosteen rind extract was performed to identify the secondary metabolites present. Antioxidant activity testing was conducted using the DPPH method with methanol as the solvent and quercetin as the positive control. The strongest IC_{50} value was found in the pure mangosteen rind extract with a value of 0.037 ppm, followed by the effervescent granules with an IC_{50} value of 0.607 ppm, and the effervescent tablets with an IC_{50} value of 2.517 ppm. A smaller IC_{50} value indicates a higher antioxidant capacity in scavenging free radicals.

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1. INTRODUCTION

Air pollution is an essential issue in the modern era. Indonesia is known as the fifth country out of 100 countries with unhealthy conditions [1]. Air pollution occurs due to contamination of the environment from chemical, physical and biological substances that trigger changes in atmospheric characteristics [2]. Free radicals are a form of reactive compounds, which are generally known as compounds that have unpaired electrons in their outer shell [3]. The presence of free radicals in the human body can cause various degenerative diseases.

Antioxidants are compounds that can neutralize, reduce and inhibit the formation of free radicals. Antioxidants have a mechanism of action to neutralize free radicals by donating electrons to free radicals. Free radicals that get donors will form paired free electrons so that it will inhibit damage in the body [1]. Antioxidants have the ability to neutralize free radicals, thus protecting the body from damage caused by oxidative stress and inhibiting the occurrence of degenerative diseases.

Mangosteen rind has *xanthone* compounds which are included in the polyphenol group. *Xanthone* has various compounds such as *mangostin, mangostano, mangostino-A, mangostino-B, trapezi loli xanthone, tovophyllin-B, α-mangostin, β-mangostin, garcinon-B, mangostano, flavonoids (epicatechin), and gartanin* [4]. *Xanthone* compounds are obtained through the extraction process using a maceration method with 96% ethanol solvent [5].

Based on research conducted by [6] with the title Antioxidant Activity Test of Ethanol Extract of *Mangosteen* Fruit rind (*Garcinia mangostana* L.) in Serum Preparation with DPPH Method, it is found that mangosteen fruit rind extract has antioxidant activity IC50 value in the final calculation is 35.80 ppm. Based on the IC50 value, it can be concluded that the ethanol extract of mangosteen fruit rind is classified as a "very strong" antioxidant.

Based on the description above, this study will be conducted on mangosteen fruit rind extraction using a maceration method. The extraction results will be formulated in the form of granules and *effervescent* tablets and then an antioxidant activity test will be conducted using the DPPH method. This study aims to compare the antioxidant activity of mangosteen rind extract, mangosteen rind extract in granule form, and mangosteen rind extract in effervescent tablets. Mangosteen rind was chosen as the active substance because of its compound content and supports the green industry because of the utilization of waste into products. The selection of extract development in effervescent form is due to efficiency in use and helps solubility. The development of granule formulations and effervescent tablets is to see if the difference in treatment will affect antioxidant activity.

2. METHODS

Tools and Materials

Laboratory glassware, Uv-Vis Spectrophotometry, analytical digital balance, refrigerator, oven, test tube, glass funnel, stirring rod, glass jar, aluminum foil, filter paper, watch glass, parchment paper, bottle, spatula.

Mangosteen rind *(Garcinia mangostana* L.*)* from the Spice and Medicinal Plants Research Center (BALITTRO*)* in Bogor city, 96% ethanol, mangosteen rind extract *effervescent* granules and *effervescent* tablets taken from previous researchers, DPPH; Methanol, HCL 2N, Dragendorff reagent, Mayer reagent, Liebermann-Burchard reagent,

acetone, boric acid, oxalic acid, acetic acid, sulfuric acid, ether, 10% iron III chloride solution, anhydrous acetic acid, sulfuric acid, chloroform, distilled water, quercetin.

Plant Determination

Plant determination is carried out to determine plant identification such as specific names or species. Plant determination was carried out at Herbarium Bogoriense, Botany Division, Biology Research and Development Center-LIPI Cibinong.

Sample Preparation

Samples of mangosteen rind (*Garcinia mangostana* L.) were obtained from the Spice and Medicinal Plants Research Center (BALITRO). This study is a continuation of previous research so that the samples obtained from previous researchers in the form of *effervescent* granules and *effervescent* tablets*.* The samples that have been obtained are then tested for antioxidant activity using the DPPH method.

Mangosteen rind Extraction

The extraction process was carried out by maceration method. The maceration method was chosen because it is the simplest and most widely used method and this method is suitable and good for small and industrial scale [7]. The maceration method was carried out using 96% ethanol solvent. A total of 1600 grams were macerated into 12 L of ethanol solvent. Maceration was carried out for 5 days. The collected residue was then macerated using 96% ethanol as much as 4 L. Re-maceration was carried out for 2 days. The filtrate obtained from the maceration results is then in the *Rotary evaporator* with a temperature of 40 ° C. So that a half thick extract is formed and then in a water bath with a temperature of 40 ° C.

Preparation of Dry Extract

The results obtained from the water bath are thick extracts. The thick extract is then oven dried at 40°C to obtain a coarse dry extract, then the dry extract is blended until smooth.

Phytochemical Screening

a. Preparation of phytochemical test solution

Preparation of test solution for phytochemical screening was carried out by dissolving 500 mg of ethanol extract of mangosteen fruit rind (*Garcinia Mangostana* L.) in 50 mL of 95% ethanol [8].

b. Flavonoid Test

A total of 1 mL of the test extract solution, moisten the remainder with acetone P, add a small amount of boric acid fine powder P and oxalic acid fine powder P, heat carefully on a water bath and avoid excessive heating. Mix the residue obtained with 10 mL ether P. Observe under UV light 366 nm; the solution fluoresces intensively yellow, indicating the presence of flavonoids [8].

c. Alkaloid Test

A total of 2 mL of the test extract solution was evaporated on a porcelain cup until the residue was obtained. The residue was then dissolved with 5 mL of HCL 2N. The solution obtained was then divided into 3 test tubes. The first tube is added with dilute acid which serves as a blank. The second tube was added Dragendorff reagent as much as 3 drops and the third tube was added Mayer reagent as much as 3 drops. The formation of an orange precipitate in the second tube and a yellow precipitate in the third tube indicates the presence of alkaloids [8].

d. Identification of tannins and polyphenols

A total of 3 mL of test extract solution is divided into 3 parts, namely tube A, tube B, tube C. Tube A is used as a blank, tube B is reacted with 10% iron (III) chloride solution,

dark blue or greenish black color indicates the presence of tannins and polyphenols, while in tube C only gelatin salt is added. If a precipitate forms on tube C, the extract solution is positive for tannins [8].

e. Steroid and triterpenoid examination

Examination of steroids and triterpenoids was carried out by the Liebermann-Burchard reaction. A total of 2 mL of test solution was evaporated in a vaporizer cup. The residue was dissolved with 0.5 mL chloroform, and added 0.5 mL anhydrous acetic acid. Furthermore, 2 mL of concentrated sulfuric acid was added through the tube wall. The formation of a brownish or violet ring on the border of the solution indicates the presence of triterpenoids, while when a blue-green ring appears, it indicates the presence of steroids [8].

Preparation of Effervescent Granule and Tablet

Table 1. Formula of Mangosteen Rind Extract Effervescent Granule and Tablet Preparation

Material	Ratio (%)
Mangosteen Rind Extract (*Garcinia mangostana* L.)	31,2
Citric Acid	27,5
Tartaric Acid	25,1
Sodium Bicarbonate	34
Polyvinylpyrrolidone (PVP)	1
PEG 6000	1
Lactose	Ad 68,8

ANTIOXIDANT ACTIVITY TEST

a. DPPH Reagent

Make a DPPH solution by weighing DPPH as much as 5 mg and then dissolving it with methanol until the limit mark of a 100 mL volumetric flask becomes a 50 ppm DPPH solution. Store in a dark container and then place in the refrigerator [4].

b. Determination of DPPH Maximum λ

DPPH 0.5 mm solution was pipetted 5 mL and then put into a 25 mL volumetric flask added with solvent until the limit mark (40 ppm), then measure the absorbance with a Uv-Vis spectrophotometer at λ 400 - 800 nm. [9].

c. Preparation of Master Solution

By dissolving 25 mg of test sample with methanol in a 25 mL volumetric flask until the limit mark (1000 ppm concentration) [9].

d. Preparation of Test solution

The mother solution was taken as much as 1 mL, 2 mL, 3 mL, and 4 mL and then diluted in a 2 mL volumetric flask (to obtain concentrations of 40 ppm, 80 ppm, 120 ppm, and 160 ppm). Furthermore, in each measuring flask added 5 mL of 0.5 mM DPPH solution (C = 40 ppm), then added methanol solvent to the limit mark [9].

e. Determination of Percent Damping

By adding the test sample, the antioxidant activity is seen by the decrease in absorbance of DPPH solution. The absorbance value of DPPH solution before and after addition is shown in % inhibition. The DPPH free antiradical capacity as percent absorbance inhibition at 517 nm peak can be calculated by the formula:

% DPPH Immersion=A Count test material A Count DPPH X 100%

f. Determination of IC50 Value

IC50 is the amount of sample concentration of activity inhibiting 50% of DPPH absorbance. To determine the IC50 price, a linear line equation is made by entering

the sample concentration value as the abscissa (x-axis) and the percent inhibition value (%) [9].

3. RESULTS

Mangosteen rind Extraction Result

The results of mangosteen rind extraction obtained a thick extract yield of 23.4% and a dry extract yield of 15.5%.

Determination Result

The determination results shown are the type of *Garcinia mangostana* L. from the *clusiaceae* tribe.

Identification of Mangosteen rind Extract

a. Phytochemical screening showed positive results for alkaloids, flavonoids, terpenoids, tannins and polyphenols.
b. Macroscopic identification shows that mangosteen skin simplisia is correct by organoleptically observing the taste, smell and color.
c. Microscopic identification showed that the simplisia had fragments of endocardium and exocarpium.

4. DISCUSSION

Mangosteen Rind Extraction

The maceration method was carried out using 96% ethanol solvent. A total of 1600 grams were macerated into 12 L of ethanol solvent. Maceration was carried out for 5 days. The maceration process will experience the process of withdrawing secondary metabolite compounds. After 5 days, the solvent and residue were separated. The collected residue was then re-macerated using 96% ethanol as much as 4 L. Remaceration was carried out for 2 days. This is done to obtain maximum secondary metabolite compounds. The re-maceration results were then separated from the residue so that the filtrate was obtained. The 96% ethanol solvent was chosen because it is a universal solvent that can attract compounds that are soluble in polar to non-polar solvents [12].

Dry Extract

The thick extract obtained is 374.14 g with a yield of 23.4% after being oven at 40°C, the dry extract is obtained as much as 249.3% with a yield of 15.5%.

Phytochemical Screening

The results of phytochemical screening testing are positive for the presence of secondary metabolite compounds in mangosteen rind extract, namely alkaloids, flavonoids, terpenoids, tannins and polyphenols. While negative for steroid compounds, glycosides and saponins. The results of phytochemical screening can be seen in Table 2. below:

Table 2. Phytochemical Screening Results

Phytochemical Test	Parameters	Results Research	Results Literature
Alkaloids	1. There is an orange-red color change (dragendorff reagent) 2. White precipitate (mayer reagent)	Positive	Positive
Flavonoids Saponins	Color change to intensive yellow fluorescence	Positive	Positive
Terpenoid Steroids	Foam on the sample	Negative	Positive
Tannins and polyphenols	1. Blue color change (steroids) 2. Color change to red (terpenoids)	1. Negative (steroids) 2. Positive (terpenoids)	Positive

Antioxidant Activity of Quercetin, Pure Extract, *Effervescent* Granule and *Effervescent* Tablet

a. Quersetin

The results obtained in the calculation of % inhibition of quercetin obtained the equation obtained y = 0.0468x + 30.575 with a correlation coefficient (R^2) of 0.9962 and IC50 results of 415.06 µg/mL which indicates the antioxidant content contained in quercetin is not strong. This happens because in this study using polar solvent methanol which causes antioxidants in quercetin does not come out or is less soluble in methanol. According to research [10] flavonoid compounds such as quercetin are more attracted/extracted to non-polar solvents. The results can be seen in Figure 1.

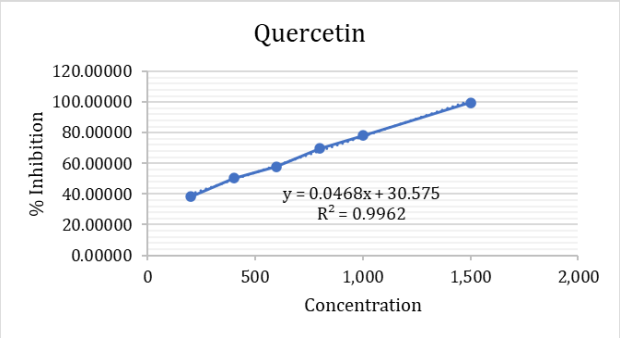

Figure 1. % Quercetin Inhibition

b. Pure Extract

In a previous study [14] obtained an IC50 value of 1.64 which has very high activity. In the pure extract, the antioxidant activity test was carried out by making a parent standard concentration of 10.1 ppm and a standard series of 0.010; 0.020; 0.030; 0.040; and 0.051 dissolved using methanol p.a. Showing the results of the IC50 value of 0.037 ppm which indicates the antioxidant activity contained therein is very strong. The acquisition of very strong antioxidant activity may be influenced by the extract treatment, namely the sample used in the form of dry extract.

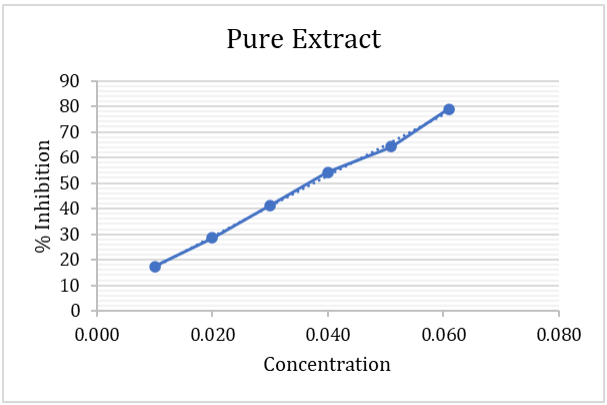

Figure 2. % Inhibition of Pure Extract

c. *Effervescent* Granule

The effervescent granules used are in accordance with those in table 1, namely mangosteen peel extract of 32.2% with a ratio of citric acid 27.5% and tartaric acid 23.1%. The results of the formulation were then tested for antioxidant activity with a standard concentration of 11 34 ppm and a standard series of 0.227; 0.567;

1.134; 1.701 and 2.268 ppm then dissolved using methanol solvent p.a. Shows the results of IC50 effervescent granules show a value of 0.607 ppm which indicates that the antioxidants contained in the sample are very strong. However, it is quite decreased compared to the pure extract sample. The shift in antioxidant activity occurred due to the presence of wet granule making materials such as the addition of citric acid with tartrate acid mixed in mangosteen fruit peel extract (*Garcinia mangostana* L.). This causes the antioxidant activity to decrease compared to the antioxidant results of the pure extract sample. This decrease in antioxidant activity is influenced by the addition of additives that cause changes in characteristics [15] and the mixing process using wet granulation. The wet granulation method involves heat, this correlates with longer exposure to heat such as boiling causing loss of antioxidant activity [16].

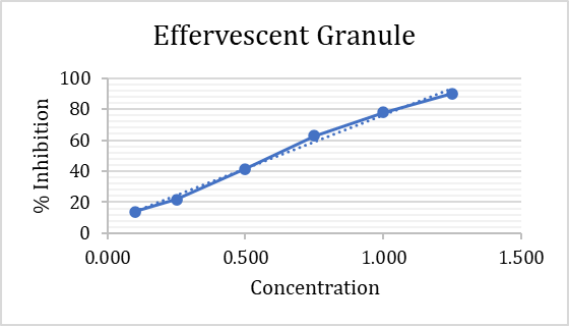

Figure 2. % Inhibition of *Effervescent* Granule

d. *Effervescent* Tablets

Antioxidant activity testing was carried out by making a standard solution of 10,000 ppm and standard series of 5000, 6000, 7000, 8000, 9000 ppm dissolved using methanol *p.a*. The results aimed at testing the antioxidant activity of *effervescent* tablets showed IC50 results of 2,517 ppm which indicates very strong antioxidant activity. The cause of the decrease in antioxidant effectiveness occurs when pressure and friction force are applied which causes a reduction in antioxidant activity. This is in accordance with what several researchers have done, namely according to researcher [11] that some antioxidant compounds undergo oxidation during the formulation process. When formed into a lozenge preparation that causes a decrease in antioxidant activity. Then strengthened by the results of research from [12] which states that the decrease in antioxidant activity of *effervescent* tablet preparations is influenced when the pH value is in an increasingly alkaline or neutral state. This is one of the causes of decreased antioxidant activity when formed into tablet preparations.

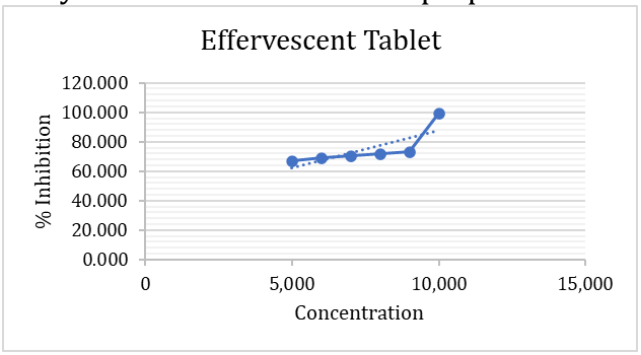

Figure 3. % Inhibition of *Effervescent* Tablets

Table 1. Comparison of Antioxidant Activity of Pure Extract, *Effervescent* Granule and *Effervescent* Tablet

Sample	Concentration	Absorbance 1	Absorbance 2	Absorbance 3	IC_{50}
Pure Extract	0,010	0,691	0,691	0,690	0,037
	0,020	0,597	0,597	0,597	
	0,030	0,491	0,492	0,492	
	0,040	0,382	0,383	0,383	
	0,051	0,300	0,300	0,298	
***Effervescent* Granule**	0,100	0,724	0,724	0,724	0,607
	0,250	0,659	0,659	0,659	
	0,500	0,493	0,493	0,493	
	0,750	0,313	0,313	0,313	
	1,000	0,187	0,187	0,187	
Tablet *Effervescent*	9000	0,328	0,328	0,328	2,517
	8000	0,342	0,342	0,345	
	7000	0,352	0,356	0,355	
	6000	0,368	0,369	0,367	
	5000	0,388	0,384	0,383	

Table 1 shows that there are differences in antioxidant activity in mangosteen rind extract, mangosteen peel extract effervescent granules, and effervescent tablets. This difference is influenced by the process in the development. Mangosteen rind extracted using 96% ethanol has very strong antioxidant activity [13], this is directly proportional to the results of antioxidant activity testing on mangosteen rind extract which is 0.037 ppm. an effervescent granule of mangosteen rind extract obtained an IC50 value of 0.607 ppm which indicates very strong antioxidant activity. An effervescent tablet of mangosteen rind extract obtained an IC50 value of 2.517 ppm which indicates very strong antioxidant activity. based on these results that with the development there is no difference in antioxidant activity but there is a decrease in IC50 value. The shift in antioxidant activity occurred in mangosteen peel extract and mangosteen peel extract effervescent granules due to the presence of excipients ingredients such as citric acid with acid. This caused the antioxidant activity to decrease compared to the antioxidant results of the pure extract sample. In effervescent tablets, there is a decrease in antioxidant activity, the cause of the decrease in antioxidant effectiveness occurs when given pressure and friction force which causes a reduction in antioxidant activity. This is in accordance with what several researchers have done, namely according to researchers [11] that some antioxidant compounds undergo oxidation during the formulation process. When formed into a lozenge preparation that causes a decrease in antioxidant activity. Then strengthened by the results of research from [12] which states that the decrease in antioxidant activity of effervescent tablet preparations is influenced when the pH value is in an increasingly alkaline or neutral state. This is one of the causes of decreased antioxidant activity if formed into tablet preparations.

5. CONCLUSION

Based on the results of the research analysis that has been done, it can be concluded that antioxidant activity in pure mangosteen fruit peel extract (*Garcinia mangostana* L.) contains very high antioxidant levels with an IC50 value of 0.037 ppm which is able to ward off free radicals well. Then the antioxidant activity in effervescent granule preparations has an IC50 value of 0.607 ppm which indicates the antioxidants in it are very high. While the antioxidant activity in effervescent tablet preparations has an

IC50 value of 2.517 ppm which indicates the antioxidants in it are very high. In the study there was a decrease in antioxidant activity which was influenced by the development process such as the addition of excipients, the mixing process between active substances and excipients, and the tablet molding process which requires friction.

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