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Original Article

ANTIOXIDANT ACTIVITY OF THE ACTIVE FRACTION OF MANGOSTEEN RIND EXTRACT (*GARCINIA MANGOSTANA*)

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ABSTRACT

Objective: Determination of the IC50 value of the active Fraction was carried out to see the antioxidant activity of the ethanol extract of mangosteen peel (*Garcinia mangosteen*) Mangosteen peel is known to have very strong antioxidant activity. The research began with maceration of mangosteen peel with ethanol solvent. Then, the mangosteen peel extract was fractionated with three solvents, namely those with different polarities, namely water, dichloromethane, and n-hexane.

Methods: The ethanol extract of mangosteen rind was further fractionated using n-hexane, dichloromethane, and water, then the solvent was removed by evaporation. The three resulting fractions were measured for antioxidant activity using the DPPH method with Quercetin as a comparison. The active Fraction with the highest IC50 value is then compared with the extract, and the comparison is seen with TLC. Next, the Fraction was tested with GC to see the remaining solvent. And then continued with determining alpha mangostin levels in all fractions using UHPLC.

Results: IC50 value for each n-hexane fraction was 50.65µg/ml, the dichloromethane fraction was 34.66µg/ml, and the water fraction was 45.72µg/ml. The results of the solvent test showed that there was no residual solvent in the dichlormethane fraction, as seen from the chromatogram results. The results of the assay showed the following results: n-Hexan fraction 25.18%, DCM fraction 31.23%.

Conclusion: The dichloromethane fraction showed the highest antioxidant activity with the best IC 50 value and had higher levels of alpha mangostin than the water and n-hexane fractions.

Keywords: Antioxidant, Dichloromethane fraction, IC50, Garcinia mangostana

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INTRODUCTION

Antioxidants are substances can neutralize free radicals by stabilizing, neutralizing or minimizing oxidation reaction in cells caused by free radical reactions. *Garcinia mangostana L*. is a plant known for its antioxidant activity that can used in traditional medicine. Antioxidant activity is expressed as the IC50 value (Inhibition Concentration 50), the concentration of the sample solution required to inhibit 50% of free radicals. The smaller the IC50 value, the higher the free radical scavenging activity [1]. In previous research aimed at optimizing the solvent extraction process using 96% ethanol, ethyl acetate and methanol, free radicals produced could damage the cells that produce the insulin dye. in the pancreas, causing insulin deficiency in the body [2]. Compounds considered to be effective in scavenging oxygen radicals are phenolic compounds because phenolic radicals have a lower electron-reducing capacity than oxygen radicals. Phenolic compounds can act as reactive oxygen-scavenging intermediates without causing further oxidation reactions [3]. One of the sources of antioxidants derived from the fruit is mangosteen (*Garcinia mangosteen* L). Based on phytochemical studies show that the peel of mangosteen fruit contains several compounds, such as xanthone derivatives. Also, tannins, triterpene, and other bioactive compounds.

Mangostin compounds are the most common compounds found in the skin of mangosteen fruit. Yellowish mangosteen compounds have benefits. Such as anti-inflammatory, antitumor, antidiabetic, cardiovascular, antifungal, antioxidant, and antiobesity [4]. The spot with an Rf value of 0.50 shows the same pattern as the standard solution, which is alpha mangostine. In addition to the alpha-mangostine stain, there are other stains with antioxidant activity. Therefore, in this study, a fractionation procedure was performed to separate the methanol extract. The separation method is performed by column chromatography. The fractions are collected from the fractionation process; each fraction will be tested qualitatively for antioxidant properties using the TLC-DPPH method and quantitatively through the IC50 value. This study aimed to determine the IC50 value of ethanol extract and its partition results from the skin of the mangosteen fruit (*Garcinia mangosteen*). Fractionation was carried out in three different solvents based on their polarity. In this research, the newest thing is to use the solvents water, dichlormethane and n-hexane. In previous research, ethyl acetate solvent was used to obtain the active fraction. In previous research, ethyl acetate solvent was used to obtain the active fraction [14].

MATERIALS AND METHODS

Ethanol extract of mangosteen peel (Garcinia mangostana) was obtained from BRIN Serpong, n-hexane technical (Merck), Aqua dest, dichlormetane (Merck), methanol, chloroform (Merck), F254 silica gel plate for TLC, quercetin, 1,1-diphenyl 2-picrylhydrazyl (DPPH). The equipment used in this research is an analytical balance, split funnel, water bath, vacuum rotary evaporator. Others such as UV-Vis spectrophotometer, vial, Eppendorf Research 1000 ul micropipette, UHPLC, GC (SHIMADZU GC-2014), sonicator.

Fractionation

The ethanol extract of mangosteen peel is fractionated with several solvents. The thick extract of mangosteen rind was then added with 500 ml of distilled water until a liquid solution was obtained, then partitioned with 500 ml of n-hexane pa, three times then partitioned with 500 ml of dichloromethane pa. The results of each fraction were dried until all the solvent was removed.

Phytochemical screening of dichlormethane fraction, n-hexane fraction and water fraction [15]

Identification of alkaloids

Dichlormethane fraction; The Fraction is dissolved in a mixture of 1 ml of 2N hydrochloric acid and 9 ml of aqua dest then heated on a tangas of water for 2 min. The solution of the test material is cooled and filtered; then its filtrate is used for the identification of alkaloids with solutions of Dragendorf, Mayer and Bouchardat. The filtrate divided into 3 parts each is inserted in a test tube then successively reacted with the above reagent.

Glycoside identification

The fractionation results were hydrolyzed with 2N hydrochloric acid, then cooled and filtered, and the filtrate was used for the Molish Test. The Molish Test is performed by adding Molish solution to the filtrate in a test tube and then stirring and flowing concentrated sulfuric acid through the tube wall.

Saponin identification

The result of fractionation is added 5 ml of hot water in the test tube then cooled and shaken vigorously for 2 min. If a steady froth is formed for not less than 10 min, 1-10 cm in height is added 1 drop of 2N hydrochloric acid.

Identify flavonoids. The Shinoda test carried out identification of flavonoids, a number of extracts were dissolved in 1-2 ml of 96% ethanol and added 0.5g of zinc powder and 2 ml of 2N hydrochloric acid, allowed to stand for 1 min, then 10 drops of concentrated hydrochloric acid were added.

Phenol identification

Phenol identification is carried out by adding 3-4 drops of iron reagent (III) chloride to several extracts dissolved in 96% ethanol.

Identification of sterols and terpenes

The dichloromethane Fraction is dissolved in ether then evaporated, the residue is added 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid will form a red-green or violet-blue color.

Determination the IC_{50} value antioxidant activity with DPPH method

All parts of the shared results were performed on antioxidant activity by comparing DPPH and quercetin as controls. Determination of IC50 value Antioxidant activity by DPPH method. The absorbance of DPPH solution was measured using an ultraviolet-visible (UV-Vis) spectrophotometer. Place 1.5 ml of DPPH into the cuvette, then add 1.5 ml of test solution or positive control solution (1:1) and leave at room temperature for 30 min. Positive control as quercetin solution. A series of test solutions and positive control solutions were measured for absorbance at the maximum wavelength [5]. Obtained as the absorbed fraction from the mangosteen peel.

Determination qualitative profile TLT-bioautografi antioxidant activity

Qualitative antioxidant activity was observed during the production of a methanolic mangosteen peel sample solution and the production of an α-mangosteen reference solution. The plates were then eluted in a bath saturated with the chloroform mobile phase:

methanol (9.5:0.5) v/v. The plate was sprayed with 1 mmol DPPH solution and left for 30 min. Then, observe with your eyes. Calculated value of Rf and blobs appear. In situ test material with antioxidant activity is yellow with a purple background [6]

Solvent contamination test in dichloromethane fraction

Gas Chromatography measures a total of 10% DCM fraction in water-ethanol under certain conditions. The sample/standard was added with ethanol until the tera mark, then homogenized and filtered with Millipore 0.45 µm, then continued to be injected into the GC. Injector temperature 180 °C, column temperature 60 °C, detectore FID, flow rate 1 ml/minute, N2 carrier gas with DB-5 column.

Determination of alpha mangostin levels in fractions

The equipment used is UHPLC, with an Xbridge C18 Column and a mobile phase of As. Phosphate: Methanol (12:88)

- 50 mg of the fraction sample was dissolved in 10 ml of methanol, then homogenized using a vortex for 20 min followed by the process of dissolving the fraction using a sonicator for 15 min.
- 10 µl is taken then dissolved in 990 µl of solvent solution (Mobile Phase) then vortexed for 30 seconds and then injected into the UHPLC [17].

RESULTS AND DISCUSSION

Characterisctic of mangostana peel extract

The extraction process by BRIN Serpong. Mangosteen peel extract obtained with the following characteristics. Evaluation of mangosteen extracts obtained water soluble ash content of 0,62±0.08% where it has met the moisture content required by Indonesian Herbal Pharmacopoeia ie less than 10%. Loss on drying 7.26±0.25, totas ash content 0.80±0.13, acid soluble ash content 0.18±0.04, total flavonoids 15.96±0.85, total phenols 29.47±1.39. In the skin of the mangosteen fruit (*Garcinia mangostana L.*) there are no volatile oil content [7]. The data results above were obtained from CoA Brin Serpong.

Phytochemical screening for dichlormethane fraction, n-hexane fraction and water fraction

From the phytochemical screening of the three fractions, the following data was obtained:

Table 1: Three fraction phytochemical screening results

Test	n-Hexan fraction	DCM fraction	Water Fraction
Flavonoid	-	+	-
Fenol	+	+	+
Tanin	-	-	-
Alkaloid	-	-	-
- Dragendorf	-	-	-
- Mayers	-	-	-
- Bouchardat	+	+	+
- Wagner	-	-	+
Steroid Terpen	-	-	+
- Liebermann-Burchard			
- Salkowski			

The DCM fraction contains flavonoids, while the n-hexane and water fractions do not. all three fractions contain phenol compounds. and the third fraction contains alkaloids from the reaction using Wagner's reagent.

Determination the IC_{50} value antioxidant activity with DPPH method

In determining the IC50 value, the t data obtained was formed after reacting with the DPPH absorption of each fraction and quercetin. The absorption data was then used to calculate the percentage of DPPH radical quenching (%P). The DPPH radical capture ratio (%P) represents the percentage of DPPH that reacts with antioxidant compounds [8]. The percent P value (%P) was analyzed using probit analysis to obtain the IC50 value. The results of antioxidant activity testing with DPPH showed an IC50 value for the water fraction of 45.75 ug/ml, the n-hexane fraction 50.64 ug/ml, and the dichlormethane fraction 34.66 ug/ml. The high anthocyanin content and high xanthone content in mangosteen peel have a strong

antioxidant effect [9]. The free radical DPPH is a quick, easy, and affordable way to test antioxidant capacities. It is frequently used to assess a compound's capacity to function as a hydrogen source and free-radical scavenger. The DPPH test depends on the stabilised free radical DPPH being eliminated. Indeed, DPPH is a crystalline dark-colored substance composed of stable free-radical particles. Specifically, it's a widely recognized radical and a well-liked antioxidant test. The DPPH radical is dark purple in solution when it is first reduced and then turned into DPPH-H; however, upon further reduction and transformation into DPPH-H, it becomes colorless or light yellow [16].

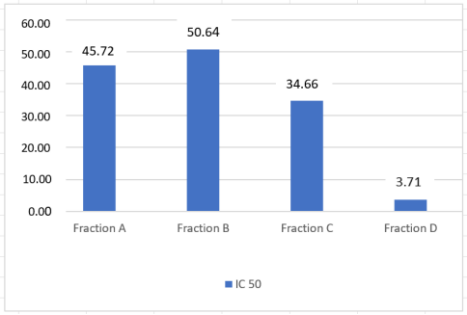

Fig. 2: Graph IC 50 for each sample

Determination qualitative profile TLT-bioautografi antioxidant activity

The fractionation is carried out using separate columns the aim of separating the compounds contained in the mangosteen extract (*Garcinia mangostana L.*). The operating principle of the separation process is based on the degree of polarity; non-polar compounds will be eluted first, followed by semi-polar and polar compounds. Based on fig. 3, we obtained several spots with antioxidant activity marked by yellow spots on a purple background. In addition to one spot with the same pattern and standard approach to the Rf value of alpha-mangostin (0.434), there were other spots with other Rfs that also showed DPPH activity. On the Extract track, there are three Points with Rf values (0.42; 0.60; 0.85) and the DCM part is two Points with Rf values (0.34; 0.85) and for standard quercetin is a single point (0.434).

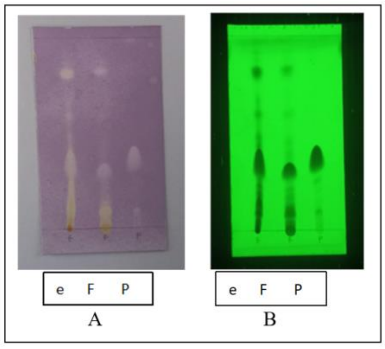

Fig. 3: Observe the chromatogram after spraying DPPH solution using stationary phase: F254 Silica gel plate and mobile phase: Chloroform: Methanol (9.5: 0.5 v/v) (Left/A) Slight phenomenon (e: extract, F: fraction DCM, P = standard) and (Right/B) UV 254 nm

Testing the antioxidant activity of ethanol extracts and their practitioner results showed that the sample had better inhibitory activity in its ethanol extract than its partition results. This is because several active compounds in the extract can synergize in inhibiting free radicals so that the IC50 value of the extract is better than the fraction.

Solvent contamination test

The DCM fraction of 10% in water-ethanol is measured by Gas Chromatography under certain conditions; the hope is that the content of DCM solvent is very small and below the standard allowed by BPOM. Based on the certificate of test results No.1362/PPM-FFUI/X/2022, the sample of 10% DCM fraction was declared undetectable to have contamination of DCM solvent; the data can be seen in the appendix. Therefore, DCM fraction samples can be used in the next research stage.

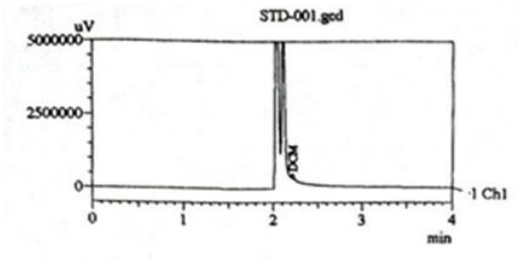

Fig. 4: Chromatogram DCM by gas chromatography

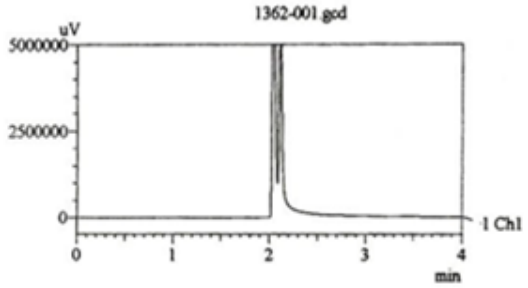

Fig. 5: Chromatogram DCM fraction by gas chromatography

From the chromatogram results, it can be seen that in the standard DCM solvent, there is a peak area, while in the DCM fraction, there is no peak area, whereas in fig. 1 and 2. In the chromatogram area table, it can be seen that there is an area in the standard DCM solvent and there is no area in the DCM fraction. It can be concluded that there was no DCM solvent contamination in the DCM fraction of mangosteen peel.

Determination of alpha mangostin levels in fractions

The water fraction did not provide a pick area when injected with 100x dilution. for the n Hexan fraction the content was 25.18% and the DCM fraction 31.23%. It can be concluded that the n-Hexan and DCM fractions contain alpha mangostin with the highest concentration in the DCM fraction.

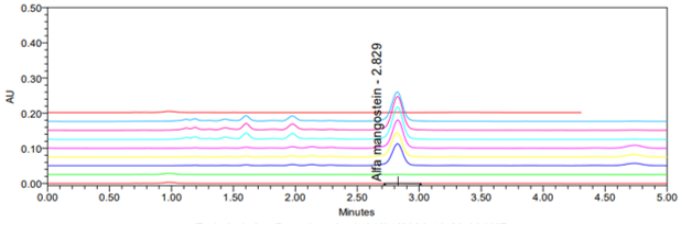

Fig. 6: Chromatogram of sample

CONCLUSION

The result of the partitioning of each Fraction obtained the IC50 value as follows: n-hexan fraction 50.65 µg/ml, DCM fraction 34.66 µg/ml and water fraction 45.72 µg/ml. Based on the IC50 value, the DCM fraction has better antioxidant activity than other fractions. And DCM fraction was declared undetectable to have contamination of DCM solvent. Therefore, DCM fraction samples can be used in the next research stage.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest with this research A. W., M. J., S. S. and B. E. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscrip

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