ORIGINAL ARTICLE

Extraction of antioxidants from fruit peel of *Artocarpus altilis*

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Abstract

Aim: Fruit peel of *Artocarpus altilis* traditionally in West Sumatra has been used and believed to be analgesic and treat other generative diseases. The aim of this study is to quantify the potential of antioxidant activity and the total phenolic and flavonoids contents from methanol extract of fruit peel *A. altilis*. All of this is intended for waste utilization and to find other natural sources of antioxidants and pharmaceutical formulations in the future. **Material and Methods:** The antioxidant activity was performed by 2,2´-diphenyl-1-picrylhydrazyl, and the total yield of phenolic and flavonoids contents was determined using spectrophotometer methods. **Results and Discussion**: Research results show that fruit peel of *A. altilis* has an inhibitory concentration 50% 479.31µg/ml, total phenolic content 6277 mg of dry weight of extract, expressed as gallic acid equivalents, and total flavonoid contents 4874 mg expressed in terms of rutin equivalent. Data from the present results show that methanol extract of fruit peel of *A. altilis* possesses significant free radical scavenging properties and clear correlation exists between the strong antioxidant activity and phenolic and flavonoids contents. **Conclusion**: The results suggest that fruit peel of *A. altilis* can be regarded as natural plant sources of antioxidants with high value.

Key words: Antioxidant activity, fruit peel, total flavonoid content, total phenolic

INTRODUCTION

Free radicals greatly affect human health and cause some degenerative and dangerous diseases such as cancer, hypertension, heart disease, and diabetes.[1] Consumption of foods containing phenolic compounds has a correlation with reduced coronary heart disease, cancer, and death. Phenolic compounds can function as antioxidants, anticancer, antiviral, and anti-inflammatory activity.[2,3] On the other side, the use of synthetic antioxidants such as butylated hydroxyanisole and tertiary butylhydroquinone is still in doubt and anxious for its safety.[4] This condition leads to the increasing interest of the community to switch using natural antioxidants derived from plant secondary metabolites. These secondary metabolites have biological activity and important pharmacological activities such as anti-allergic, antibiotic, anticarcinogenic, and antioxidative.[5,6]

Flavonoids are one of the most important phenol class compounds found in nature.[7] Flavonoid compounds contribute to antioxidant activity by breaking free radicals in the body.[8] The activity of these antioxidants is due to the presence of phenolic compounds such as quercetin and routine.[9,10]

More than 4000 species and varieties of flavonoids have been identified from flowers, fruits, and leaves.[11] Some research results show that the flavonoid compound has a biological activity related to its antioxidant activity. Natural antioxidants in crude extracts or isolated products are very effective in preventing damage caused by oxidative stress.[12] The toxicity profile of medicinal plants has not been evaluated, but generally it can be concluded that drugs derived from plant products are safer than synthetic products.[13,14]

One of biodiversity and medicinal properties is *Artocarpus altilis*. Plants from *Moraceae* family are widespread in the

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area of West Sumatra, Indonesia, and have traditionally been used as an analgesic, antidiabetic, and anti-inflammatory. Research conducted by Kolar, 2011, mentioned that fruit *A. Altilis* contains antioxidant compounds of phenolic, flavonoids and tannins with high levels.[15] The chemical compounds contained in the fruit *A. altilis* also have been used to treat cancer, diabetes, anti-inflammatory, analgesic, antipyretic, etc.[16] This study aims to investigate antioxidant activity, phenolic content, and total flavonoid from methanol extract of fruit peel of *A. altilis,* to find natural antioxidants in pharmaceutical formulas, and to utilize waste from the fruit of *A. altilis.*

MATERIALS AND METHODS

Raw Materials

Fresh peels of *A. altilis* were procured from Sungai Geringging Pariaman, West Sumatera, Indonesia.

Chemicals

The different chemicals such as ethanol, methanol, and diphenyl picrylhydrazyl (DPPH) from Sigma chemicals, Folin–Ciocalteu reagent (Merck), and gallic acid, quercetin, rutin, sodium carbonate, aluminum chloride, sodium acetate, and ferric chloride (Merck) were used during the investigation.

EKSPERIMENTAL PROCEDURE

Extraction of Antioxidant from Fruit Peels of *A. altilis*

The dried powders of peels were extracted by maceration method using methanol as a solvent. 100 g of the dried powder was macerated for 3 × 3 days at 25°C. Extract was filtered through Whatman filter paper No. 41 for the removal of peel particles and concentrated under vacuum at 40°C. The dry extract was stored at 4°C. The residues obtained after filtration were weighed to obtain the extraction yield.

Extraction yield (%) = (Weight of the residue)/(total weight of the peel powder) ×100[17]

Determination of DPPH Radical Scavenging Activity of Antioxidant Extract

Determination of antioxidant activity of *A. altilis* extract was performed by DPPH method according to Molyneux, 2004.[18] Before analysis, serial dilutions of the methanolic extracts of the samples were prepared. Diluted sample (0.2 mL) and DPPH working solution (50 µM) were added to a microcentrifuge tube. After vortexing, the tubes were left in the dark for 30 min at room temperature (23°C). The absorbance was then measured against methanol at 515 nm in 3 ml cuvettes using a spectrophotometer. The decrease in absorbance of a sample was calculated in comparison to a blank sample (0.2 mL methanol and 3.8 mL DPPH). The relative decrease in absorbance was then calculated as follows:

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample})/Abs_{control})] \times 100$$

Determination of Total Phenolic Content (TPC)

The total phenol content is determined according to Folin-The Ciocalteu reagent method of Singleton *et al*. (1965) with modified.[19] 0.2 mL extract, with concentration 150 µg/mL, added 15.8 mL aquadest, and 1 mL of Folin–Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 8 min. Then, 3 mL of 10% sodium carbonate solution was added and further incubated for 2 h at room temperature, and the absorbance was measured at 765 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (GAE) (mg of gallic acid/g of extracted compound).

Determination of Total Flavonoid Content (TFC)

The flavonoid content was determined according to aluminum chloride colorimetric method.[20] The reaction mixture with a final volume of 5 mL consists of 0.5 mL of sample (1 mg/ml), 1.5 mL methanol, 0.1 mL (10%) of aluminum chloride, and 0.1 mL (1 M) of potassium acetate, and 2.8 mL aquadest was incubated at room temperature for 30 min. The absorbance of all the samples was measured at 415 nm. Rutin was used as positive control. Flavonoid content is expressed in terms of rutin equivalent (mg/g of extracted compound).

RESULTS AND DISCUSSION

The extraction of *A. altilis* fruit peel using the maceration method using methanol as a solvent. The selection of maceration methods for the extraction process is due to several reasons. First because of the simplicity of the process and the second does not require high temperatures so it can reduce the possibility of damage to the content of chemical compounds that have antioxidant activity contained in the skin of *A. altilis* fruit.[21] Maseration results (maserate) in the form of dark blue solution evaporated using a rotary evaporator. The vacuum process that occurs during the solvent evaporation allows the solvent to evaporate at a temperature below its boiling point and the process can work faster. Evaporation of methanol solvent can be carried out below its boiling point at 55°C. This process is carried out at these temperatures to keep the active compound contained undamaged by heating.[22,23]

The phytochemical screening of the extracts was performed using *in vitro* method by reacting the sample with specific reagent solution to determine the secondary metabolite content of the extract.[24] Table 1 shows the result of phytochemical screening of the fruit peel of *A. altilis*

Based on phytochemical screening, it is known that methanol extract of fruit peel *A. altilis* has a secondary metabolite content of alkaloid compounds, flavonoids, phenols, tannins, and glycosides.

The antioxidant activity of fruit peel of *A. altilis* was performed using DPPH method. This method was chosen because it is a common and simple method to test antioxidant activity *in vitro* using the sample in small amounts and the processing time is relatively short.[25] DPPH radical scavenging activity assay aims to assess the ability of DPPH to be a stable free radical, and when reacting with the antioxidant compound, the stability will be reduced and transformed into a 2,2´-diphenyl-1-picrylhydrazyl, compound. Free radicals are initially purple and with the presence of antioxidants will turn into light yellow [Figure 1].[26]

The DPPH color change is caused by the active compound in the sample donating its hydrogen atom to the DPPH free radical, so it reduces to a more stable form of DPPH-H (1,1-diphenyl-2-picrylhydrazyl). From measurement of methanol extract *A. altilis* fruit peel indicates that the higher the concentration used, the absorbance value obtained also decreases. This decrease in absorbance value occurs at 517 nm wavelength.

Table 1: Phytochemical screening results

Secondary metabolites	Reagent	Information
Alkaloids	Mayer and Dragendorff	+
Flavonoids	HCL and Mg	+
Tannin	$FeCl_3$	+
Phenolic	$FeCl_3$	+
Saponin	Aquades	-
Glycosides	Liebermann–Burchard	+

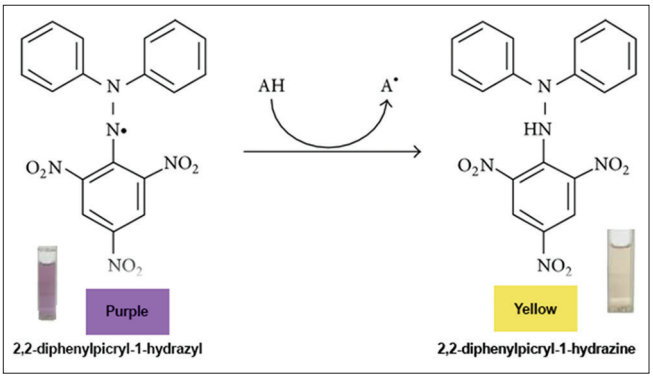

Figure 1: Schematic of DPPH free radical reactions with antioxidants

The results of determination of antioxidant activity of methanol extract fruit peel of *A. altilis* can be seen in Table 2.

Table 2 shows a decrease in absorbance values ranging from 0.4 to 0.2 for each increase in extract concentration. Scavenging activity from fruit peel of *A. altilis* occurs due to the compounds of polyphenols and flavonoids.

Antioxidant activity was seen from the decrease in absorbance value of DPPH free radicals caused by samples at various concentrations and increase in percentage value of inhibition concentration. Visually visible also can be seen the change of color purple DPPH to yellow after 30 min of incubation.

The value of free radical scavenging activity is expressed as inhibitory concentration (IC_{50}) which is the amount of concentration of test compounds that can reduce free radicals by 50%. The smaller the value of IC_{50}, the activity of free radical scavenging will be higher.[27] Free stable radicals (DPPH) are mixed with antioxidant compounds that have the ability to donate hydrogen so that free radicals present in DPPH can be scavenged.[28]

IC_{50} value of methanol extract fruit peel of *A. altilis* obtained based on the calculation of linear regression equation is shown in Table 1, where the regression equation of methanol extract obtained in Table 1 is $\hat{y}=5.423+0.093x$ and $r=0.9845$. The coefficient y in this equation is calculated as the value of IC_{50}, while the coefficient x is the concentration of the extract to be determined its value. Nilai the value of x obtained is the amount of concentration required to absorb 50% of free radicals DPPH. The value of r = 0.9845 which is close to +1 (positive value) illustrates that between the concentration of the extract and the linear antioxidant activity. Increased concentrations of the extract will also increase the antioxidant activity. This can be seen from the relationship curve of methanol extract concentration of fruit peel *A. altilis* to the percentage of inhibition as shown in Figure 2.

IC_{50} value of methanol extract fruit peel of *A. altilis* based on the calculation obtained is equal to 479. 31 µg/mL. According to Molyneux (2004), a substance with an IC_{50} value between 200 and 1000 µg/mL is less active but still potentially as an antioxidant substance. The active antioxidant compounds contained in the methanol extract of fruit peel of *A. altilis* in the form of crude extracts, so it is still bound to a glycoside group. Glycoside groups that bind to flavonoids can decrease antioxidant activity. Antioxidant activity will increase with increasing hydroxyl groups and will decrease with the presence of glycoside groups.[29] Flavonoid compounds found in nature are generally very rarely found in the form aglycon of plavonoid, commonly found in the form of flavonoid glycosides.[30]

The methanol extract of fruit peel *A. altilis* contains phenol compound which is a compound containing hydroxyl group (-OH), bonded directly to an aromatic hydrocarbon

Table 2: Antioxidant activity of methanol extract fruit peel *A. altilis*

Concentration (µg/mL)	Absorbance	% inhibition	Equation (ŷ=a+bx)	IC (µg/ml)
Blank	0.531	0	ŷ=5.423+0.093x r=0.9845	479.31
100	0.460	13.37099		
200	0.397	25.23540		
300	0.341	35.78154		
400	0.321	39.54802		
500	0.251	52.73069		

A. altilis: Artocarpus altilis

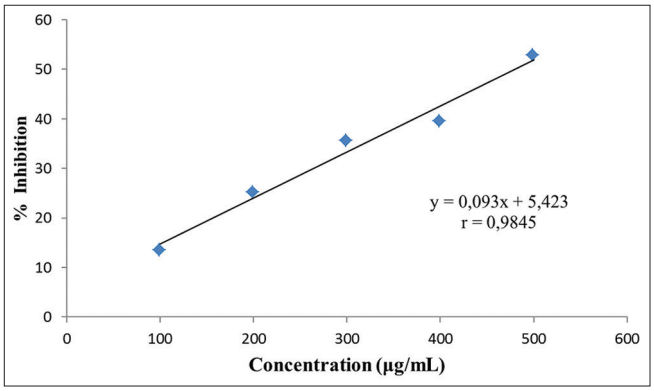

Figure 2: Linear regression curve extract methanol fruit peel of *A. altilis*

ring group. In plants, phenol compounds are simple phenols, *benzoquinon*e, phenolic acids, acetophenone, naphthoquinone, xanthone, coumarin, bioflavonoid, stilbene, tyrosine derivatives, hydroxycinnamic acid, flavonoids, lignans, and tannins.[31] Natural phenol compounds that are antioxidants can be classified in two groups, namely, lipophilic and hydrophilic groups (including phenol compounds). The antioxidant activity of phenol compounds is formed due to the ability of the phenol compounds to form phenoxide ions which can give one electron to free radical as shown in Figure 3.

Antioxidant compounds of phenol (FI-OH) react with free radicals (FI-OH•) forming ROOH and a radical phenol compound (FI-OH•) which is relatively unreactive. Furthermore, the phenolic compounds are radical (FI-OH•) which can react again with free radicals (ROO•) forming a non-radical compound.[32]

One of the natural antioxidants is gallic acid (3, 4, 5-trihydroxybenzoic acid). Gallic acid is included in the phenolic compounds and has strong antioxidant activity. Determination of TPC can be performed using Folin–Ciocalteu reagent. This method is based on the reducing strength of the phenolic hydroxy group. All phenolic compounds including simple phenols can react with Folin–Ciocalteu reagent. The aromatic ring present in the phenol compound (phenolic hydroxyl group) can reduce phosphomolybdate phosphotungstate to molybdenum to form a blue color. The total phenolic compound is expressed in GAE as the sum milligram of gallic acid in 1 g of sample.[33,34]

The results of the measurement of the calibration of gallic acid on the Folin–Ciocalteu reagent can be seen in Figure 4.

Linear regression equation ŷ=0.42212+0.0010832x, R^2=0.949, SD=0.0099, LoD=27.42 µg/mL,

and LoQ=91.39 µg/ml. Information: SD: Standard deviation, LoD: Limit of detection, LoQ: Limit of quantification

TPC was calculated on the basis of equivalent to gallic acid (mg GA/g extract) using the regression equation ŷ=0.42212+0.0010832x with value R^2=0.949. This can be interpreted that 94.9% of absorbance is affected by concentration, while the rest is influenced by other factors such as temperature, light, storage, and chemicals.

Phenolic in fruits and vegetables have a lot of attention because of the potential of antioxidant activity. Phenolic compounds undergo complex oxidation-reduction reactions with phosphotungstic acid and phosphomolybdate contained in the Folin–Ciocalteu reagent. However, some chemical groups of amino acids, proteins, organic acids, sugars, and amine aromatic can react with the reagent thus affecting the observation.[35] One method to minimize this effect is by drying. This drying process aims to remove ascorbic acid, proteins, and sugars that can interfere with the withdrawal of the active substance.

The results show that fruit peel of *A. altilis* has a high TPC that is 6277 mg/g EAG, using a standard gallic acid curve (R^2=0.949). This gives meaning that polar compounds in the fruit peel of *A. altilis* can dissolved well in methanol.[36] The total content of phenol in the sample is determined by the Folin–Ciocalteu method based on the ability of the phenolic compounds in the extract reacts with the phosphomolybdic-phosphotungstatic acid contained in the Folin–Ciocalteu reagent. This reaction produces a blue molybdenum tungstate compound. The more blue the color intensity of the solution indicates the total content of phenol in the sample is greater.

The reaction between the phenol compound and the Folin–Ciocalteu reagent in an alkaline. To create an alkaline

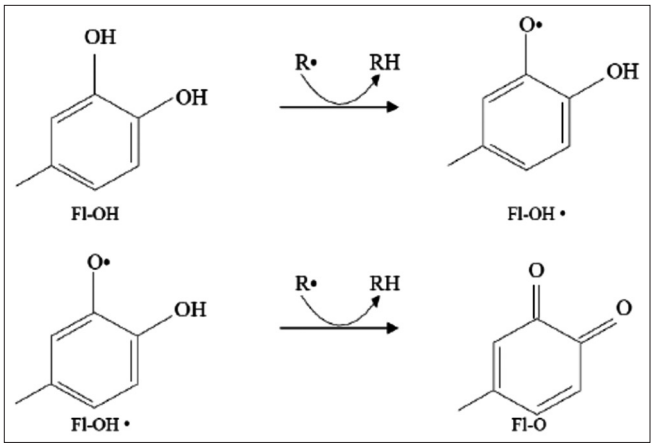

Figure 3: The mechanism of free radical scavenging by flavonoids (Kandaswami and Middleton, 1997)

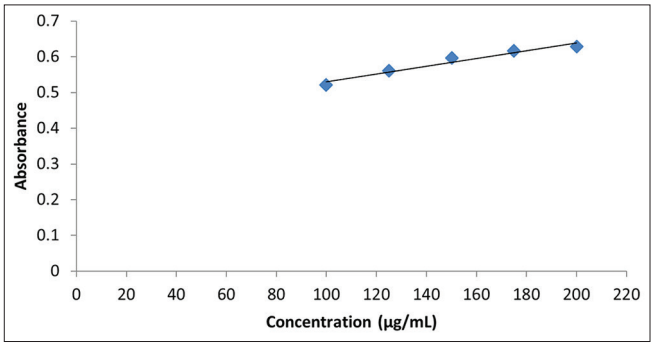

Figure 4: Gallic acid calibration curve in Folin–Ciocalteu reagent

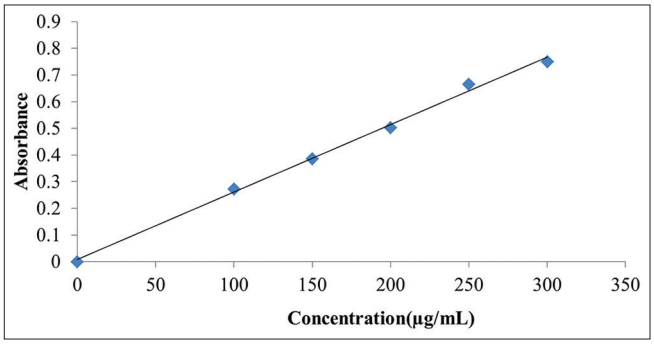

Figure 5: Routine calibration curve

atmosphere, 10% sodium carbonate is used, so that the protons present in the phenolic compound can dissociate into phenolic ions. In alkaline, hydroxyl groups in phenolic compounds react with Folin reagents forming a blue complex with unknown structure and can be detected by a spectrophotometer. The blue color formed is directly proportional to the concentration of phenolic ions formed.

Determination of TFC in A. altilis fruit using Chang method 2002, with standard routin at wavelength 415 nm. Standard routine solutions are mixed with aluminum trichloride and sodium acetate as specific reagents which form a yellow complex.[37]

From the calibration curve we get the linear regression equation $\hat{y}$ = 0,079+0,0025x. From regression equation obtained TFC extract methanol skin of *A. altilis* fruit equal to 48174 mgQE/g calculated as routin. Next, we calculate the data validation parameters useful to prove that the parameters are eligible to use. The main purpose of data validation is to ensure that the analytical methods used can provide valid results and trustworthy (high trust level). Based on the data obtained can be detected limit of detection (LoD) and limit of quantification (LoQ), where the price of LoD obtained is 6.58 µg/mL, which means at that concentration the sample can still be detected by the tool used. While the price of LoQ is obtained 21.93 µg/which describes the accuracy of the analysis. The results of this show that there is a positive relationship between the total content of phenol with the content of flavonoids on antioxidant activity so that the fruit peel of *A. altilis* has the potential as a source of natural antioxidants that can be developed into pharmaceutical products.

CONCLUSION

Antioxidants were extracted from the fruit peels of *A. Altilis* has high antioxidant activity and phenolic content may prove to be a better substitute in place of synthetic antioxidants in extending the shelf life of food product by preventing the peroxide formation in the product containing fat and oil. The total phenolic and flavonoid content fruit Peel of *A. altilis* can be used as a source of natural antioxidants. In addition, natural antioxidants are safe and impart health benefit to the consumer.

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