Comparison of Antioxidant Activity of Ethanolic, Methanolic, n-Hexan, and Aqueous Extract of Parkia speciosa Peel based on Half -Maximal Inhibitory Concentration Through Free Radical Inhibition

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Abstract. The objectives of this study was to determine the half maximum inhibitory concentration (IC_{50}) from four types of *Parkia speciosa* peel extracts (ethanol, methanol, n-hexane, and aqueous) through DPPH free radical inhibition. First Parkia's peel extract made by drying the Parkia's peel that has been sorted, then crushed and mashed with a blender. Parkia's powder then macerated for 3 replication using each type of solvent and then solvent evaporation was carried out using a rotary vacuum evaporator. The evaporated extract produced then tested for antioxidant activity using the IC_{50} method and phytochemical screening was performed to analyze the potential content of functional compounds. The results showed that all types of solvents dissolve alkaloid compounds (except water extract), flavonoids, saponins, tannins, and phenols. IC_{50} values produced from the four types of petai bark extract using methanol, ethanol, water, and n-hexane solvents sequentially were 76.92; 111; 136; and 201 ppm. Methanol extract had the lowest IC_{50} value of 76.92 ppm which resulted that the methanol extract of petai skin had a strong (active) antioxidant strength compared to others.

Keywords: Antioxidant, Exctract, Parkia's peel, IC_{50}

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1. Introduction

The use of natural materials with bioactive compounds has recently expanded. This is relating to the increase of various degenerative diseases caused by free radicals such as heart disease, arteriosclerosis, cancer, and symptoms of aging. Problems related to the function and ability of antioxidants in the body as inhibitor of cell oxidation chain due to highly reactive free radicals [1][2]. The use of traditional plants as plants that have functional values for health has been widely used and

researched in Indonesia. The content of compounds such as phenolic compounds, flavonoids, and terpenes in traditional plants functions as antioxidants, anti-inflammatory, anti-microbial and others.

Antioxidants based on the source are divided into 2 types of natural antioxidants and synthetic antioxidants [3]. Synthetic antioxidants may have negative effects if consumed for a long time. meanwhile naturalantioxidant is widely used as an ininhibitor to free radicals in the body. The limited production of endogenous antioxidants has an impact to the body to need additional exogenous antioxidants to inhibit free radicals. Therefore, exploration of natural sources of antioxidant have been continuously improved. One of the Indonesian plants source which has the potential to be explored is petai.

Petai (*Parkia speciosa*) is one of the abundant plant in Indonesia because it is easy to grow anywhere. *Parkia speciosa* peel (Parkia's peel) is a part of the petai plant which is not used and is usually thrown away as waste. Parkia's peel have potential benefit such as antioxidants, antidiabetic, and antiangiogenic. Potential benefit from Parkia'speel may become from its contain such as phenolic compounds and flavonoids in large quantities. The antioxidants contained in the extract of petai seeds and peels after being fractionated with several solvents showed that the ethyl acetate fraction of petai peel had the greatest antioxidant potential, with an IC_{50} value of 85.92 ppm. While IC_{50} from petai seeds had the greatest activity with IC_{50} value was 136.29 ppm [4].

The functional compounds found in Parkia's seed and peel extract influenced by various factors, one of them is the type of solvent used and the extraction temperature [5]. Types of solvents have different levels of polarity and solubility for certain types of compounds, while the extraction temperature determines the optimal temperature of the extraction process so that the maximum extraction is obtained. Therefore, the use of Parkia's peel as a source of natural antioxidants is very promising and it is necessary for further investigation the factors affecting antioxidant extraction process of Parkia's peels. Based on the reasons, it is necessary to carry out research on the comparative test of the antioxidant activity of the ethanol, methanol, aqueous, and n-hexane extracts of Parkias peel using the inhibitory concentration 50 (IC_{50}) test method.

2. Methods

2.1. Sample preparation

Parkia speciosa peel was separated from the seeds and then sliced to obtain uniform size. The peel then dried in a cabinet dryer at 50^{o}C, for 48 hours until completely dry. The dried peel was smoothed with a grinder and sieved with a 70 mesh sieve. The resulting powder was ready for extraction.

2.2. Extraction

The extraction process was carried out by the maceration method in an erlenmeyer and wrapped in aluminum foil. A total of 100 grams of parkiosa peel powder are macerated in Erlenmeyer for 24 hours. After 24 hours of maceration then filtered and obtained macerate, maceration is repeated up to 3 times. After the maceration process ends, the maserate or filtrate were evaporated until a concentrated sample of each type of solvent is obtained using a rotary vaccum evaporator at 40^{o}C. The concentrated sample was obtained then analyzed for phytochemical screening and testing for antioxidant DPPH.

2.3. DPPH Antioxidant Assay

Quantitative measurements of radical scavenging assay were carried out according to the method described by [5]. The quantitative measurement of radical scavenging properties was carried out in universal bottle. The reaction mixture contained 50 µl of sample at concentration ranging from 0; 20; 40; 60; 80; 100 ppm and 5 ml of a 0.04% (w/v) solution of DPPH in 80% methanol. Gallic acid was used for comparison or as a positive control. The DPPH solution in the absence of sample was used as control and the 80% methanol was used as blank. Discolourations were measured at 517 nm by using spectrophotometer (HITACHI U-1900 spectrophotometer 200V) after incubation for 30 min in the

darkroom. Measurement was performed at least in triplicate. The percentage of the DPPH free radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = ((A_0 - A_1)/ A_0) \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the *Parkia speciosa* peel extract [6].

2.4. Determination of Half maximal Inhibitory concentration

The actual decrease in absorption induced by the test was compared with the positive controls. The IC_{50} (concentration providing 50% inhibition) values were calculated use the dose inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging effect.

2.5. Phytochemical Screening (Qualitative analyses)

- Alkaloid Identification
  0.5 grams of sample extract was dissolved in 5 ml hydrochloric acid, then filtered. The filtrate obtained was used as a sample solution. About 1 ml sample solution then added with 2 drops of Mayer LP reagen, a positive result showed by the formation of a yellow precipitate.
- Flavonoid Identification
  A total of 0.5 grams sample extract was added with 2 drops of NaOH solution. The formation of an intense yellow color by adding dilute acids indicates the presence of flavonoids.
- Saponin identification
  A total of 0.5 grams of sample extract was added with 2 ml of distilled water, then shaked for 10 seconds. Positive results were showed by formation of a stable foam for not less than 10 minutes
- Tanin identification
  A total of 0.5 grams of sample extract was added with 2 drops of 1 % gelatin solution in NaCl. the formation of white pellet indicates the presence of tannin
- Fenolic identification
  A total 0.5 grams of sample extract was added with 3 drops of $FeCl_3$ solution. The presence of Fenolic compound indicated by formationof greensh blue color of the sample solution.

2.6. Statistical Analysis

All the data analysis was replicated and showed as mean ± SD. Analysis of Variance were perfomed using one-way analysis of Variance (ANOVA). Significant differences between means were determined by Duncan's Test and if P value less than 0.05 were considered statistically significant. All data were analysed using SPSS 17 version programme.

3. Results and Discussion

The dried Parkiosa's peel powder was subjected to normal temperature (37^{o}C) condition of maseration process. as a result, methanolic, ethanolic, n-hexane and aqueous exctracts were obtained. The extract was analyzed for phytocemical screening, antioxidant activity based on half maximum inhibitory concentration (IC_{50}).

3.1. Yield Extract

The different types of solvents would determine the yield extract. The results of the *Parkia speciosa* peel yield extract's using various types of solvent can be seen in Table 1.

Table 1. Effect of various types of solvents on the yield percentage of the extract

Sample	powder weight (g)	final weigt ekstrak(g)	Yield (%)
Methanolic extract	20	4,56	22,80
Ethanolic extract	20	3,27	16,35

Aqueous extract	20	4,99	24,95
n-hexane extract	20	3,05	15,25

Parkia's peel dry powder was extracted by maceration for 24 hours using various types of solvents. The maserate obtained after the extraction process then concentrated with a rotary vaccum evaporator to remove the solvent, so that a concentrated extract will be obtained. In general, the extract yield ranged from 15.25 to 24.95%. The extract yield of the four types of extracts had a high enough value, in which the yield of water extract was higher than methanolic extract. This may be caused by compounds from Parkia's peel that dissolve in water more than other solvents. Methanol and ethanol are also universal solvents capable of binding or dissolving compounds derived from natural materials, both non-polar, semi-polar and polar [7].

3.2. Phytocemical screening

Phytochemical screening was carried out to determine the potential content of compounds qualitatively extracted from the material with various types of solvents, so that potential antioxidant activity can be identified. The results of phytochemical's screening are as shown in Table 2.

Tabel 2. Results of Phytochemical Screening Parkia's peel Extract using various types of solvents

Compound group	Sample extract			
	Metanolic	Ethanolic	Aqueous	n-Heksan
Alkaloid	+	+	-	+
Flavonoid	+	+	+	+
Saponin	+	+	+	+
Tanin	+	+	+	+
Fenol	+	+	+	+

The results of phytochemical screening of Parkia's peel extract with various types of solvent showed that there were compounds derived from the alkaloids (except aqueous extract), flavonoids, saponins, tannins, and phenols. Parkia's peel contains secondary metabolite compounds in the form of phenolic compounds and their derivatives that have the potential as antioxidants [8][9]. Secondary metabolic compounds that have the potential to act as antioxidants are phenolic compounds, such as phenyl propanoids, flavonoids, anthocyanins, tannins, melanins, simple monocyclic phenols, and lignins [10].

3.3. Half Maximum inhibitory concentration (IC_{50})

Quantitative antioxidant activity testing was carried out by measuring DPPH radical activity by spectrophotometry method at an absorption wavelength of 517 nm. The IC_{50} (inhibitory concentration 50) value indicates that the ability of the extract or compound to reduce or inhibit free radicals by 50%. Determination of the IC_{50} activity can be seen in Table 3.

Table 3. Determination of half maximum inhibitory concentration of each sample

Sample Concentration (ppm)	% inhibiton of methanolic extract	IC_{50}	% inhibiton of ethanolic extract	IC_{50}	% inhibiton of n-hexan extract	IC_{50}	%Inhibiton of aqueous extract	IC_{50}
0	-		-		-		-	
20	9,724		9,681		8,388		9,124	
40	20,186	76,9	17,009	111	21,063	201	16,295	136
60	33,677		25,308		35,611		21,974	
80	54,312		35,217		43,296		30,581	
100	68,325		45,748		56,002		37,129	

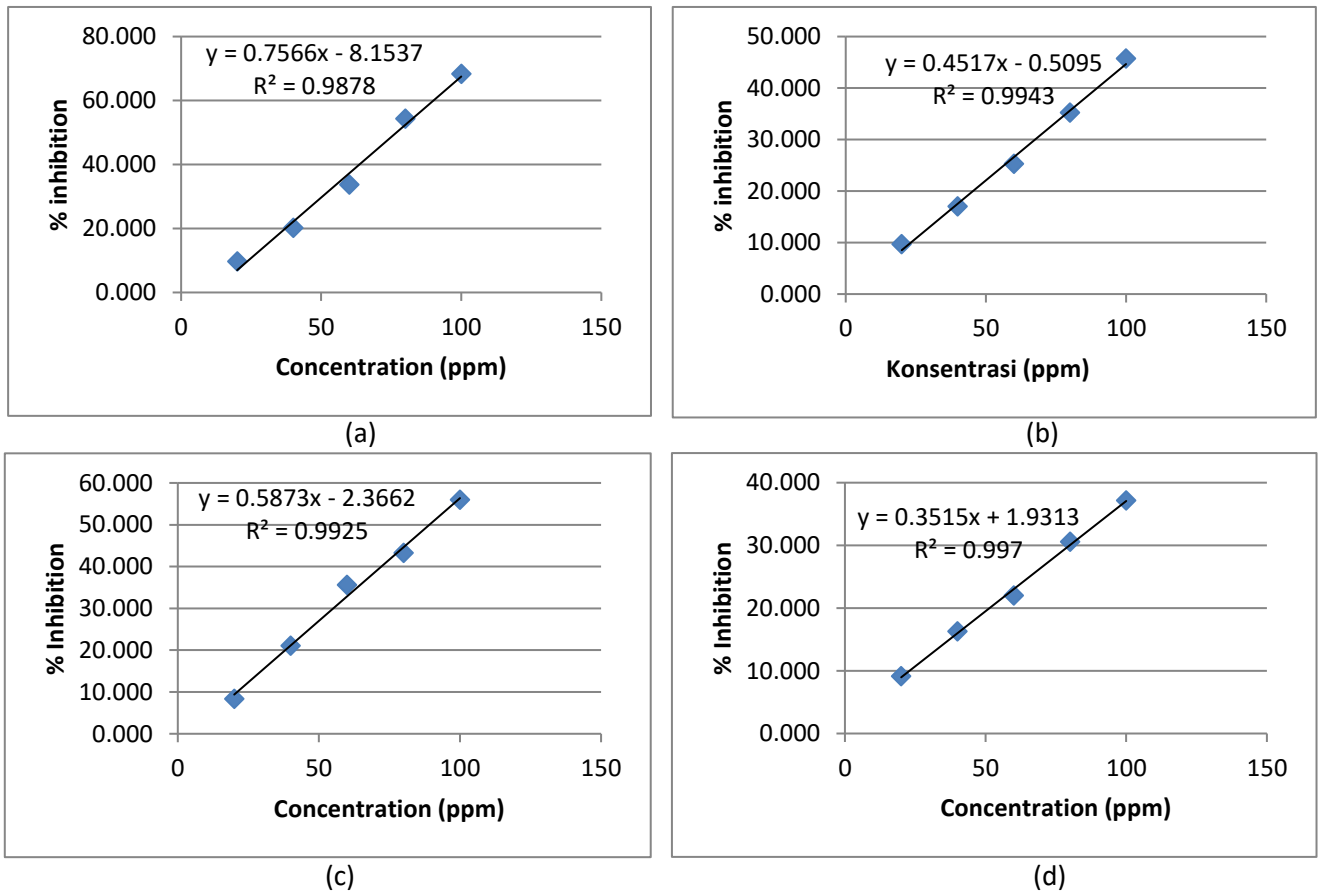

Figure 1. Regretion curve of DPPH analysis based on Typical solvent (a) methanolic extract, (b) ethanolic extract, (c) n-hexane extract, (d) aqueous extract

Antioxidant properties of Parkia's peel extracts was evaluated to find a new natural source of antioxidant. DPPH radical is a commonly used reagent for evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay [11]. The principle of this assay in the color change of DPPH solution from purple to yellow as the radical is quenched by the antioxidant [12]. The colour changes can be measured quantitatively by spectrophotometer at 517 nm.

Based on Figure 1 (a), the linear equation value for methanolic extract y = 0.756x-8.153, so the calculation of the IC_{50} value for methanoic extract obtained the following values: y = 0.756x-8.153 (for y = 50), then the x value is 76.92 ppm. The IC_{50} value of the methanol extract can be categories as active antioxidant power (50-100 ppm) [13]. Furthermore, based on the calculation of the regression curve In the ethanol extract sample (Figure 1.b), the regression equation y = 0.451x - 0.509 (assuming the value of y = 50) is obtained, the x value is 111 ppm. The IC_{50} value of the Parkia's peel ethanolic extract showed that the extract had moderate IC_{50} antioxidant activity (101-250 ppm). The value obtained is classified as having the ability to reduce moderate radicals, it happened because the possibility of the extract obtained has low purity or is in the form of crude extract.

The correlation curve between the concentration of aqueous extracts used in reducing free radical DPPH (Figure 1.d) was calculated as% inhibition against free radicals. So that the regression equation y = 0.351x + 1.931 (y value = 50) is obtained, then the x value (IC50) is 136 (ppm). The IC50 value ranging from 101-250 ppm had sufficient or moderate antioxidant reducing power [13]. While, the

calculation of IC_{50} from n-hexane extract of Parkia's peel had a value about 201 ppm. The result Showed that n-hexane extract has the highest IC_{50} value compared to the other solvents. so that in the determination of IC_{50} n-hexane extract has a low qualification of antioxidant strength (> 200 ppm).

The comparison value of IC_{50} among four typesof solvent usedin this research, the methanolic extract exhibit a significant dose dependent inhibition of DPPH activity with 50% of inhibition (IC_{50}) at concentration of 76.9 ppm (Figure 1.a). Basically, a higher DPPH radical-scavenging activity is associated with a lower IC_{50} value. There are studies have been carried out to evaluate the antioxidant activity of *Cassia* species using DPPH assay [14] and reported that, particularly *C. fistula* exhibited higher antioxidant activity compared to *C. spectabilis* [15]. Whereby the present study proof that, the *Parkia speciosa* peel extract has the potential compound(s) react as antioxidant which is suitable to develop a drugs for the prevention of human disease related to free radical mechanism.

4. Conclusion

The methanolic extract of *Parkia speciosa* peel had the highest IC_{50} activity value compared to the three types of extracts with a value of 76.92 ppm, while the ethanol extract, aqueous extract, and n-hexane extract were 111; 136; and 201 ppm, respectively. The IC_{50} value in the range between 50-100 has strong antioxidant activity, while the IC50 value in the range 101-250 includes moderate ability.

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