

Antioxidant Activities, Phenolic and Flavonoid Contents of Methanolic Extract of *Stelechocarpus burahol* Fruit and its Fractions

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ABSTRACT: Kepel or *Stelechocarpus burahol* is an exotic fruit originating from Java, Indonesia. This research was intended to evaluate the antioxidant activities of extracts and fractions of kepel fruit pulp (KFP) based on radical scavenging capability towards 2,2'-azinobis(3-ethylbenzo thiazoline-6-sulphonic acid) diammonium salt (ABTS) and 2,2'-diphenildiphenyl-1-picrylhydrazil radical (DPPH). In this study, the radical scavenging activities were also correlated with total phenolics and flavonoid contents. Among the evaluated samples, the ethyl acetate fraction exhibited the highest antiradical antioxidant activity towards ABTS radical with IC₅₀ of 0.35 µg/ml, lower than that of vitamin C (as positive control). It also showed highest antiradical activity using DPPH based assay. Methanolic extract had total phenolic content of 58.28 ± 0.37% wt/wt gallic acid equivalent, higher than its fraction. Meanwhile the petroleum ether soluble fraction revealed flavonoid content (76.06 ± 11.9%) as rutin equivalent, among the extract and fractions evaluated. Based on coefficient (R²) values, phenolics and flavonoids contents contributed to 73.09% and 30.99% towards antiradical scavenging activities, respectively.

Key words: Kepel fruit pulp, ABTS, phenolics content, flavonoid content, DPPH.

INTRODUCTION

Kepel or known as Burahol with scientific name of *Stelechocarpus burahol* is one of the plants originating from Java, Indonesia. This plant is also grown in Southeast Asia throughout Melanesia. Kepel is used traditionally as perfume by aristocratic ladies in Java, especially by the consorts of sultans of Jogjakarta. The fruit pulp of kepel is diuretic and able to prevent inflammation in kidney. It is also used in cosmetics as deodorant to make sweat fragrant. The formula containing fruit pulp is known as "awetayu" (remain beautiful).¹

Some biological activities of kepel have been reported namely anti-hyperuricemic and xanthine oxidase inhibitor,² anti-inplantation,³ anticancer,⁴

antiseptic,⁵ anti-inflammation,⁶ and antioxidant activities.^{7,8} It was reported that ethanol extract of kepel leaves did not induce the toxic effect on male and female Sprague-Dawley rats. The pseudo-lethal dose of 50% (pseudo-LD₅₀) value of ethanolic extract of kepel leave was higher than 5000 mg/kg body weight.⁹ Due to its beneficial activity of kepel, Purwantiningsih *et al.*¹⁰ have standardized kepel leaves to ensure the safety, efficacy and the quality of the kepel products.

Previous study reported that aqueous extract of kepel leaves exhibited radical scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl, the active flavonoid component is tentatively identified as flavon with hydroxyl group on C-3, C-7, C-3', C-4' and methyl on C-5.⁷ Two phenatrene lactams (aristolactam BI and aristolactam BII) were also isolated from stem bark of kepel.⁴ In this study, the methanolic extract and fractions of fruit pulp of kepel were evaluated for its antioxidant activities *in vitro*.

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Antioxidants are defined as any compounds or materials capable of delaying or preventing the oxidation of lipids, proteins, or other molecules by inhibiting initiation or propagation of free radical reaction.¹¹ There are two sources of antioxidants, synthetic and natural. The synthetic antioxidants such as propyl gallate, *ter*-butylhydroquinone butylhydroquinone, butylated hydroxytoluene, and butylated hydroxyanisole are very effective in inhibiting oxidation reactions, however, some scientist reported the toxic effects of synthetic antioxidants,¹² as a consequence, the demand for natural antioxidants coming from plants has increased tremendously because of the consumers concern regarding the safety of synthetic antioxidants.¹³ In the recent years, the exploration of underutilized part of fruit such as rambutan peel,¹⁴ mango peel¹⁵ are proposed as natural antioxidant sources in order to reduce plant wastes.

Natural antioxidants derived from plants, are mainly due to phenolics and flavonoids contained in plants. For this reason, determining antioxidant activity is frequently correlated with the total contents of phenolics and flavonoids, as suggested by some authors.¹⁶⁻¹⁸ In this study, the phenolics and flavonoid contents were correlated with antioxidant activities of extracts and fractions of kepel fruit pulp.

MATERIALS AND METHODS

Kepel fruit was obtained from several locations around Gadjah Mada University (UGM), Yogyakarta. Its identification was performed in Laboratory of Pharmacognosy, Department of Pharmaceutical Biology, UGM. 2,2'-azinobis(3-ethylbenzo thiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu reagent, gallic acid and potassium persulphate were purchased from Sigma (Aldrich, USA). The other solvents and reagents used were of analytical grade obtained from E. Merck (Darmstat, Germany).

Preparation of methanolic extract and fractions of kepel fruit pulp. Preparation of extract and fractions were performed as that described by Permatasari and Rohman.¹⁴ The fruit pulp of kepel was cleaned and cut into small pieces using commercial cutter, blended and subjected to maceration process using methanol as extracting solvent (1: 10) for three days. Macerate was filtered and evaporated using vacuum rotary evaporator to obtain methanol extract of kepel fruit pulp. The methanolic extract was added to warm distilled water and was subsequently fractionated using petroleum ether. The residue of methanol extract was then fractionated again using chloroform to obtain chloroform fraction. The residue was finally extracted using ethyl acetate (Figure 1). The methanolic extract as well as the fractions of petroleum ether, chloroform, ethyl acetate and water were subjected to assays antioxidant activity and phenolics and flavonoid contents.

Antioxidant activity. The antioxidant activity of extract and fractions of kepel fruit pulp was performed using the decolorization assay with ABTS⁺ radical cation according to Re et al.¹⁹ The solution of ABTS (7 mM) and potassium persulphate (2.45 mM) were mixed in ratio 1:1 and allowed to stand in the dark for 12-16 hrs to produce stock solution of ABTS radical cation (ABTS⁺). This solution was further diluted with methanol to attain absorbance of 0.600-0.800 at 734 nm. The ABTS⁺ working solution (3 ml) and 30 μ l of blank, standard or sample were mixed and the absorbance was measured at 734 nm after 6 min using a spectrophotometer. The blank was run with methanol-80. A standard curve was prepared using Trolox solution (0.3-1.5 mM).

Antiradical activity. Antiradical activity of samples toward 2,2'-diphenyl-1-picrylhydrazil picrylhydrazyl (DPPH) was evaluated using spectrophotometer visible at 517 nm according to Blois method as described by Permatasari and Rohman.¹⁴ The stable DPPH radical (0.1 mM) in methanol was prepared, and then 1 ml of this solution was mixed with 3 ml of sample at different

concentrations. A control, containing 1 ml of DPPH radical solution and 3 ml of methanol was prepared. The mixture was at ambient temperature for 20 min, and the absorbance was subsequently measured at

517 nm against blank of methanol. The ability of extract and fractions to scavenge the DPPH radical was calculated using the formula:

$$\% \text{ Radical scavenging} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

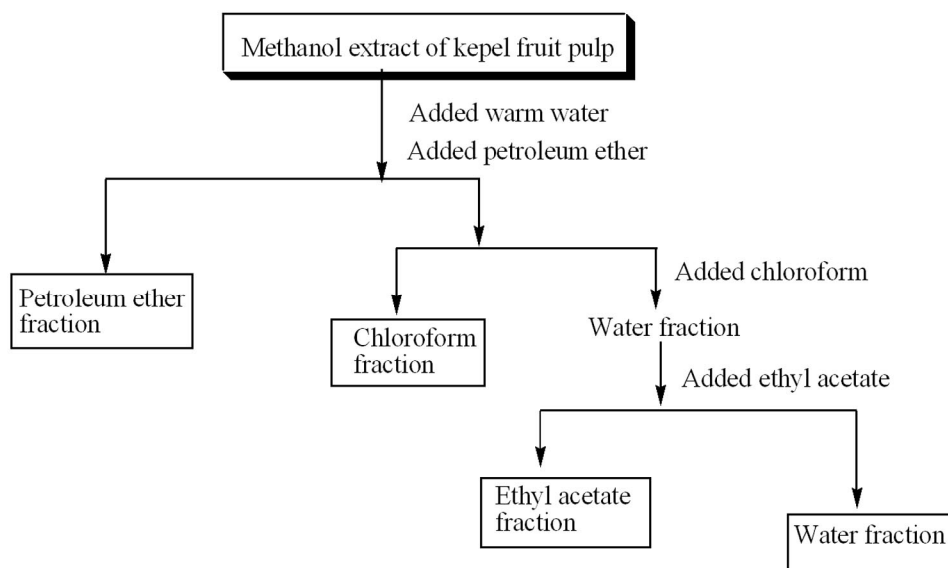


Figure 1. Extraction and the fractionation step of kepel fruit pulp.

The percentage of antioxidant activity was plotted against the sample concentrations ($\mu\text{g/ml}$) to obtain IC_{50} , defined as the concentration of the samples necessary to cause 50% scavenging of DPPH radical, calculated by an equation generated from linear regression.²⁰

Determination of total phenolics. The concentration of total phenolics was determined using Folin-Ciocalteu according to Chun *et al.*²¹ Total phenolic contents of samples are expressed as gram gallic acid equivalent/100 gram dry samples. The sample was analysed in triplicate.

Analysis of flavonoid content. Flavonoid contents of samples were determined using aluminium chloride colorimetric method according to Zou *et al.*²² An aliquot of diluted sample solution was mixed with 2 ml distilled water and was subsequently added with 0.15 ml NaNO_2 (5%) and allowed to stand for 5 min. After that, 0.15 ml AlCl_3 (10%) was

added and allowed to stand for 6 min. The mixture was added with 2 ml NaOH (4%) solution and distilled water was added to make final volume of 5.0 ml. The mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus a prepared water blank. Total flavonoid contents of extracts and fractions were expressed as gram rutin equivalent/100 g dry material.

Data analysis. All data were analysed in triplicate and expressed as mean \pm standard deviation using Excel (Microsoft Inc., USA).

RESULTS AND DISCUSSION

Antioxidant assay using free radical scavenging is the most popular methods reported by some investigators. Two radical scavenging methods were used in this study, namely 2,2'-diphenyl-1-picrylhydrazil picrylhydrazyl (DPPH) radical and

2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt cation radical (ABTS⁺). The intensity of both radicals was reduced when added with compounds or samples capable of donating their hydrogen radical such as phenolics and flavonoids. Therefore, these compounds are known as antioxidant. The reduction of colour intensity is expressed with decreasing absorbance values. For DPPH assay, the solvent used is methanol for the fact that methanol offers the best sensitivity.²³

The antiradical scavenging activities toward ABTS radical of extracts and fractions of KFP are shown in table 1. The activities were expressed as IC₅₀ (concentration of extracts having capability to scavenge 50% radical). The lower the IC₅₀, the more active the samples is as antioxidant. Among the evaluated samples, ethyl acetate fraction exhibited the highest antiradical activity with IC₅₀ of 0.35 µg/ml. This IC₅₀ value was lower than that of vitamin

C (as positive control) and higher than other evaluated samples. Therefore, it can be stated that ethyl acetate fraction has higher antioxidant activity than vitamin C (IC₅₀ = 0.760 µg/ml) and others. The ethyl acetate fraction also revealed the highest antiradical activities, among extract and fractions evaluated, in our previous studies, namely in *Morinda citrifolia*,²⁰ red fruit (*Pandanus conoideus* Lam)¹⁶ and *Phyllanthus urinaria* L.²⁴ Ethyl acetate is semi-polar solvent, as a consequence some semi-polar compounds such as phenolics and flavonoids were more extracted in this solvent. Table 2 compiled the antiradical activities of methanolic extract and its fraction (petroleum ether, chloroform, ethyl acetate, and water) of KFP using DPPH radical. Again, ethyl acetate fraction had the highest antiradical activities with (IC₅₀ 1.30 ± 0.02 µg/ml), compared to other fractions and positive control vitamin C (IC₅₀ = 3.55 ± 0.04 µg/ml).

Table 1. The antiradical activity of methanol extract and its fraction using ABTS radical assay.

Samples	Regression equation	Correlation coefficient	IC ₅₀ (µg/ml)
Vitamin C (positive control)	$y = 33.564x + 24.503$	0.9945	0.760
Methanol extract	$y = 2686.44x + 13.60$	0.9870	1.40
Chloroform fraction	$Y = 740.7x + 4.32$	0.9870	6.20
Ethyl acetate	$Y = 8100x + 21.88$	0.9761	0.35

Table 2. The antiradical activity of methanol extract and its fraction using DPPH radical assay.

Samples	IC ₅₀ (µg/ml)
Vitamin C (positive control)	3.55 ± 0.04
Methanol extract	7.47 ± 0.13
Petroleum ether fraction	1.90 ± 0.02
Chloroform fraction	5.80 ± 0.03
Ethyl acetate	1.30 ± 0.02

A group of compounds responsible for antiradical scavenging activities are phenolics and flavonoid, due to the capability of phenolics and flavonoids to donor hydrogen radical into ABTS and DPPH radicals, therefore some antioxidant activities from natural sources are correlated with the total contents of phenolics and flavonoids.¹⁸ Table 3

showed the total content of phenolics and flavonoids in extract and fractions of KFP. Methanolic extract had total phenolic content of 58.28 ± 0.37% wt/wt gallic acid equivalent, higher than its fraction, meanwhile, petroleum ether exhibited the highest flavonoid content (76.06 ± 11.9%) as rutin equivalent, among extract and fraction evaluated.

Figure 2 exhibited the correlation between IC_{50} values, either using ABTS radical or DPPH radical, with phenolics (A) and flavonoid contents (B) of extracts and fractions. The coefficient determination

Table 3. Contents of total phenolics and total flavonoid of methanolic extract and fractions of kepel fruit pulp.

Extract or fraction	Phenolic contents (mean \pm SD)*	Flavonoid contents (mean \pm SD)**
Methanolic extract	58.28 \pm 0.37	49.81 \pm 0.37
Petroleum ether fraction	48.61 \pm 1.97	76.06 \pm 11.95
Chloroform fraction	24.72 \pm 0.98	60.47 \pm 1.82
Ethyl acetate	43.15 \pm 2.10	71.00 \pm 4.10
Water fraction	20.69 \pm 0.56	19.99 \pm 0.16

*expressed as % wt/wt gallic acid equivalent; **expressed as % wt/wt rutin equivalent.

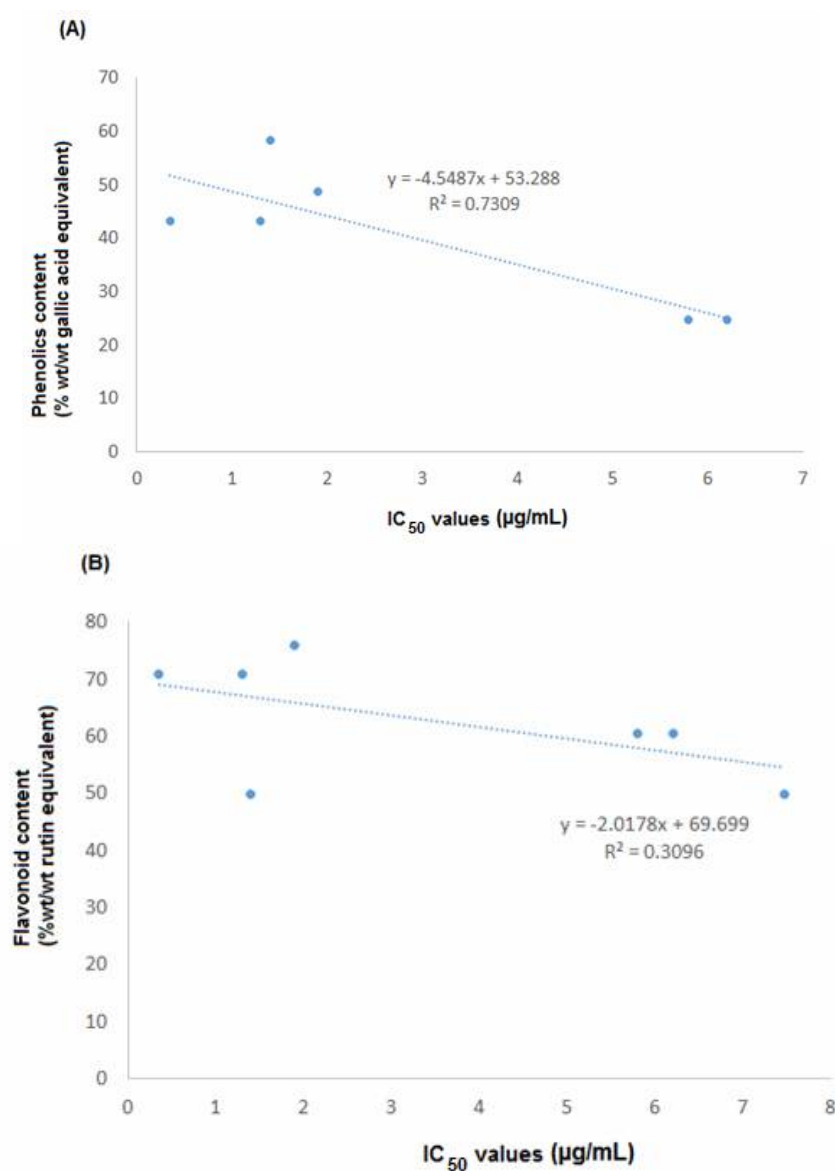


Figure 2. The correlation between IC_{50} values and phenolic contents (A) as well as IC_{50} values and flavonoid contents (B).

(R^2) value is used as parameter describing the contribution of phenolics and flavonoid contents toward radical scavenging activity.¹⁸ The R^2 values obtained were 0.7309 and 0.3096 for correlation between IC_{50} values with phenolics and flavonoid contents, indicating that phenolics and flavonoids contents contributed to 73.09% and 30.99% toward antiradical scavenging activities, respectively. In addition, some other secondary metabolites such as alkaloids may contribute toward those activities.

The correlation between the antioxidant capacity and total content of phenolics as well as flavonoid were investigated by some authors. Kriaa *et al.*²⁵ reported that the linear relationship between the total antioxidant activities with total phenolic contents ($R^2 = 0.687$) and with flavonoid contents ($R^2 = 0.773$) of the methanol extract of three varieties of date palm. The phenolics contents also statistically correlated ($p < 0.05$) with antioxidant activity with R^2 for such correlation of 0.52.²⁶ The interrelationship of antioxidant activities with the phenolics and flavonoid contents in some extracts of wild were reported. The multivariate of principal component analysis (PCA) was used to describe such correlation. PCA can identify that the phenolics and flavonoid contents are closely correlated with antioxidant activities.²⁷

CONCLUSION

The methanolic extract and its fraction of kepel fruit pulp (KFP) exhibited strong antiradical activities toward 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzo thiazoline-6-sulphonic acid) diammonium salt cation (ABTS⁺) radicals. The fractions of ethyl acetate and petroleum ether had higher antiradical activity than vitamin C used as positive control. The antioxidant activities of KFP were highly correlated with the contents of phenolics and flavonoids present in KFP.

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