Research Article

Optimization of the Ultrasound Assisted Extraction of *Phaleria macrocarpa* (Scheff.) Boerl. Fruit Peel and its Antioxidant and Anti-Gout Potential

Candra Irawan[1], Maman Sukiman[2], Ismail[3], Imalia Dwi Putri[1], Andita Utami[3,*], Avisani Dewanta[3], Aulia Noviyanti[1]

ABSTRACT

Aims: This study aimed to obtain the skin extract of *Phaleria macrocarpa* (Scheff.) Boerl. through the application of ultrasound-assisted extraction (UAE) with variations in time and amplitude to produce optimal extraction conditions. The extract's potential as an antioxidant with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition parameters, and its potential as an anti-gout. **Results:** The yield of crude ethanol extract of *Phaleria macrocarpa* (Scheff.) Boerl rind obtained from the UAE process ranged from 18 to 21%. The phytochemical test results of *Phaleria macrocarpa* fruit peel extract contain phenolic compounds, tannins, saponins, and alkaloids. Extract B was treated for 35 minutes and had an amplitude of 65% with an antioxidant activity IC_{50} of 52.01 ± 0.06 mg/L and a reduction of uric acid level of 90.49 ± 0.08. **Conclusion:** The ethanol extract of the fruit peel of *Phaleria macrocarpa* from the UAE has the potential as a source of antioxidants and anti-gout.

Key words: *Phaleria macrocarpa* (Scheff.) Boerl., Ultrasound-assisted extraction, Antioxidant, Anti-gout.

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INTRODUCTION

Indonesia is one of the developing countries in Asia with the highest number of gout patients. Gout affects approximately 1.7 percent of Indonesia's population.[1,2] When uric acid levels in the blood exceed 6.8 mg/dL, monosodium urate (MSU) crystals will form and accumulate in joints, tendons, and other tissues. The accumulation of crystals causes gout.[3,4] Uric acid levels that are too high can cause inflammation in blood vessel cells,[5,6] and are linked to hyperlipidemia, cardiovascular disease, hypertension, kidney disease, and diabetes.[3,7] Several therapies can be tried to treat gout. The most effective therapy is to reduce uric acid levels, one of which is using xanthine oxidase (XO) inhibitors.[8] Allopurinol is an effective XO inhibitor and is used as the main drug for the treatment of gout. However, side effects such as renal and gastrointestinal toxicity, hepatitis, and allergic reactions have been frequently reported.[9] Therefore, it is very important to find an effective and safe XO inhibitor for pharmaceutical applications.

Medicinal plants are sources of active compounds that are critical in drug development due to their diverse and unique chemical structures.[10] One of the native plants of Indonesia is *Phaleria macrocarpa*. *Phaleria macrocarpa* fruit is empirically believed to be an effective medicine to treat several diseases such as high blood pressure, diabetes, gout, and so on. In addition, various studies report that secondary metabolites of this plant such as tannins, saponins, phenolic compounds, flavonoids, terpenoids and alkaloids play a major role as antioxidants, anti-inflammatory, antimicrobial agents and also have cytotoxic activity.[11-13]

The selection of an extraction method to obtain plant extracts is a critical first step in the study of medicinal plants. Maceration is one of the most common extraction methods. The disadvantage of this method is that it necessitates a lengthy extraction time and the use of a large amount of solvent.[14] Various extraction techniques have been developed in order to obtain methods that are more environmentally friendly, reduce solvent use, prevent possible compound degradation due to heat use, shorten extraction times, increase reaction rates, and increase extract yield and quality.[15] One of them is the ultrasonic sonification method.[16,17]

The use of ultrasonic waves passed through the solvent causes a cavitation effect, which produces a mechanical effect, allowing greater penetration of the solvent into the sample matrix and increasing the contact surface area between the solid and liquid phases. As a result, the solute diffuses rapidly from the solid phase into the solvent.[17] In this method, the possibility of degradation of the targeted compound is relatively low.[15] This extract will be prepared using the ultrasound-assisted extraction method in this study. Variations in time and amplitude conditions were carried out in the extraction in order to obtain optimum conditions for obtaining the yield with the highest antioxidant and anti-uric acid activity.

METHODS

Simplicia setup

The plants used were previously determined by the correctness of identity in the Herbarium Bogoriense, Botany field of the Indonesian Institute of Sciences Biology Research Center, Cibinong, Bogor Regency, West Java. *Phaleria macrocarpa* fruit peel samples (Figure 1) were obtained farmers in Bogor. Simplicia was mashed using a blender then the simplicia powder was stored in a dry container, closed, identified and protected from direct sunlight.

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Figure 1: Fruit peel samples of *Phaleria macrocarpa.*

Extraction of simplicia

The procedure refers to the research that has been carried out by Irawan using the UAE method.[18] The fruit peel dry powder was weighed at approximately 7 grams and put into a 250 mL beaker glass for 4 repetitions. Then, added with 70% technical ethanol, the solvent was added until the dry fruit peel powder was submerged and stirred until everything was mixed. Fruit peel simplicia was extracted with UAE using time variation parameters (minutes) and amplitude (%) at 30 minutes-60%; 35 minutes-65%; 45 minutes-60%; and 45 minutes-65%. The extraction results were filtered to separate the liquid extract from the dregs and put into a beaker which had been weighed. Each beaker is 250 mL. The filtrate in the 250 mL beaker obtained was removed by removing the ethanol solvent by evaporation using an oven at a temperature setting of 40 ^{o}C and left until all the ethanol had evaporated to obtain a fruit peel yield. The extract without solvent was weighed and the percent yield value was calculated.

Phytochemical screening

Phytochemical screening was carried out on each variation of time (minutes) and amplitude (%) of fruit peel extraction using the UAE method. Phytochemical screening tests carried out included tests for alkaloids, flavonoids, phenols, saponins, and tannins.[19]

DPPH method antioxidant test

Test for antioxidants: the DPPH method refers to the method used by Irawan.[20] An amount of 5 mg of stem bark extract was dissolved with methanol pa in a 5 mL measuring flask, resulting in a sample solution with a concentration of 1000 mg/L. DPPH 39 mg/L solution was added to five 5 mL measuring flasks, then each was put into five 5 mL measuring flasks, then each was added 2 mL of DPPH 39 mg/L solution, then measured with methanol pa, and homogenized (sample concentrations of 20, 40, 80, 160 mg/L). The solution was incubated for 30 minutes at room temperature (25°C), then the absorbance of the solution was measured using a visible light spectrophotometer at a wavelength of 515 nm. The work is carried out in five repetitions. The same work was carried out to make a comparison solution of BHT with concentrations of 4, 32 and 64 mg/L.

Antioxidant activity is expressed as percent inhibition (% inhibition) with the following equation:

$$\%\ Inhibition = \frac{(A_{blank} - A_{sample})}{A_{blank}} x100\%$$

Details:

A_{blank} = Absorbance without sample

A_{sample} = Absorbance of the sample

The % inhibition value associated with the concentration in ppm (mg/L) will produce a linear equation (Y = bX + a). The IC_{50} value was obtained from the calculation when the % inhibition was 50%.

Uric acid test

The uric acid test procedure refers to the method that has been used by Irawan.[20] A total of 5 mg of extract was dissolved with methanol pa in a 5 mL measuring flask, resulting in a sample solution with a concentration of 1000 mg/L. The solution was piped at 40 μL, then placed in a 5 ml measuring flask with 40 μL of uric acid standard (6 mg/dL) and allowed to stand for 5 minutes. The solution was treated with 0.25 mL of 1 TBHBA uric acid reagent for 5 minutes before being treated with 62.5 mL of 1 TBHBA uric acid reagent for 30 minutes at 20-25°C. The absorbance of the solution was measured using a visible spectrophotometer at 513 nm of absorption. The same treatment was carried out on uric acid standards, allupurinol as a positive control, and blanks. The absorbance was recorded, and then the uric acid level in the sample was calculated.

RESULT AND DISCUSSION

Ultrasonic-Assisted Extraction

Fruit peels were extracted using the UAE method with ethanol 70% as a solvent. In this extraction stage, variations are made on the amplitude parameters used in the ultrasonic probe and the extraction time. The amplitude variations used are 60% and 65%, while the time variations used are 30, 35 and 45 minutes. The yield of fruit peel extraction can be seen in Table 1.

Table 1 shows that the A-C samples produced yields of relatively the same amount, which was around 21%. At the time of extraction, and the amplitude used was 45 minutes to 65%, the yield decreased to 18.47%. Research that has been done shows that increasing the amplitude can increase the extraction yield. Increasing the amplitude can increase the penetration of solvent into the cell, so that the cell wall breaks more easily. As a result, higher yields are obtained. The higher the amplitude used, the greater the energy used and the greater the heat generated. As a result, it can cause a decrease in yield and damage to the metabolite compounds contained in simplicial.[21]

Phytochemical screening

The results of the phytochemical screening test for ethanolic fruit peel extracts with several variations in time and amplitude can be seen in Table 2. Based on the results of phytochemical screening, the compounds contained in the ethanolic extract of *Phaleria macrocarpa* fruit peel include saponins, phenols, tannins, and alkaloids.

Table 1: **Yield (%) of crude extract from UAE process.**

Sample	The Various Parameter of Time (Minutes)-Amplitude (%)	Sample Weight (g)	Crude Extract Weight (g)	Yield (%)
A	30-60	7.2582	1.5848	21.83
B	35-65	7.2575	1.5449	21.29
C	45-60	7.2581	1.5485	21,33
D	45-65	7.1535	1.3212	18.47

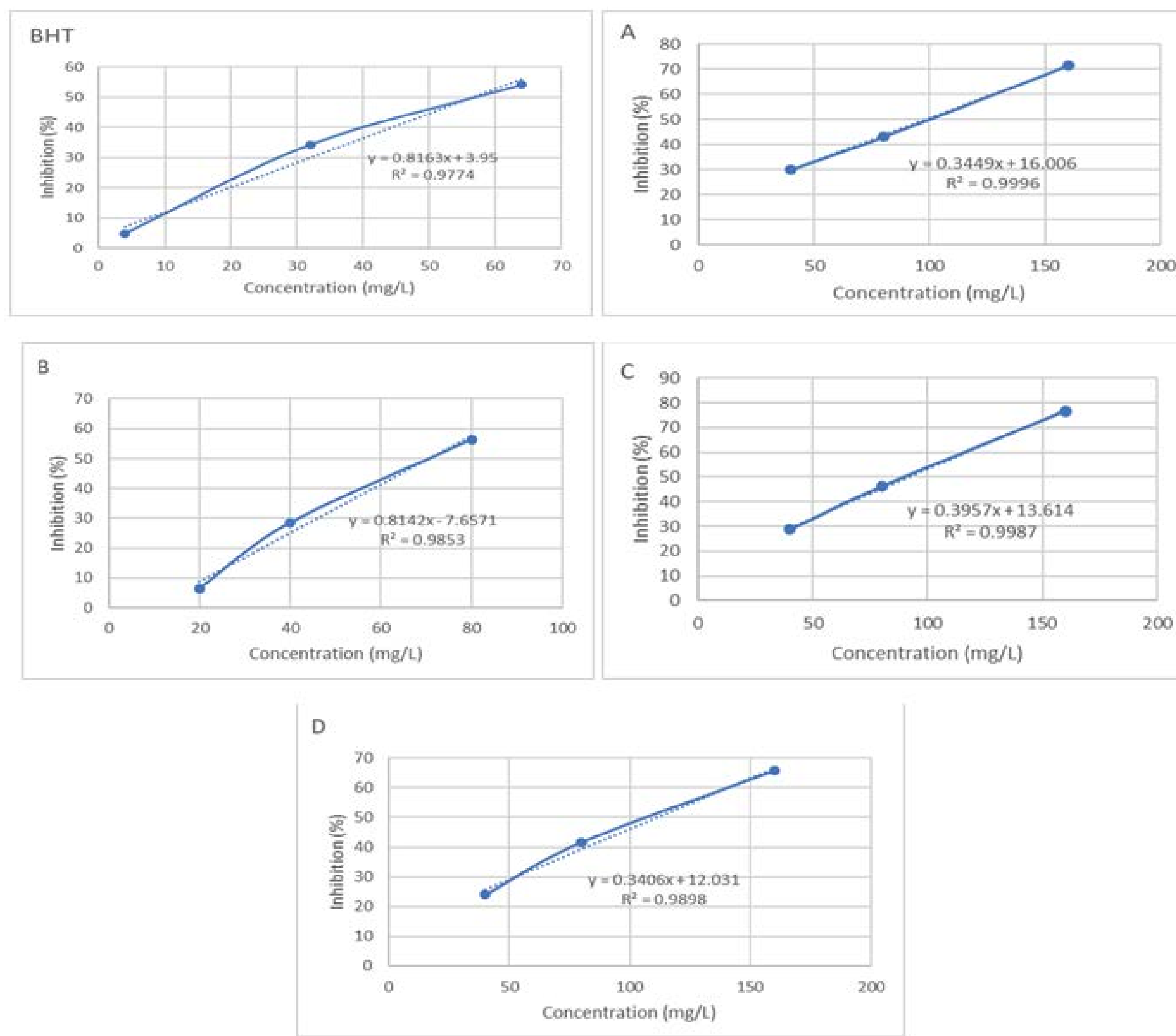

Figure 2: DPPH radical scavenging activities (%) of BHT, (A) Ethanolic extracts of *Phaleria macrocarpa* fruit peel in 30 minutes extraction time-60% of amplitude, (B) with 35 minutes extraction time-65% of amplitude, (C) with 45 minutes extraction time-60% of amplitude and (D) with 45 minutes extraction time-65% of amplitude.

Table 2: Phytochemical screening results of ethanolic extract of *Phaleria macrocarpa* fruit peels.

No	Phytochemical Groups	Various Parameter			
		Time (Minutes) – Amplitude (%)			
		30 – 60 A	35 – 65 B	45 – 60 C	45 – 65 D
1	Saponins	+++	+++	++	+++
2	Phenols	++++	++++	++++	++++
3	Tannin	++++	++++	++++	+++
4	Flavonoids	-	-	-	-
5	Alkaloids				
	• Dragendrof's	++	++	++	++
	• Mayer	++	++	++	++

Description: ++++ = Very Strong Reaction, +++ = Strong Reaction

++ = Medium Reaction, + =Weak Reaction, - = No Reaction

Saponins have been reported to have potential as natural antioxidants.[22] Several biological effects of phenolic compounds have been studied, including antioxidant, anti-carcinogenic, alpha-glucosidase activity inhibitor, anti-inflammatory, and free radical scavenging.[23-25] Tannins have been reported to have high antioxidant, immunomodulatory, and antibacterial activity.[26-28] Alkaloids have been reported to have various pharmacological activities, including anticancer, antihyperglycemic, and antibacterial antioxidant

activities, so they are widely used as natural medicines to treat various diseases.[28-30]

Antioxidant activity of the DPPH method

The concentrations of *Phaleria macrocarpa* fruit peel extract evaluated were in the range of 20 to 160 mg/L. DPPH is a free radical that is stable at room temperature and in methanol and produces a purple solution. When free radicals react with antioxidants, their free radical properties are lost because the chain is broken and the color changes from purple to light yellow.[31] The color change of the solution is caused by the presence of a component that donates a hydrogen atom. Antioxidants reduce DPPH radicals to a more stable form, namely DPPH-H. The reaction between DPPH and antioxidants will produce reduced forms of DPPH and antioxidant radicals.[32,33] Changes in the color of the solution affect the absorbance of DPPH. The higher the concentration of antioxidant components in the solution, the lower the absorbance of DPPH.[34] The absorbance of the sample solution was measured by visible light spectrophotometry at a wavelength of about 520 nm.[33,35]

The antioxidant activity test results with DPPH are expressed as % inhibition (Table 3), which is then linked to a series of sample or standard concentrations to produce a curve, as shown in Figure 2. The regression equation of BHT and ethanol extract of *Phaleria macrocarpa* fruit peel with treatments A, B, C, and D obtained were $y = 0.8163x + 3.95$; $y = 0.3449 + 16.006$; $y = 0.8142 + 7.6571$; $y = 0.3957 + 13.614$; and $y = 0.3406x + 12.031$, respectively. From this equation, the IC_{50} values for BHT, treatment extracts A, B, C, and D were 56.41 ± 0.09, 98.82 ± 0.1, 52.01 ± 0.06, 92.07 ± 0.1, and 111.82 ± 0.2 mg/L, respectively. In general, BHT has better DPPH inhibitory activity than the ethanol extract of *Phaleria macrocarpa* fruit peel. The antioxidant activity of *Phaleria macrocarpa* fruit peel extract with treatments A, B and C was included in the strong category because the IC_{50} value was in the range of 50-100 mg/L.[36]

The results showed that the extract of *Phaleria macrocarpa* fruit peel with an extraction treatment of 35 minutes to 65% amplitude had higher antioxidant activity than the other three extracts. The high antioxidant activity is correlated with the results of phytochemical screening. Alkaloid, phenolic, and tannin compounds are present in the extract. Tannins and other phenolic compounds have OH-groups, while alkaloids have NH-groups whose hydrogen atoms can be donated to free radicals so that they become non-radical compounds. These results indicate that the ethanolic extract of *Phaleria macrocarpa* fruit peel has the potential as an alternative source of natural antioxidants.

Anti-uric acid potential activity test

To determine the ability of *Phaleria macrocarpa* fruit peel extract to reduce uric acid, an anti-uric acid activity was tested using the in vitro method with TBHBA reagent and pure uric acid as a standard. The principle of the in vitro anti-uric acid test are the oxidation of uric acid into allantoin and peroxide compounds by the uricase enzyme. Then the peroxidase compound produced reacts with 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) to produce a quinonemin compound which can be measured by a visible light spectrophotometer at a wavelength of 546 nm.[37] The reduction of uric acid (%) after being incubated with allopurinol and various *Phaleria macrocarpa* fruit peel extracts is shown in Table 4.

Table 3: Antioxidant activity test results of the DPPH method.

Sample	Concentration (mg/L)	Mean % Inhibition ± SD	IC_{50} ± SD
	4	4,9232 ± 0,06	
BHT	32	34,3681 ± 0,02	56.41 ± 0,09
	64	54,1862 ± 0,09	
	40	30,1126 ± 0,08	
A	80	43,1285 ± 0,07	98.56 ± 0,1
	160	71,3415 ± 0,08	
	20	6,3321 ± 0,05	
B	40	28,3537 ± 0,03	52.01 ± 0.06
	80	56,3321 ± 0,06	
	40	28,7758 ± 0,08	
C	80	46,2711 ± 0,1	92.07 ± 0.1
	160	76,5947 ± 0,04	
	40	24,0619 ± 0,07	
D	80	41,6745 ± 0,05	111.82 ± 0.2
	160	65,7364 ± 0,07	

Table 4: The reduction of uric acid (%) after incubation with allopurinol and various of *Phaleria macrocarpa* fruit peel extracts.

Sample	Absorbance	Concentration of Uric Acid (mg/L)		Reducing Uric Acid Level (%)
Uric Acid Standard (0.5 mg/L)	0.0602	0.5000		
		React with sample	The rest of reaction	
Allopurinol (0.5 mg/L)	0.0584	0.0146 ± 0.0004	0.4854 ± 0.0004	2.92 ± 0.09
A (30 minutes - 60%)	0.0070	0.4420 ± 0.0007	0.0580 ± 0.0007	88.4 ± 0.1
B (35 minutes - 65%)	0.0057	0.4524 ± 0.0004	0.0475 ± 0.0004	90.49 ± 0.08
C (45 minutes - 60%)	0.0056	0.4538 ± 0.0008	0.0462 ± 0.0008	90.76 ± 0.2
D (45 minutes - 65%)	0.0064	0.4466 ± 0.001	0.0534 ± 0.001	89.33 ± 0.2

Based on the results of the study, various extracts of the *Phaleria macrocarpa* fruit peel have activity that has the potential to reduce uric acid better than allopurinol. The highest reducing uric acid level (%) value resulted in extract C of 90.76 ± 0.2 followed by extract B of 90.49 ± 0.08, but the extraction time for extract C was 15 minutes longer than for extract B causing, energy consumption to be greater. In addition, the antioxidant activity test in condition B (IC_{50} 52.01 ± 0.06) was higher than in condition C (IC_{50} 92.07 ± 0.1). Therefore, the optimum condition chosen for this extraction is an extraction time of 35 minutes with an amplitude of 65% (B).

Guava juice has been reported to have high antioxidant activity. These antioxidants can scavenge free radicals and inhibit the formation of xanthine oxidase, which consequently can prevent an increase in uric acid levels in blood creatine.[38] Polyphenols and flavonoids lower uric acid levels by acting as antioxidants and inhibiting free radicals, inhibiting several enzymes such as xanthine oxidase, cyclooxygenase, and lipoxygenase.[39] Polyphenols are substrates for xanthine oxidases. The presence of polyphenols causes a decrease in uric acid production because xanthine oxidase tends to oxidize polyphenols rather than xanthine.[40]

CONCLUSION

Based on the results of the study, it can be concluded that the phytochemical test results of *Phaleria macrocarpa* fruit peel extract using UAE contain phenolic compounds, tannins, saponins and alkaloids. The antioxidant activity of the DPPH method is strong and has a high potential for reducing uric acid. The optimum extraction of extract B was treated for 35 minutes and had an amplitude of 65% with an antioxidant activity IC_{50} of 52.01 ± 0.06 mg/L and a reduction of uric acid level of 90.49 ± 0.08 %.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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GRAPHICAL ABSTRACT

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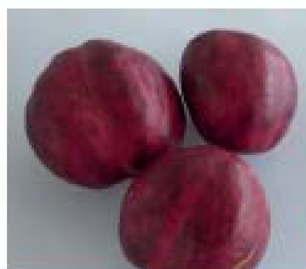
Graphical Abstract



***Phaleria macrocarpa* (Scheff.) Boerl.**

Simplicia Set Up and Extraction with UAE Methods using parameters variation of time and amplitude

Sample	The Various Parameter of Time (Minutes)-Amplitude (%)	Sample Weight (g)	Crude Extract Weight (g)	Yield (%)
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Phytochemical Screening

No	Phytochemical Groups	Various Parameter: Time (Minutes) – Amplitude (%) 30 – 60 A	35 – 65 B	45 – 60 C	45 – 65 D
1	Saponin	+++	+++	++	+++
2	Phenols	++++	++++	++++	++++
3	Tannin	++++	++++	++++	+++
4	Flavonoids	-	-	-	-
5	Alkaloids				
	• Dragendorff's	++	++	++	++
	• Mayer	++	++	++	++

Antioxidant Test with DPPH Methods

Sample	Concentration (mg/L)	Mean % Inhibition ± SD	IC_{50} ± SD
BHT	4	4.9252 ± 0.06	56.41 ± 0.09
	32	34.5681 ± 0.02	
	64	54.1882 ± 0.09	
A	40	38.1126 ± 0.08	98.56 ± 0.1
	80	43.1281 ± 0.07	
	160	71.3415 ± 0.09	
B	20	6.5121 ± 0.05	52.01 ± 0.06
	40	28.5937 ± 0.03	
	80	56.3821 ± 0.06	
C	40	28.7758 ± 0.08	92.07 ± 0.1
	80	46.2711 ± 0.1	
	160	78.5947 ± 0.04	
D	40	24.0019 ± 0.07	111.82 ± 0.2
	80	41.6745 ± 0.05	
	160	65.7844 ± 0.07	

Anti-Uric Acid Potential Activity Test

Sample	Absorbance	Concentration of Uric Acid (mg/L): React with sample	The rest of reaction	Reducing Uric Acid Level (%)
Uric Acid Standard (0.5 mg/L)	0.0602	0.5000		
Allopurinol (0.5 mg/L)	0.0504	0.0146 ± 0.0004	0.4854 ± 0.0004	2.92 ± 0.08
A (30 minutes – 60%)	0.0079	0.4420 ± 0.0007	0.0580 ± 0.0007	88.4 ± 0.1
B (35 minutes – 65%)	0.0057	0.4524 ± 0.0004	0.0475 ± 0.0004	90.49 ± 0.08
C (45 minutes – 60%)	0.0056	0.4538 ± 0.0008	0.0462 ± 0.0008	90.76 ± 0.2
D (45 minutes – 65%)	0.0064	0.4466 ± 0.001	0.0534 ± 0.001	89.33 ± 0.2

The antioxidant activity of the DPPH method is strong and has an high potential for reducing uric acid. The optimum extraction of extract B was treated for 35 minutes and had an amplitude of 65% with an antioxidant activity IC_{50} of 52.01 ± 0.06 mg/L and a reduction of uric acid level of 90.49 ± 0.08 %.

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