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Research Article

# Application of Ultrasound-Assisted Extraction on the Stem Bark of *Rhinachantus Nasutus* (L.) Kurz, Total Phenolic, and Its Potential as Antioxidant and Inhibitor of Alpha-Glucosidase Enzyme Activity

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## ABSTRACT

**Aims:** This study aims to obtain a stem bark extract of *Rhinachantus nasutus* (L.) Kurz through the application of ultrasound-assisted extraction (UAE) and reveal: the total phenolic content in the extract; The extract's potential as an antioxidant with copper-reducing strength parameters, and its potential as an antidiabetic by inhibiting alpha-glucosidase activity. **Results:** The crude ethanol extract of *R. nasutus* stem bark obtained from the UAE process was 7.4896 g with a yield of 4.99%. The high total phenolic content, namely 677.3343±0.0007 mg GAE / g sample, the antioxidant activity test using the CUPRAC method gave an $IC_{50}$ value of 18.43±0.20 mg / L. In addition, the ethanol extract of stem bark has a high ability to inhibit the activity of the alpha-glucosidase enzyme with an $IC_{50}$ value of 10.95±0.28 mg / L. **Conclusion:** The ethanol extract of the stem bark of *R. nasutus* from UAE has the potential as a source of antioxidants and antidiabetic.

**Key words:** Alpha-glucosidase enzyme, Antidiabetic, Antioxidant, *Rhinachantus nasutus* (L.) Kurz, Total phenolics content, Ultrasound-assisted extraction.

## INTRODUCTION

Medicinal plants play a significant role in human health. This is related to the content of chemical compounds in medicinal plants, which can produce physiological effects on the human body. Today, traditional medicine derived from various medicinal plants is widely studied and used as the basis for finding new compounds to treat multiple diseases. Important bioactive compounds in plants are phenolic compounds, alkaloids, tannins, and flavonoids [1].

The World Health Organization (WHO) reports that the number of people with diabetes mellitus increases every year. In 2014, the percentage of people with diabetes mellitus in adults worldwide was 8.5% or 422 million sufferers[2], and it is predicted that it will be 700 million by 2045[3]. Diabetes is a metabolic disease characterized by an uncontrolled increase in glucose levels in the blood (hyperglechemicals). This occurs due to impaired insulin secretion, insulin action, or both. Other factors are genetics, age, and obesity. The chronic hyperglycemic state of diabetes is associated with long-term damage, impaired function, and various organs' failure, especially the eye; kidney; nerve; heart; and blood vessels[4,5].

Several alpha-glucosidase inhibitors, such as acarbose, have been used clinically to treat diabetes[6]. This medicine may cause side effects such as flatulence, stomach cramps, vomiting, and diarrhoea. Therefore, it is necessary to find alternative drugs that can inhibit alpha-glucosidase activity without side effects[7]. Various studies have been conducted to identify natural sources that can inhibit alpha-glucosidase activity. Natural ingredients such as cereals, Pometia pinnata, strawberries, blueberries, broccoli sprouts, and protein in egg whites have been reported to exhibit alpha-glucosidase inhibitory activity[8,9,10].

*Rhinacanthus nasutus* (L.) Kurz is a flowering plant in the acanthaceae family spread across Southeast Asia, India and China[11,12]. *R. nasutus* is known by various names, such as Snake Jasmine, Rangchita Dainty, Spurs, Palakjuhi, Juhipani, Gajkarni, Uragamalli, Nagamalli, Nagamulla, Puzhukkolli, Nagamalle, Nagamallige, Doddapatike, Juipana, Dadmari, Palakjuhi, and Yudhikaparni[12]. Another name is Bai He Ling Zhi in China[13], Thong-pan-chung in Thailand[14], while in Indonesia it is known as "Manukan" and Cengkerang is used by the people of Menggala, Kabupaten Tulang Bawang, Lampung. Roots, stems, and leaves of *R. nasutus* are used in traditional medicine such as diabetes eczema, eczema, scabies, leprosy, herpes, pulmonary tuberculosis, hepatitis, hypertension, and obesity[15]. *R. nasutus* leaf extract has been reported to have more significant alpha-glucosidase inhibition activity than acarbose[16]. It has never been previously reported regarding alpha-glucosidase inhibiting activity from the bark of *R. nasutus*.

A crucial first step in the study of medicinal plants is selecting an extraction method to obtain plant extracts. One of the most widely used extraction methods is maceration. This method's weakness is that it requires a long extraction time and the use of large amounts of solvent[17]. Maceration using hexane, ethyl acetate, methanol, and distilled water was

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carried out on the dried powder of the leaves of *R. nasutus*[18]. Various extraction techniques have been developed to obtain methods that are more environmentally friendly, reduce solvent use, prevent possible degradation of compounds due to heat use, shorten extraction times, increase reaction rates, and increase the yield and quality of extracts[19]. One of them is the sonification method using ultrasonic[20,21].

The use of ultrasonic waves passed in the solvent will cause a cavitation effect which gives a mechanical effect, thus allowing greater penetration of the solvent into the sample matrix, increasing the contact surface area between the solid and liquid phases. As a result, the solute quickly diffuses from the solid phase to the solvent[21]. In this method, no chemicals can prevent the possibility of chemical degradation of the targeted compounds[19]. The use of the UAE extraction method on the bark of *R. nasutus* has not been previously reported.

# 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. The stem bark samples of *R. nasutus* (Figure 1) were obtained from the researchers' private plants planted in Bogor. The bark that is sampled is one year old. The stems were washed with water, and then the bark was collected and dried in the open air without sunlight for two weeks. Simplicia was weighed and mashed using a blender and sieved with a 40-mesh sieve. Simplicia powder is stored separately in dry, closed, identified containers and protected from direct sunlight until extraction is carried out.

## Extraction of Simplicia

The simplicia powder of the stem bark of *R. nasutus* was weighed as much as 150 g, then 500 mL of 70% ethanol solvent was added. The mixture was sonicated using a vibrating ultrasonic probe for 30 minutes at room temperature with an amplitude of 0.6 m. The extract without the solvent was weighed, and the percent yield was calculated.

## Total Phenolic

The total phenolic content of the extract was determined by the Folin – Ciocalteu method[22]. Briefly 400 µL of crude extract (1 mg / mL) that were made up to 6 mL with distilled water, mixed thoroughly with 1 mL of Folin – Ciocalteu reagent for 3 min, followed by the addition of 2,5 mL of 10% (w / v) sodium carbonate, measured with distilled water in a 10 mL measuring flask, then homogenized. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from gallic acid calibration curves (concentrations 0, 4, 6, 8, and 10 mg / L). The results were expressed as mg of gallic acid equivalent per g dry weight.

**Figure 1:** The Stem Bark Samples of R. nasutus.

## Antioxidant Activity Test of the Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Method

A total 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 1,000 mg / L. Solution pipette 20; 40; 60; 80; and 100 µL, then each was put into five 5 mL measuring flasks, then added 1 mL of CUPRAC solution, then measured with methanol pa, and homogenized (sample concentrations 4, 8, 12, 16; and 20 mg / L). The solution was incubated for 30 minutes at 37°C, the absorption of the solution was measured using a visible light spectrophotometer at a wavelength of 459 nm[23]. The work is carried out in three repetitions. The same work is done for the BHT comparators by pipetting 1.25; 2.5; and 3.75 µL of BHT solution of 1,000 mg / L (BHT concentrations of 0.25; 0.5; and 0.75 mg / L). Reducing activity can be calculated with the following equation:

$$\%.\ Reduction\ Activity = \frac{(A_{sample} - A_{blank})}{A_{sample}} x\ 100\%$$

Details :

$A_{blank}$ = Absorbance without sample

$A_{Sample}$ = Absorbance of sample

The calculated value is entered into a linear equation (Y = bX + a) with the ppm concentration (mg / L) as the abscissa (X-axis) and the % value of the reduction as the ordinate (Y-axis). The $IC_{50}$ value is obtained from the calculation when the % reduction is 50%.

$$IC50 = \frac{50 - a}{b}$$

## Alpha-Glucosidase Inhibitor Activity Test

The alpha-glucosidase inhibition test was carried out on a blank solution (test solution without sample/standard), acarbose solution as a comparison standard (positive control), stem bark extract as a sample. The comparison standard and the stem bark ethanol extract sample were weighed and dissolved with a phosphate buffer pH 6.8. Samples that were not dissolved with phosphate buffer were first dissolved with DMSO at a maximum of 10%. The standard solution and sample were diluted into several concentrations. A total of 30 µL of standard solution and 17 µL of samples were added to the para-Nitrofenil-α-D-glucopyranoside (PNPG) substrate. The solution was incubated for 5 minutes at 37°C, and 17 µL of the alpha-glucosidase solution was added. The solution was incubated again at 37°C for 15 minutes, then added 100 µL of 200 mM sodium carbonate. The absorbance of the solution was measured with a microplate reader at λ 405 nm[24]. The same procedure was carried out for the control test, but with a difference after the first incubation, 100 µL of 200 mM sodium carbonate was added first, and 17 µL of the alpha-glucosidase solution was added after the second incubation. The absorbance of the solution was measured with a microplate reader at λ 405 nm.

# RESULT AND DISCUSSION

## Ultrasonic-Assisted Extraction

The extraction of *R. nasutus* stem bark was carried out by sonification method using an ultrasonic probe with ethanol solvent at 25°C and an amplitude of 0.6 m for 30 minutes. The irradiation cross-sectional area depends on the vibrating horn dye's depth and can be used to adjust

the irradiation intensity. The ultrasonic configuration of this vibrating horn system can be used for the need to damage plant cell tissue, thereby increasing the ability of solvents to penetrate cells and produce higher extracts and an efficient process[25,26]. Table 1 shows the crude ethanol extract of *R. nasutus* skin of 7.4896 g with a yield of 4.99%.

## Phenolic Content of Ethanol Extract

Determination of total phenol levels in the stem bark of *R. nasutus* in this study using the Folin - Ciocalteu method. The Folin-Ciocalteu reagent contains phosphomolybdic acid and phosphotungstic acid, which phenolic compounds will reduce to form a bluish-purple complex molybdenum-tungsten combination[27,28]. Gallic acid is used as a standard because it is stable and is a derivative of hydroxybenzoic acid[29]. The standard curve of gallic acid can be seen in Figure 2 with the linear regression equation y = 0.0996x + 0.0455 and an R2 value of 0.9829. The total phenol content in the stem bark of *R. nasutus* obtained from this equation was 677.3343±0.0007 mg GAE / g of the sample.

The total phenol in plants usually correlates with antioxidant activity. The higher the phenol content, the higher the antioxidant activity[30]. Phenolic compounds can donate protons to inhibit free radicals so that the radicals will become stable. This stable radical is formed due to resonance in the aromatic ring, which causes the delocalization of electrons[31,32].

## Antioxidant Activity of the CUPRAC Method

The CUPRAC method is a method for determining antioxidant activity based on single electron transfer (SET)[33]. The reagent Cu (II) -neocuproin (Cu (II) - $(Nc)_2$) is used as an oxidizing agent. This method's principle is the reduction of Cu $(Nc)_2^{2+}$ reagents by antioxidants to form Cu $(Nc)^{2+}$. The observation is that the colour changes from blue to yellow. The absorbance can be read at 450 nm. The absorbance read at a specific wavelength correlates with the concentration of antioxidants in the sample[23].

**Table 1: Weight of Ethanol Extract and Yield Resulting from the UAE Process.**

| Sample | Weight (g) | Yield (%) |
|---|---|---|
| **Dry sample** | 150.000 | 4.99 |
| **Ethanolic Extract** | 7.4896 | |

**Table 2: Antioxidant Activity Test Results of the CUPRAC Method.**

| Sample | Concentration (mg/L) | % Reduction Power | $IC_{50}$ (mg/L) |
|---|---|---|---|
| BHT | 0.25 | 15.71±0.46 | 0.73±0.02 |
| | 0.5 | 33.53±0.28 | |
| | 0.75 | 51.00±0.77 | |
| Ethanolic Extract | 4 | 25.17±1.44 | 18.43±0.20 |
| | 8 | 35.10±0.27 | |
| | 12 | 41.49±0.44 | |
| | 16 | 45.27±0.38 | |
| | 20 | 51.86±0.15 | |

**Table 3: Results of Alpha-Glucosidase Enzyme Activity Inhibition Test.**

| Sample | Concentration (mg/L) | % Inhibition | $IC_{50}$ (mg/L) |
|---|---|---|---|
| Acarbose | 30 | 35.31±0.07 | 98.67±0.13 |
| | 60 | 41.50±0.27 | |
| | 90 | 47.23±0.15 | |
| | 120 | 56.53±0.15 | |
| | 150 | 60.11±0.15 | |
| Ethanolic Extract | 10 | 48.22±0.39 | 10.95±0.28 |
| | 25 | 59.89±,1.14 | |
| | 50 | 70.65±0.1 | |
| | 100 | 74.95±0.03 | |

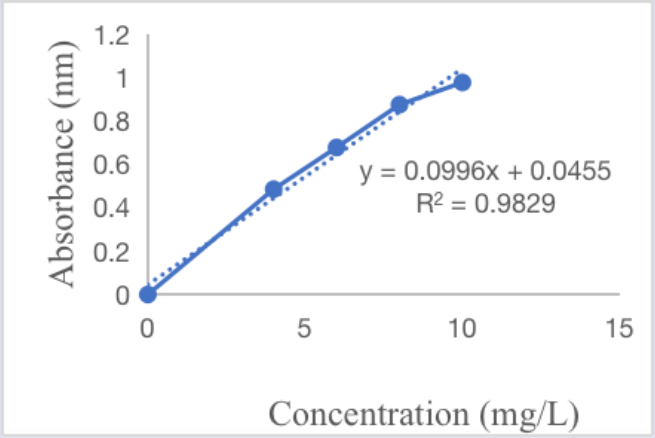


**Figure 2:** Curve for Gallic Acid Used as a Standard.

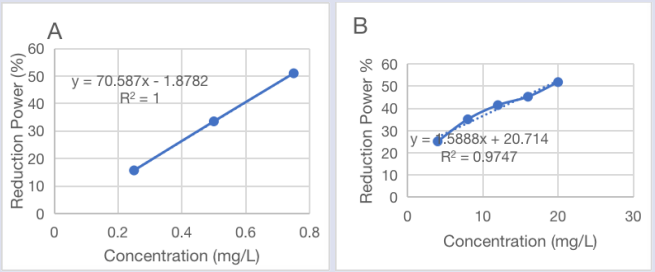


**Figure 3:** Cupric Reducing Ability of (A) BHT and (B) R. nasutus Stem Bark Ethanol Extract.

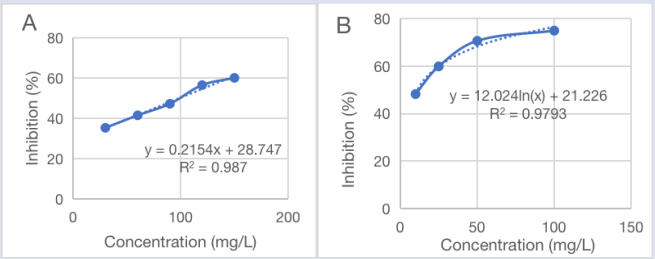


**Figure 4:** Graph of the Relationship between Concentration and % Inhibition for IC50 Determination. (a) Acarbose and (b) *R. nasutus* Stem Bark Ethanol Extract.

The CUPRAC test results are expressed as % reduction power (Table 2), which is then linked to a series of sample or standard concentrations to produce a curve, as shown in Figure 3. The regression equation for BHT and ethanol extract of *R. nasutus* stem bark obtained was y = 70,587 x - 1.8782 and y = 1.5888 + 20.714. From this equation, the $IC_{50}$ value for BHT was 0.73±0.02 mg / L and for the ethanol extract of *R. nasutus* stem bark was 18.43±0.20 mg / L. This $IC_{50}$ value describes each sample's antioxidant capacity at the time of the reducing % value of 50. In general, BHT has a better reducing ability to $Cu^{2+}$ than the ethanol extract of *R. nasutus* stem bark, but the reduction power of ethanol extract is included. In the very strong category because the $IC_{50}$ value is less than 50 mg / L[34].

The results showed that the ethanol extract of the stem bark of *R. nasutus* has the potential as an alternative source of natural antioxidants. The

reaction mechanism that occurs in the CUPRAC method involves oxidation and reduction reactions. This mechanism correlates with the redox properties of antioxidant compounds in plants. This property plays an important role in trapping and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[35,36].

### Potential Inhibition of Alpha-Glucosidase Enzyme Activity in Ethanol Extract

Based on the alpha-glucosidase enzyme activity inhibition test, acarbose solutions at concentrations of 30, 60, 90, 120, and 150 mg / L had % inhibition of 35.31±0.07, 41.50±0.27, 47.23±0.15, 56.53±0.15, and 60.11±0.15, respectively. The ethanol extract of *R. nasutus* stem bark at concentrations of 10, 25, 50, and 100 mg / L had % inhibition of 48.22±0.39, 59.89±1.14, 70.65±0.1, and 74.95±0.03, respectively (Table 3). The relationship between the concentration and % inhibition of acarbose and ethanol extract is made in graphical form (Figure 4), so that the regression equations are y = 0.2154x + 28.747 and y = 12.024lnx + 21.226, respectively. From this equation, the $IC_{50}$ value for acarbose was 98.67±0.13 mg / L and for the ethanol extract of *R. nasutus* stem bark was 10.95±0.28 mg / L.

Based on these data, the ethanol extract of *R. nasutus* stem bark has higher alpha-glucosidase enzyme inhibiting activity than acarbose standard. The total phenolic data results, phytochemical screening, antioxidant activity tests, and alpha-glucosidase inhibitory activity tests were related to one another. The antioxidant and antidiabetic properties of the stem bark ethanol extract are related to the presence of phenolic groups that can donate hydrogen atoms to free radicals to become less reactive. Phenolic compounds can also act as competitive inhibitors of carbohydrate digesting enzymes (alpha-glucosidase enzymes) through hydrophobic interactions. These carbohydrates are not quickly hydrolyzed into glucose molecules[10].

## CONCLUSION

The crude ethanol extract of *R. nasutus* stem bark obtained from the UAE process was 7.4896 g with a yield of 4.99%. The bark extract's total phenolic content was 677.3343±0.0007 mg GAE / g sample, the antioxidant activity test using the CUPRAC method gave an $IC_{50}$ value of 18.43±0.20 mg / L. The presence of phenolic compounds is thought to have a role in high antioxidant activity. Also, the ethanol extract of stem bark has an increased ability to inhibit the alpha-glucosidase enzyme activity with an $IC_{50}$ value of 10.95±0.28 mg / L. It can be concluded that the ethanol extract of the stem bark of *R. nasutus* from the UAE has the potential as a source of antioxidants and antidiabetic.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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## GRAPHICAL ABSTRACT

The Stem Bark Samples of *R. nasutus*

Simplicia Set up and Extraction with UAE Methods using Ethanolic Solvent

The crude ethanol extract of *R. nasutus* stem bark of 7.4896 g with a yield of 4.99%.

Total Phenolic Content

Antioxidant Test with CUPRAC Method

Alpha-Glucosidase Inhibitor Activity Test

The $IC_{50}$ value for BHT was 0.73±0.02 mg / L and for the ethanol extract of *R. nasutus* stem bark was 18.43±0.20 mg / L.

The total phenol content in the stem bark of *R. nasutus* obtained from this equation was 677.3343±0.0007 mg GAE / g of the sample.

The $IC_{50}$ value for BHT was 0.73±0.02 mg / L and for the ethanol extract of *R. nasutus* stem bark was 18.43±0.20 mg / L.

**Conclusion:** The ethanol extract of the stem bark of *R. nasutus* from the UAE has the potential as a source of antioxidants and inhibitor of alpha-glucosidase enzyme activity.

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