Potential of *Rhinachanthus nasutus* (L.) Kurz Leaves Extract as an Antioxidant and Inhibitor of α-Glucosidase Activity

Candra Irawan¹,², Berna Elya¹*, Muhammad Hanafi³, Fadlina Chany Saputri¹

ABSTRACT

**Aims:** The goal of this study is to learn more about the antioxidant and antidiabetic properties of *Rhinachanthus nasutus* (L.) Kurz (RnLK) leaf extract. The Ultrasound-Assisted Extraction (UAE) technique was used to extract the leaf material, and the solvent used was ethanol with a 70% concentration. The total phenol content (TPC) of the extracted material was determined. The Cupric Ion Reducing Antioxidant Capacity (CUPRAC) method was used to examine antioxidant activity, whereas α-glucosidase activity was used to test antidiabetic action. **Results:** The ethanol extract of RnLK leaves yielded 8.36%, with a TPC of 607.1±0.2 mg GAE/g sample. The IC₅₀ value for leaf extract antioxidant activity was 19.1±0.1 mg/L. Furthermore, the leaf extract inhibits α-glucosidase activity and has an IC₅₀ value of 81.3±3 mg/L, making it an antidiabetic. **Conclusion:** The ethanolic extract of RnLK leaves can be used as an alternative antioxidant and antidiabetic material, according to the findings of this study.

**Key words:** RnLK, UAE, CUPRAC method, Anti-diabetic, α-glucosidase activity.

INTRODUCTION

Antioxidants play an essential role in life because they can neutralize or destroy free radicals such as reactive oxygen species (ROS) before damaging cells. Oxidation by ROS causes cell membranes disintegration, membrane proteins damage, and DNA mutations. Oxidative stress may result if this condition lasts, which occurs when the body's ability to neutralize free radicals outnumbers its ability to eliminate them. This oxidative stress can cause various degenerative diseases such as cancer, inflammation, arthritis, atherosclerosis, Alzheimer's disease, Parkinson’s disease, neurodegeneratives, and diabetes mellitus. Diabetes mellitus (DM) is a long-term metabolic disease marked by high blood glucose levels (hyperglycemia). DM is caused by damage to the pancreas, resulting in decreased insulin secretion, insulin action, or both. As a result, blood glucose concentrations increase, glucose utilization by cells decreases, and fat and protein utilization also increase. By 2030, it is predicted that people living with diabetes in the world will reach 578 million.

One of the pharmacological therapies of DM is oral hypoglycemic drugs such as α-glucosidase inhibitors. Currently, the drugs that are widely used for the treatment of diabetes are acarbose, miglitol, voglibose, and emiglitate. α-glucosidase inhibitory activity has been discovered in natural fertilizers like cereals, blueberries, strawberries, broccoli sprouts, sardines, and egg white. People in Southeast Asia, India, and China have long utilized *R. nasutus* (L.) Kurz as a traditional medicine. Diabetes, eczema, leprosy, pulmonary TB, scabies, herpes, hepatitis, hypertension, and obesity are all treated with the roots, stems, and leaves of RnLK. The activity of RnLK stem bark extract in inhibiting α-glucosidase has been reported to be even higher than that of the acarbose standard.

The dry powder of RnLK leaves has been extracted by maceration, but this method has disadvantages as the volume of solvent used is relatively large and the extraction time is long. Prospective extraction techniques have been intensively introduced to overcome this limitation, one of which is ultrasonic extraction. It's the first time the ultrasound-assisted extraction (UAE) approach has been used on RnLK leaves.

METHODS

Simplicia setup

Plant identification has been reported in previous publications. Leaf samples of RnLK can be seen in Figure 1. The leaf samples used were six months old. The sources of sampling locations and preparation of leaves for simplicia are similar to previous reports.

Simplicia’s extraction

Extraction of leaf simplicia of RnLK referred to the previously reported procedure. The difference is

---

**Correspondence**

Berna Elya

Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy Universitas Indonesia, Depok, West Java, INDONESIA.

E-mail: bernaelya@farmasi.ui.ac.id

**History**

- Submission Date: 12-04-2022;
- Review completed: 29-05-2022;
- Accepted Date: 13-06-2022.

**DOI**: 10.5530/pj.2022.14.110

**Article Available online**

http://www.phcogj.com/v14/i3

**Copyright**

© 2022 Phcogj.Com. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International license.
that in the study, the leaf sample used was 50 g and the volume of 70% ethanol was 400 mL.

**Total Phenolic Content (TPC)**

The previously modified Folin-Ciocalteu technique was used to determine the total phenolic content of RnLK leaf extract. The procedure used is similar to that done by Irawan, et al. The difference is in the standard series of gallic acid concentrations used. In this study, the concentration of gallic acid used was 0, 2, 4, 6, 8 mg/L.

**CUPRAC method for antioxidant activity testing**

The CUPRAC method for antioxidant assay refers to the technique used by Apak and has been modified by Irawan, et al. The ethanol extract solution of RnLK leaves was tested for antioxidant activity by the CUPRAC method using the same procedure as previously used by Irawan, et al.

**Activity of an α-glucosidase inhibitor**

Budiarso’s technique for determining α-glucosidase inhibitory activity is known as α-glucosidase inhibitory activity testing. Samples of leaf ethanol extract were dissolved in phosphate buffer pH 6.8 with acarbose as reference. Following that, the standard and sample solutions were dilute to various concentrations. In 17 μL of para-Nitrophenyl-D-glucopyranoside (PNPG) substrate, 30 μL of standard and sample solution were added. After 5 minutes of incubation, the solution was spiked with 17 μL of α-glucosidase solution. After that, it was incubated for another 15 minutes. The incubation was done at 37°C. After that, 100 μL sodium carbonate 200 mM was added, and the solution’s absorbance was measured at 405 nm with a microplate reader.

**RESULTS AND DISCUSSION**

**UAE**

UAE of leaves with 70% ethanol as a solvent resulted in a crude extract of 5.8520 g with a yield of 8.36%. The use of UAE extraction compared to other extraction methods such as maceration, air subcritical, and the microwave-assisted extraction (MAE) method was reported to be more effective because it reduced the degradation of phenolic compounds.

**Total Phenolic Content (TPC)**

The Folin-Ciocalteu method was used to determine the total phenolic content of RnLK leaves, with gallic acid as a reference component. Samples of leaf extract were dissolved in phosphate buffer pH 6.8 with acarbose as reference. The principle of this method is the oxidation reaction of phenolic compounds in alkaline conditions by Folin-Ciocalteu reagent to produce a blue complex. The number of phenolic compounds contained in the sample will be proportional to the increase in the intensity of the blue hue. The findings of the standard absorbance measurement of gallic acid and the linear equation can be seen in Figure 2. The TPC in the RnLK leaves produced was 607.1±0.2 mg GAE/g sample.

Generally, phenolic compounds have activity as antioxidants. The higher the total phenolic content in natural ingredients, the higher the antioxidant activity. The presence of substituted hydroxy groups in the ortho and para positions against the -OH and -OR groups of aromatic compounds can cause free radical inhibition. Phenolic compounds will donate a proton to free radicals, causing free radicals to turn into stable radicals.

**CUPRAC method for antioxidant activity testing**

Antioxidants decrease the copper (II) neocuproin ions to copper (I) neocuproin ions. The content of antioxidants in the sample coincides with an increase in yellow color intensity. BHT solutions at doses of 0.25, 0.5, and 0.75 mg/L had percent reduction powers of 15.71 ± 0.5, 33.53 ± 0.3, and 51 ± 0.8, respectively, according to the CUPRAC method’s antioxidant activity test. At doses of 4, 8, 12, 16, and 20 mg/L, the extract of RnLK leaves had percent reduction powers of 13.39 ± 1.9, 25.42 ± 0.4, 34.52 ± 0.6, 44.30 ± 0.2, and 50.34 ± 0.5, respectively (Table 1). Figure 3 shows the link between BHT concentration and percent reduction power (y = 70.587x-1.8787 and y = 2.3196x + 5.7595, respectively). BHT had an IC50 of 0.74±0.02 mg/L, while the extract of leaves had an IC50 of 19.1 ±0.1 mg/L, according to the equation.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>% Reduction Power</th>
<th>IC50 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>15.71 ± 0.5</td>
<td>0.74 ± 0.02</td>
</tr>
<tr>
<td>0.5</td>
<td>33.53 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>51 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13.39 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>25.42 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1: Data about the CUPRAC method’s antioxidant activity test results.**

For peroxide digestion, quenching singlet and triplet oxygen, as well as trapping and neutralizing free radicals, this redox characteristic is important for peroxide digestion, quenching singlet and triplet oxygen, as well as trapping and neutralizing free radicals. The IC50 value of BHT was lower than that of the ethanol extract, indicating that BHT had better reducing power to Cu2+ than the RnLK leaves extract. Because the IC50 value of ethanol extract was less than 50 mg/L, it had a very high reduction power. These findings suggested that the ethanol extract of RnLK leaves could be used as a natural antioxidant alternative. The oxidation and reduction characteristics of phenolic compounds in natural materials might generate the antioxidative effect. This redox characteristic is important for peroxide digestion, quenching singlet and triplet oxygen, as well as trapping and neutralizing free radicals.
Leaves ethanol extract inhibits α-glucosidase activity

The percentage inhibition of α-glucosidase activity against acarbose solution and ethanol extract of RnLK leaves is shown in Table 2. Figure 4 shows the equation obtained from plotting the data between the concentration of acarbose or sample extract with the percent inhibition. Acarbose and leaf extract yielded IC\textsubscript{50} values of 98.7±0.1 mg/L and 81.3±2.6 mg/L, respectively. The IC\textsubscript{50} value of the ethanol leaves extract of RnLK was lower than the acarbose standard, indicating that the ethanol leaves extract inhibited α-glucosidase more effectively than the acarbose standard. The higher activity of inhibiting α-glucosidase from RnLK leaves extract correlated with the high total phenolic content and antioxidant activity. Natural material’s antioxidant and anti-diabetic qualities are linked to the presence of phenolic chemicals. Phenolic chemicals can also inhibit α-glucosidase by competing with carbohydrate-digesting enzymes. As a result, the hydrolysis of carbohydrates to glucose molecules takes longer.  

CONCLUSION

The study’s major discovery is that the UAE extract from RnLK leaves has significant antioxidant activity and acts as an antidiabetic via blocking α-glucosidase. The UAE procedure yielded 5.8520 g of crude ethanol extract of RnLK leaves, with an 8.36 % yield, with TPC of 607.1±0.2 mg GAE/g sample. The CUPRAC method’s antioxidant activity test produced an IC\textsubscript{50} of 19.1±0.1 mg/L. High antioxidant activity was linked to the presence of phenolic chemicals. Furthermore, the ethanolic leaf extract has the active ability to inhibit α-glucosidase activity, which was indicated by an IC\textsubscript{50} of 81.3±2.6 mg/L. The ethanol extract of RnLK leaves has the potential to be an antioxidant and anti-diabetic source.

ACKNOWLEDGEMENT

The Ministry of Research and Technology/National Research and Innovation Agency of Indonesia funded this research with a Doctoral Dissertation Research Grant 2021 [Nomor: NKB-295/UN2.RST/HKP.05.00/2021].

CONFLICTS OF INTEREST

There are no conflicts of interest declared by the authors.

REFERENCES

Irawan C, et al.: Potential of Rhinacanthus nasutus (L.) Kurz Leaves Extract as an Antioxidant and Inhibitor of α-Glucosidase Activity


GRAPHICAL ABSTRACT

Rhinachanthus nasutus (L.) Kurz Leaves (RnlK)

Simplicia Set Up and Extraction with UAE Methods

UAE of leaves with 70% ethanol as a solvent resulted in a crude extract of 5.8520 g with a yield of 8.36%.

Total Phenolic Content

The TPC in the RnlK leaves extract produced was 607.1±0.2 mg GAE/g sample.

CUPRAC Method for Antioxidant Activity Testing

BHT had an IC50 of 0.74±0.02 mg/L, while the extract of leaves had an IC50 of 19.1±0.1 mg/L, according to the equation.

Activity of an α-Glucosidase inhibitor

Acarbose and leaf extract yielded IC50 values of 98.7±0.1 mg/L and 81.3±2.6 mg/L, respectively. The IC50 value of the ethanol leaves extract of RnlK was lower than the acarbose standard, indicating that the ethanol leaves extract inhibited α-glucosidase more effectively than the acarbose standard.

The study's major discovery is that the UAE extract from RnlK leaves has significant antioxidant activity and acts as an antidiabetic via blocking α-glucosidase. The UAE procedure yielded 5.8520 g of crude ethanol extract of RnlK leaves, with an 8.36% yield, with TPC of 607.1±0.2 mg GAE/g sample. The CUPRAC method's antioxidant activity test produced an IC50 of 19.1±0.1 mg/L. High antioxidant activity was linked to the presence of phenolic chemicals. Furthermore, the ethanolic leaf extract has the active ability to inhibit α-glucosidase activity, which was indicated by an IC50 of 81.3±2.6 mg/L.
Irawan C, et al.: Potential of *Rhinachanthus nasutus* (L.) Kurz Leaves Extract as an Antioxidant and Inhibitor of α-Glucosidase Activity

### ABOUT AUTHORS

Candra Irawan is a Doctoral Pharmacy Student at the Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, West Java 16424, Indonesia and Lecturer at the Politeknik AKA Bogor. He has research experience in the field of Phytochemistry and Natural Product.

Berna Elya is a Professor and Lecturer at the Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, West Java 16424, Indonesia. She develops works in the area of Pharmacognosy, Phytochemistry, and Natural Product.

Muhammad Hanafi is a Researcher Professor at the Research Center for Chemistry, Indonesian Institute of Sciences, PUSPITEK area, Serpong, South Tanggerang, Banten, Indonesia and Lecturer at the Faculty of Pharmacy, Universitas Pancasila, Srengseng Sawah, Jakarta, Indonesia. He has research experience in the field of Natural Product.

Fadlina Chany Saputri is an Associate Professor and Lecture at Department of Pharmacology, Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, West Java 16424, Indonesia. She has experience in the area of pharmacology and herbal medicine, working in drug discovery for metabolic disorder and degenerative diseases (such as diabetes mellitus, hypertension, hyperlipidemia, atherosclerosis, etc).

---

**Cite this article:** Irawan C, Elya B, Hanafi M, Saputri FC. Potential of *Rhinachanthus nasutus* (L.) Kurz Leaves Extract as an Antioxidant and Inhibitor of α-Glucosidase Activity Pharmacogn J. 2022;14(4): 373-378.