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Research Article

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

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ABSTRACT

Aims: The goal of this study is to learn more about the antioxidant and antidiabetic properties of *Rhinachantus 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 IC_{50} value for leaf extract antioxidant activity was 19.1±0.1 mg/L. Furthermore, the leaf extract inhibits α-glucosidase activity and has an IC_{50} 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.

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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.[1] This oxidative stress can cause various degenerative diseases such as cancer, inflammation, arthritis, atherosclerosis, Alzheimer's disease, Parkinson's disease, neurodegeneratives, and diabetes mellitus.[1-4]

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.[5,6] By 2030, it is predicted that people living with diabetes in the world will reach 578 million.[7]

One of the pharmacological therapies of DM is oral hyperglycemic drugs such as α-glucosidase inhibitors.[8] Currently, the drugs that are widely used for the treatment of diabetes are acarbose, miglitol, voglibose, and emiglitate. This medication might induce stomach pain, including gas, stomach cramps, diarrhea, nausea, and vomiting.[9] α-glucosidase inhibitory activity has been discovered in natural fertilizers like cereals, blueberries, strawberries, broccoli sprouts, sardines, and egg white.[9,10]

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.[11,12] The activity of RnLK stem bark extract in inhibiting α-glucosidase has been reported to be even higher than that of the acarbose standard.[13]

The dry powder of RnLK leaves has been extracted by maceration,[14] but this method has disadvantages as the volume of solvent used is relatively large and the extraction time is long.[15] Prospective extraction techniques have been intensively introduced to overcome this limitation, one of which is ultrasonic extraction.[16] 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.[13]

Simplicia's extraction

Extraction of leaf simplicia of RnLK referred to the previously reported procedure.[13] The difference is

Figure 1: The leaves samples of *R. nasutus*.

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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.[13,17] The procedure used is similar to that done by Irawan, *et al*. The difference is in the standard series of gallic acid concentrations used.[13] 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.*[13,18] 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.*[13]

Activity of an α-glucosidase inhibitor

Budiarso's technique for determining α-glucosidase inhibitory activity is known as α-glucosidase inhibitory activity testing.[19] 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.[20-22]

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.[23,24] 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.[25] 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.[26] The presence of substituted hydroxy groups in the ortho and para positions against the -OH and -OR groups of an aromatic compound can cause free radical inhibition. Phenolic compounds will donate a proton to free radicals, causing free radicals to turn into stable radicals.[27,28]

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.[18] 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,

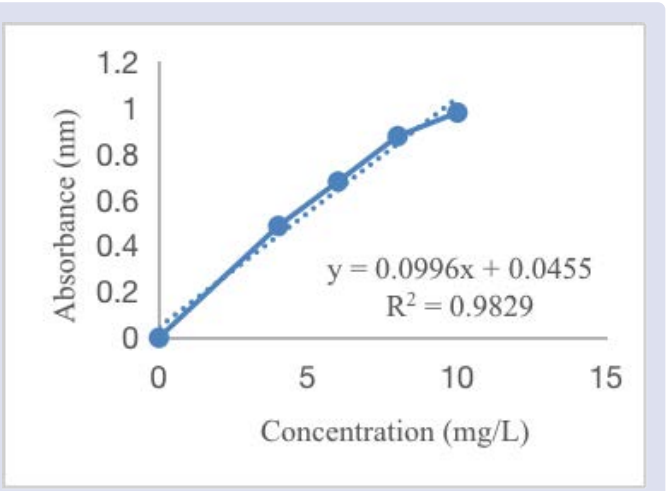

Figure 2: Gallic acid's reference curve.

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

Sample	Concentration (mg/L)	% Reduction Power	IC_{50} (mg/L)
BHT	0.25	15.71 ± 0.5	0.74 ± 0.02
	0.5	33.53 ± 0.3	
	0.75	51 ± 0.8	
Ethanolic Extract	4	13.39 ± 1.9	19.1 ± 0.1
	8	25.42 ± 0.4	
	12	34.52 ± 0.6	
	16	44.30 ± 0.2	
	20	50.34 ± 0.5	

Table 2: The α-glucosidase activity inhibition test findings.

Sample	Concentration (mg/L)	% Inhibition	IC_{50} (mg/L)
Acarbose	30	35.3 1± 0.07	98.7 ± 0.1
	60	41.50 ± 0.27	
	90	47.23 ± 0.15	
	120	56.53 ± 0.15	
	150	60.11±0.15	
Ethanolic Extract	10	23.72 ± 0.08	81.3 ± 2.6
	25	34.72 ± 0.03	
	50	42.41 ± 0.10	
	100	53.68 ± 0.63	

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 IC_{50} of 0.74±0.02 mg/L, while the extract of leaves had an IC_{50} of 19.1±0.1 mg/L, according to the equation.

The IC_{50} value of BHT was lower than that of the ethanol extract, indicating that BHT had better reducing power to Cu^{2+} than the RnLK leaves ethanolic extract. Because the IC_{50} value of ethanol extract was less than 50 mg/L, it had a very high reduction power.[29] 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.[30,31]

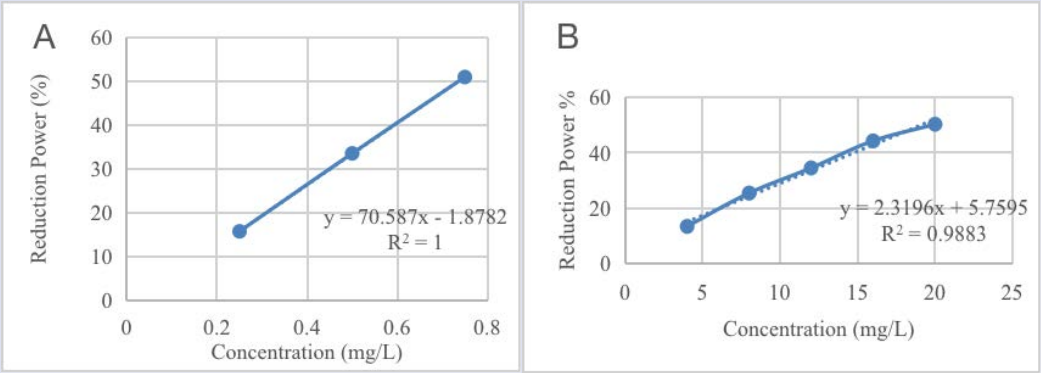

Figure 3: IC_{50} determination graph (A) BHT and (B) ethanol extract of RnLK leaves.

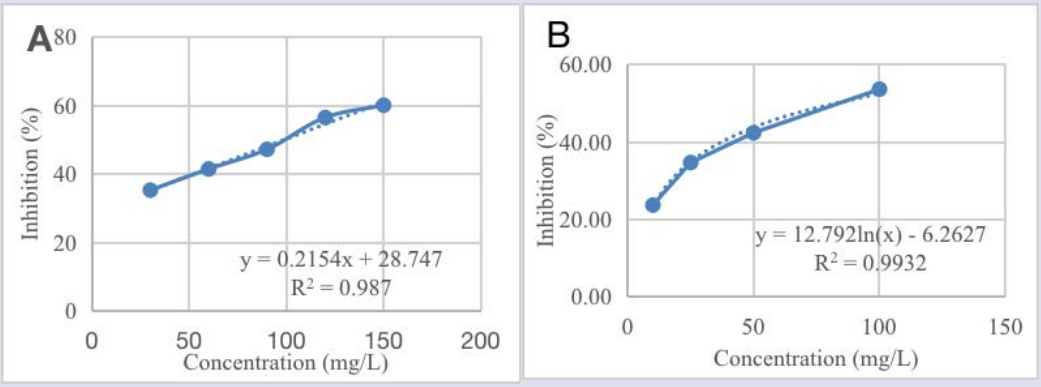

Figure 4: Graph of determination of IC_{50} for (A) acarbose and (B) ethanol extract of *R. nasutus* leaves.

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_{50} values of 98.7±0.1 mg/L and 81.3±2.6 mg/L, respectively. The IC_{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.[9]

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 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 IC_{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.

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CONFLICTS OF INTEREST

There are no conflicts of interest declared by the authors.

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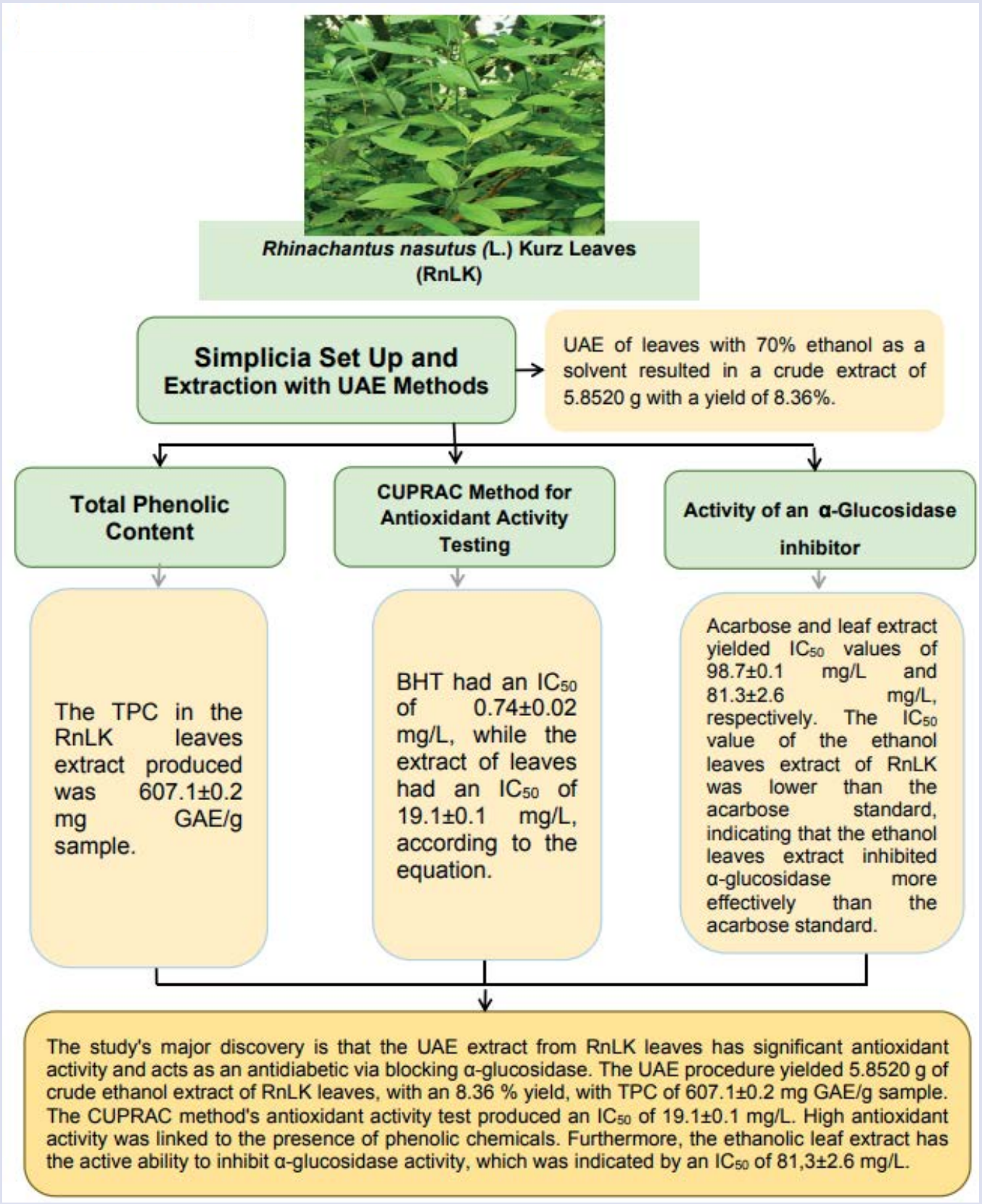
GRAPHICAL ABSTRACT



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