Pharmacogn J. 2022; 14(5): 521-526
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Original Article

Phytochemical Screening, Antioxidant Activity, and Anti-Inflammatory Potential of *Rhinachantus nasutus* (L.) Kurz Flower Ethanol Extract

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History

- Submission Date: 25-07-2022;
- Review completed: 11-08-2022;
- Accepted Date: 07-09-2022.

DOI : 10.5530/pj.2022.14.129

Article Available online

http://www.phcogj.com/v14/i5

ABSTRACT

Aims: The purpose of this study was to determine the content of the secondary metabolite compound in the flower extract of *Rhinachantus nasutus* (L.) Kurz (RnK); The potential of the extract as a radical scavenger of 2,2-diphenyl-1-picrylhydrazyl (DPPH); and its potential as an anti-inflammatory by inhibiting protein denaturation with bovine serum albumin (BSA). **Results:** Phytochemical screening results on the ethanolic extract of *R. nasutus* flowers revealed the presence of steroid glycosides, alkaloids, flavonoids, phenolics, and tannins. The extract has a strong ability to scavenge DPPH radicals with an IC_{50} value of 77.07 ± 0.05 mg/L. Besides that, the ethanol extract has very strong anti-inflammatory activity, with an IC_{50} value of 13.88 ± 0.2 mg/L. **Conclusion:** According to these findings, the ethanolic extract of R. nasutus flower can be used as an alternative anti-inflammatory drug.

Key words: RnK, BSA, 2,2-diphenyl-1-picrylhydrazyl, Anti-inflammatory.

INTRODUCTION

Inflammation is a chronic disease symptom that causes pain, swelling, redness, and tissue and organ dysfunction.[1] Inflammation is typically treated with steroidal or nonsteroidal anti-inflammatory drugs, but long-term use of anti-inflammatory drugs causes hormonal disruptions and gastric ulcers.[2] Alternative medicines based on natural ingredients are required to address this issue.

Medicinal plants have been widely used as preventive and curative efforts to treat various diseases and have therapeutic effects on inflammation. Herbal plant research and development has demonstrated their potential as a source of new drugs. In contrast to modern allophatic drugs, which have a single active substance with a single target pathway of action, herbal medicines have multiple active molecules that work synergistically with multiple targets of action.[3]

In traditional medicine, a medicinal plant known as *Rhinachantus nasutus* (L.) Kurz (RnK) is frequently employed.[4,5] The ultrasonic extraction method of ethanol extract from RnK bark was found to have high antioxidant activity.[6] The body requires antioxidants to prevent oxidative stress, which can lead to a variety of degenerative diseases such as diabetes, cancer, inflammation, and others.[7-10]

In this case, the ultrasonic extraction was applied to create RnK flower extract. Extraction with this method was the first to be performed on RnK flower extract. The resulting extract was then determined by the class of secondary metabolites contained and showed antioxidant activity through scavenging of DPPH radicals and anti-inflammatory properties.

METHODS

Simplicia setup

The identification of plants, source of sampling sites, and preparation of flowers for simplicia have been previously reported.[6] The flower samples used are two days old from blooming, and the flower samples can be seen in Figure 1.

Simplicia's extraction

The extraction procedure and conditions are the same as those used by Irawan[6]. The flower simplicia powder was extracted in the UAE. The solvent used is 70% ethanol.

Phytochemical screening

The RnK flower's crude ethanol extract was subjected to phytochemical screening using previously described techniques.[11-14]

DPPH radical scavenging test

The DPPH method was used to test the antioxidant activity of an ethanol extract solution of RnK flower, which was previously used by Irawan.[15]

Protein denaturation inhibition method for anti-inflammatory test

Anti-inflammatory tests were carried out using the protein denaturation inhibition method with bovine serum albumin (BSA). This method refers to the modified Williams procedure.[16]

A sample extract solution of 1,000 mg/L was pipetted to 80 µL, 120 µL, and 160 µL, then each was put into a 5 mL volumetric flask, calibrated with a 0.2% BSA solution in tris buffered saline (TBS). The solution was heated for 5 minutes in a water bath at 72 °C after 30 minutes of incubation at 25 °C. At

Cite this article: Irawan C, Elya B, Hanafi M, Saputri FC. Phytochemical Screening, Antioxidant Activity, and Anti-Inflammatory Potential of *Rhinachantus nasutus* (L.) Kurz Flower Ethanol Extract. Pharmacogn J. 2022;12(5): 521-526.

Figure 1: *R. nasutus* flower sample.

room temperature, the solution was cooled for 25 minutes. A visible spectrophotometer set to 660 nm was used to measure absorption. The experiment was repeated five times, also on the blank, and the positive control was sodium diclofenac (concentrations of 0.5, 0.75, and 1.0 mg/L).

RESULT AND DISCUSSION

UAE

The yield of RnK flower ethanol extract obtained in this study was 14.30%. Extraction using the UAE method of ripe banana peels (*Musa balbisiana* Colla) gives a yield of 11.80%,[17] while research on *Phaleria macrocarpa* (Scheff.) Boerl rind gives a yield of 18-21%.[18] In this study, the yield of RnK flower extract produced by the UAE method was comparable to the yield of similar extraction methods on ripe peels of *Musa balbisiana* Colla and *Phaleria macrocarpa* (Scheff.) Boerl fruit peels.

Phytochemical screening

The results of phytochemical screening on the ethanolic extract of RnK flowers showed positive results for steroid glycosides, alkaloids, flavonoids, phenolics, and tannins. Steroid glycosides and alkaloids outperformed other compounds, while sterol triterpenes produced negative results. Alkaloids have been demonstrated to have a wide range of pharmacological effects, such as hypoglycemic, antimalarial, and anticancer effects, making them popular as natural healing agents.[19-22] Many studies have proven that flavonoid compounds have activity as antioxidants, inhibitors of alpha-glucosidase activity, anticarcinogenic, cardiovascular, hyperglycemic, anti-inflammatory, antiallergic, analgesic, antibacterial, and antidepressant activities.[19,20,23] The biological effects of phenolic substances have been demonstrated to range widely, including anti-carcinogenic, anti-inflammatory, and antioxidant properties.[19,20,24] Tannins have been demonstrated to have antibacterial and antioxidant properties.[22,25,26] Steroid glycosides have been shown to have a significant effect on heart rate, act as a diuretic, and inhibit alpha-glucosidase activity.[19,20,27]

DPPH radical scavenging test

One method for determining the antioxidant activity of natural ingredients is the DPPH radical scavenging test. Antioxidants react with DPPH, causing the free radical properties to be lost due to the broken chain and the color to change from purple to light yellow.[8] Antioxidants donate hydrogen atoms to DPPH radicals, which results in the formation of stable DPPH-H compounds and antioxidant radicals.[28,29] The lower the absorption of the solution, the higher the antioxidant content.[30] BHT solutions at doses of 2 to 8 mg/L had percent inhibition of 34.16 ± 0.04, 51.23 ± 0.01, and 71.33 ± 0.04, respectively, according to the DPPH radical scavenging test, at doses of 8 to 128 mg/L, the extract of RnK flower had percent inhibition of 15.00 ± 0.4, 22.32 ± 0.09, 37.84 ± 0.03, 49.40 ± 0.04, and 67.86 ± 0.07, respectively (Table 2). Figure 2 depicts the standard line equations of BHT or RnK flower extract (y = 6.0248x + 24.12 and y = 0.4192x + 17.691, respectively). BHT had an IC_{50} of 4.30 ± 0.004 mg/L, while the extract of flower had an IC_{50} of 77.07 ± 0.05 mg/L, according to the equation.

The smaller the IC_{50} value indicates, the greater the antioxidant activity because the concentration required to reduce DPPH radicals by 50% is getting smaller.[31] The antioxidant activity of the ethanol extract of the RnK flower was classified as strong because the IC_{50} value was in the 50-100 mg/L range,[32] but BHT's general capacity to inhibit DPPH activity was higher than the ethanol extract's. The high antioxidant activity correlates with phytochemical screening results. Alkaloids, phenolic compounds, tannins, and flavonoids are found in RnK flower extract. Tannins and flavonoids contain OH, while alkaloids contain NH, both of which can give up hydrogen atoms to free radicals. As a result, radicals were transformed into non-radicals.

Protein denaturation inhibition method for anti-inflammatory test

The method of inhibiting BSA protein denaturation can be used as an initial screening for anti-inflammatory activity before anti-inflammatory tests using experimental animals, as in Williams' study.[16] When BSA is heated, it will be denatured, namely through changes in the secondary and tertiary structures. This indicates that albumin is damaged when heated, causing the body to be considered a foreign material. As a result, the body struggles with inflammatory mechanisms.[33,34]

In this investigation, heat had an impact on protein denaturation. The kinetic energy increases as the temperature rises. The protein is harmed as a result of the protein's constituent molecules moving or

Table 1: The results of phytochemical screening of ethanol extract of the flower *R. nasutus*.

Secondary Metabolite	Test Results
Alkaloids Substances:	
Dragendrof Test	+++
Meyer Test	++
Flavanoids Substances	++
Phenolic Substances	++
Saponins Substances	-
Tannins Substances	++
Steroid Glycosides Substances	++++
Sterol Triterpenes Substances	-

Table 2: Data about the DPPH radical scavenging test results.

Sample	Concentration (mg/L)	% Inhibition	IC_{50} (mg/L)
BHT	2	34.16 ± 0.04	4.30 ± 0.004
	4	51.23 ± 0.01	
	8	71.33 ± 0.04	
Ethanolic Extract	8	15.00 ± 0.4	77.07 ± 0.05
	16	22.32 ± 0.09	
	32	37.84 ± 0.03	
	64	49.40 ± 0.04	
	128	67.86 ± 0.07	

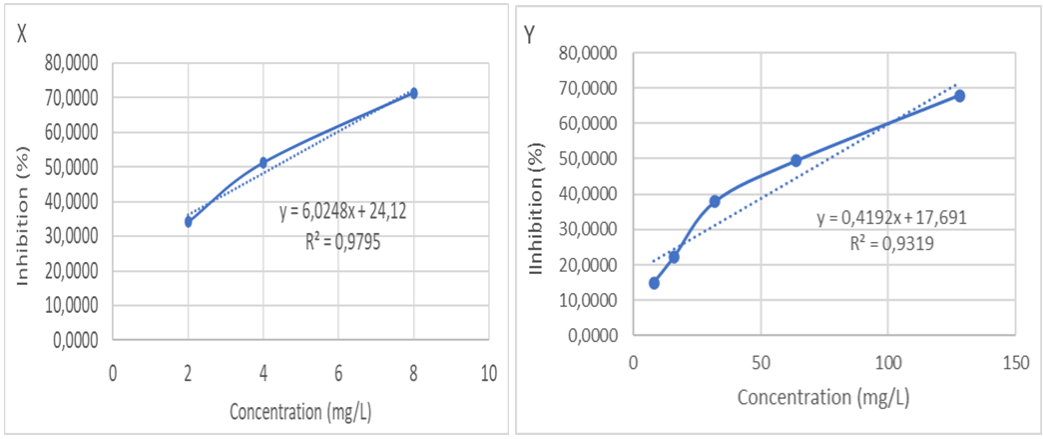

Figure 2: A graph showing the IC_{50} determination for (X) BHT and (Y) *R. nasutus* flower extract.

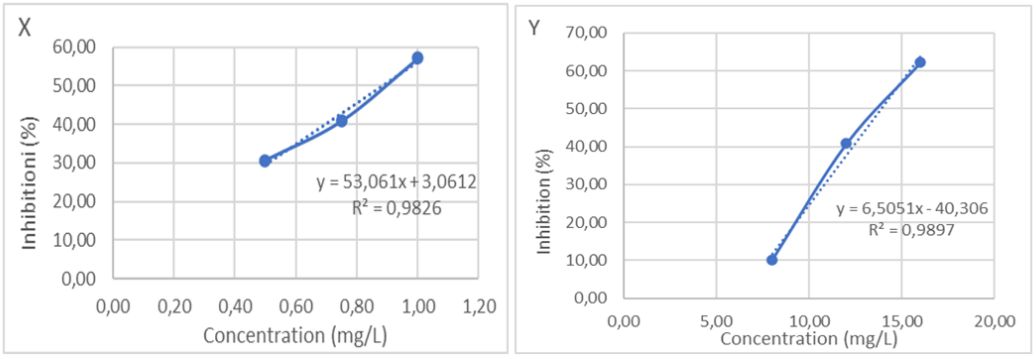

Figure 3: A graph showing the IC_{50} determination of for (X) sodium diclofenac and (Y) *R. nasutus* flower extract.

Table 3: Data on anti-inflammatory test results.

Sample	Concentration (mg/L)	% Inhibition	IC_{50} (mg/L)
Sodium Diclofenac	0.50	30.61 ± 1.4	0.88 ± 0.02
	0.75	40.82 ± 1.8	
	1.00	57.14 ± 1.3	
Ethanolic Extract	8	10.20 ± 0.5	13.88 ± 0.2
	12	40.82 ± 1.8	
	16	62.24 ± 1.4	

vibrating rapidly as a result of this. Protein denaturation takes place permanently and does not change. Denaturation of a protein reduces its liquid solubility, allowing it to settle more easily. Proteins in the body are prone to denaturation brought on by the creation of free radicals, which trigger the release of inflammatory mediators and hence trigger inflammation. The mechanism of action of diclofenac sodium is by inhibiting the synthesis of prostaglandins where the systemic effect of inflammation is fever.[35-37]

Sodium diclofenac solutions at doses of 0.5, 0.75, and 1 mg/L had percent inhibition of 30.61 ± 1.4, 40.82 ± 1.8, and 57.14 ± 1.3, respectively, according to the protein denaturation inhibition method for anti-inflammatory test, at doses of 8, 12, and 16 mg/L, the extract of RnK flower had percent inhibition of 10.20 ± 0.5, 40.82 ± 1.8, and 62.24 ± 1.4, respectively (Table 3). Figure 3 depicts, in the form of a line equation, the relationship between the concentration of sodium diclofenac or flower extract and the percent inhibition (y = 53.061x + 3.0612 and y = 6.5051x – 40.306, respectively). Sodium diclofenac had an IC_{50} of 0.88 ± 0.02 mg/L, while the extract of flower had an IC_{50} of 13.88 ± 0.2 mg/L, according to the equation.

The high anti-inflammatory activity of RnK flower extract was in line with the results of phytochemical screening and was also in line with high antioxidant activity. The antioxidant activity of natural ingredients is related to the presence of phenolic chemicals. Denatured albumin acts as an antigen involved in immune reactions such as type III hypersensitivity, serum sickness, glomerulonephritis, which is an inflammatory-based autoimmune disease. An agent that inhibits albumin denaturation or stabilizes albumin >20% can then be considered to have anti-inflammatory properties and may be subject to further anti-inflammatory testing.[16,38,39]

CONCLUSION

Steroid glycosides, alkaloids, flavonoids, phenolics, and tannins were detected in phytochemical analyses of the ethanolic extract of RnK flowers. The IC_{50} for antioxidant activity determined by the DPPH technique was 77.07 ± 0.05 mg/L. Furthermore, the extract has very strong anti-inflammatory activity, with an IC_{50} value of 13.88 ± 0.2 mg/L. According to these findings, the ethanolic extract of *R. nasutus* flower can be used as an alternative anti-inflammatory drug.

ACKNOWLEDGEMENT

The Ministry of Research and Technology/National Research and Innovation Agency of Indonesia funded this research with a Doctoral Dissertation Research Grant 2022 [Nomor: 021/E5/PG.02.00.PT/2022 dan 142. /PKS/WRIII-DRP/UI/2022 and NKB-782/UN2.RST/HKP.05.00/2022].

CONFLICTS OF INTEREST

According to the authors, there are no conflicts of interest in this study.

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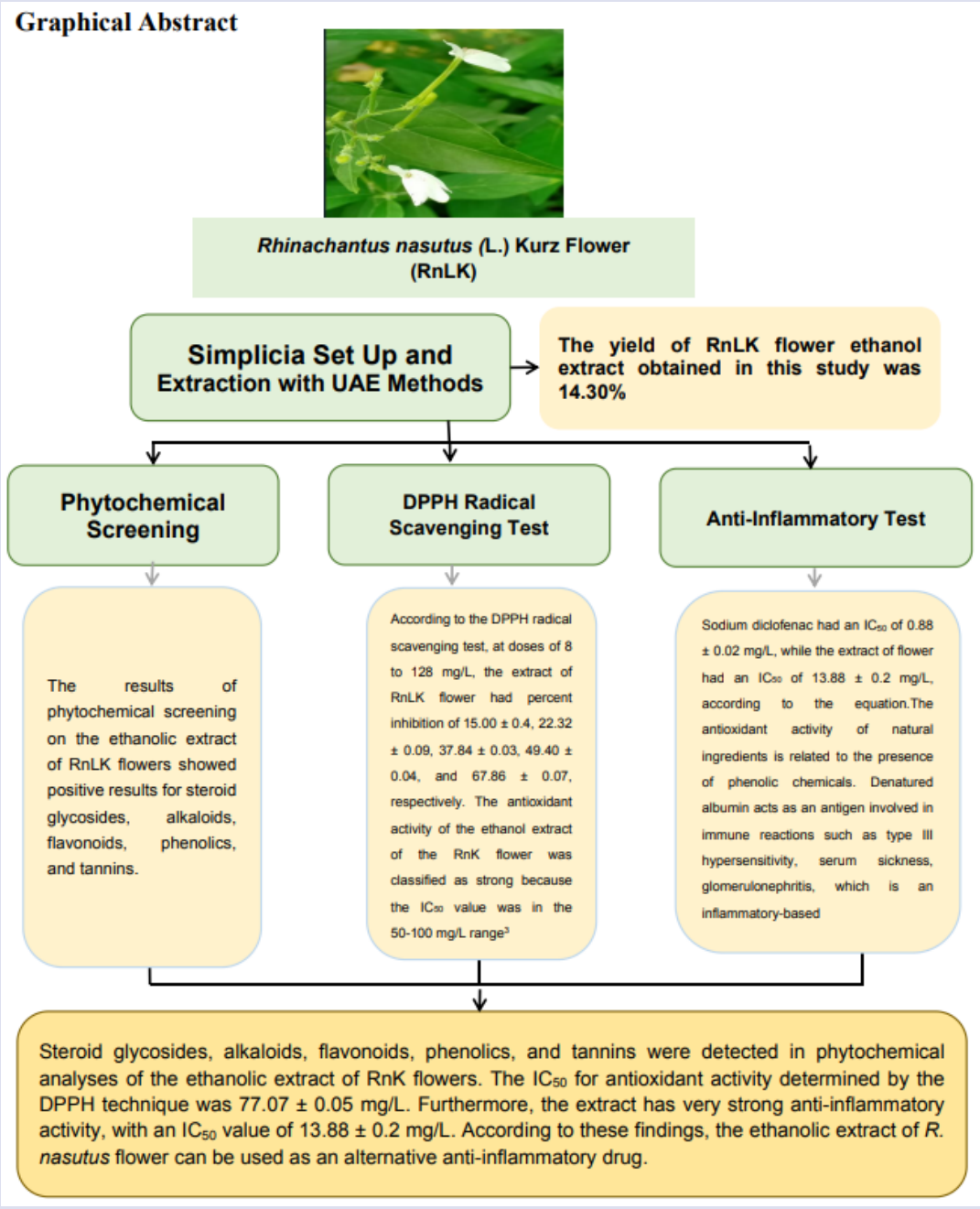
GRAPHICAL ABSTRACT



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Cite this article: Irawan C, Elya B, Hanafi M, Saputri FC. Phytochemical Screening, Antioxidant Activity, and Anti-Inflammatory Potential of *Rhinachantus nasutus* (L.) Kurz Flower Ethanol Extract. Pharmacogn J. 2022;12(5): 521-526.