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Research Article

# The Ethanolic Extract of *Rhinachantus nasutus* (L.) Kurz Flower has Antioxidant, Anti-Gout, and Antibacterial Potential

**Candra Irawan[1,2], Berna Elya[1,*], Muhammad Hanafi[3], Fadlina Chany Saputri[1]**

## ABSTRACT

The goal of this research was to explore the potential of *Rhinachantus nasutus (L.)* Kurz (RnLK) flower extract as an antioxidant utilizing the ferric reducing antioxidant power (FRAP) method; the possibility that it might be used as a treatment for gout by employing the 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) technique, as well as the possibility that it could be used as an antibacterial agent against *E. coli* and *B. subtilis*. **Results:** The $IC_{50}$ value for the extract's ability to serve as an antioxidant is 8.62±0.006 mg/L, indicating that it is quite effective. In addition, the extract of ethanol possesses highly potent anti-gout properties, being capable of bringing about a 81.95±0.1% reduction in uric acid levels. In spite of this, the antibacterial properties of *E. coli* as well as *B. subtilis* bacteria were not particularly robust. **Conclusion:** The RnLK flower has the potential to produce alternative chemicals with the ability to reduce blood uric acid levels, but according to the results of the test, the antibacterial activity has little impact on *E. coli* and *B. subtilis*.

**Key words:** RnLK, TBHBA, FRAP, Antibacterial.

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## INTRODUCTION

The *Rhinachantus nasutus* (L.) Kurz (RnLK) plant has a long history of application in the field of traditional medicine. The ethanol extract of RnLK leaves and bark has been proven in prior study to have a strong antioxidant activity and to operate as an alpha glucosidase inhibitor.[1,2] It was discovered that flowers extracted in ethanol contain steroid glycosides, alkaloids, flavonoids, phenolics, and tannins, as well as significant DPPH radical scavenging properties and the potential to be employed as anti-inflammatory medications.[3]

There is evidence that certain compounds, such as steroid glycosides, alkaloids, flavonoids, phenolics, and tannins, possess antibacterial characteristics.[4,5] Natural substances that inhibit the growth of bacteria are increasingly being utilized in place of synthetic antibiotics in the treatment of diseases.[6] The dosage is lower than what is suggested for the medication, and when treatment is discontinued prior to the bacteria being entirely eradicated by the antibiotic, the bacteria develop resistance to antibiotics as a result of the situation. As a consequence of this, different medication molecules that are capable of overcoming the challenge posed by bacterial resistance are necessary.[7-9]

Gout is an inflammatory joint illness that can significantly reduce one's quality of life. When there is an excess of uric acid in the body, the body's fluids become saturated, which leads to the development of the disease in the joints.[10] Allopurinol is by far the most common medicine prescribed for the treatment of gout. In addition to its beneficial effects, allopurinol can cause side effects such as nephritis, toxicity, and allergic reactions in some individuals. These drugs include a risk of causing allergic responses as well as hepatitis in some people. For this reason, it is essential to treat gout with alternative medicines like allopurinol, which are efficient but have few adverse effects compared to other treatments.[11]

Several pharmaceutical companies are in the process of transitioning to the usage of treatments that are derived from natural ingredients including antibiotics and medications that treat gout. In this study, we used an ethanolic extract of RnLK flowers to explore *in vitro* the antioxidant, antibacterial, and anti-uric acid characteristics of the extract.

## METHODS

### Sample preparation

The ethanolic extract of RnLK flower was utilized in this investigation. This extract has been created in the past and reported on in several publications.[3]

### FRAP test

The FRAP approach, which has been used in the past by Putri,[12] was utilized in order to evaluate the degree to which an ethanol extract solution of the RnLK flower possessed antioxidant properties.

### Uric acid test

Ismail's technique for measuring uric acid levels was the basis of the test described.[13]

### Antibacterial test

Paper discs about 6 mm in diameter were used in a disc diffusion test to determine whether or not *E. coli* and *B. subtilis* had any antibacterial effects. The antibacterial testing was repeated twice. After inoculating culture medium (Mueller Hinton Agar) with a pathogen suspension, the paper discs were submerged in 100 mg/L samples. Incubation was carried out for 2x24 hours at 37°C. The diameter of the inhibition zone on the paper disc was observed and measured.[14,15]

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## RESULT AND DISCUSSION

### Antioxidant capacity with FRAP method

The transformation of ferric iron, which has the oxidation state $Fe^{3+}$ into ferrous iron, which has the reduction state $Fe^{2+}$, was necessary for the success of this approach. The antioxidants that are present in the RnLK ethanol extract are largely responsible for the decrease that happens as a consequence of this. When placed in an acidic environment, this $Fe^{2+}$ ion will react with 1,10 phenantroline to generate a complex in the red range. During the course of this process, substances that donate electrons to act as antioxidants will contribute to the reaction's overall success.[16,17]

A curve can be generated by connecting the findings of an antioxidant activity test carried out using the FRAP method with a standard concentration series or sample, as shown in Figure 1. This connects the results of the test to the curve. The regression equations for gallic acid and ethanol extract of RnLK flower are shown to be y = 28,886x + 28,647 and y = 2,845x + 25,462 respectively in Figure 1. The $IC_{50}$ values for gallic acid and the ethanol extract of the RnLK flower, according to this equation, were 0.74±0.004 mg/L and 8.62±0.006 mg/L, respectively.

Gallic acid in general has the ability to reduce $Fe^{3+}$, which is superior to the ethanolic extract of RnLK flowers. Due to the fact that its $IC_{50}$ value is lower than 50 mg/L, the reducing power of ethanol extract is considered very strong.[18]

There was a correlation found between the high antioxidant activity and the results of the phytochemical screening. Alkaloids, phenolic chemicals, tannins, and flavonoids were found in the ethanol extract of RnLK flower, as well as flavonoids, according to a prior[3]. Polyphenols include both flavonoids and tannins as constituents. It has been demonstrated in a number of studies that there is a correlation between the amount of phenolic compounds found in plants and the antioxidant activity of those plants.[19-22] Reduction and oxidation processes will take place between the plant antioxidant molecules and the FRAP reagents. Singlet quenching, the destruction of triplet oxygen or peroxide, and the capture and neutralization of free radicals are all within their capabilities.[23,24]

### Antigout activity

In this study, the *in vitro* approach employing TBHBA reagent and uric acid pure analysis as a reference was utilized to assess whether or not the

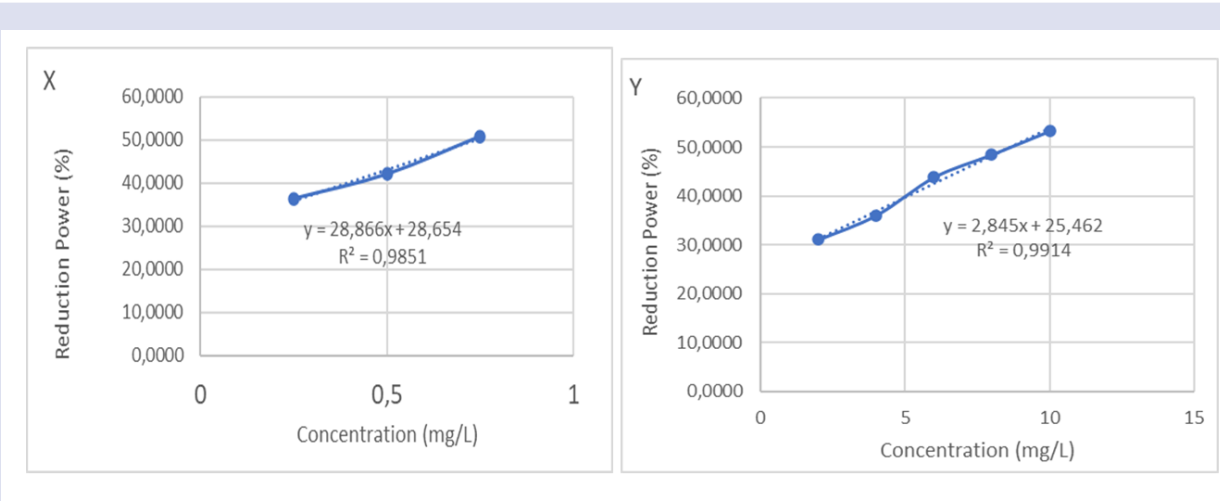


**Figure 1:** The following graph illustrates the relationship between concentration and percentage of reduction power for the $IC_{50}$ determination: (X) gallic acid; (Y) ethanol extract of RnLK flower.

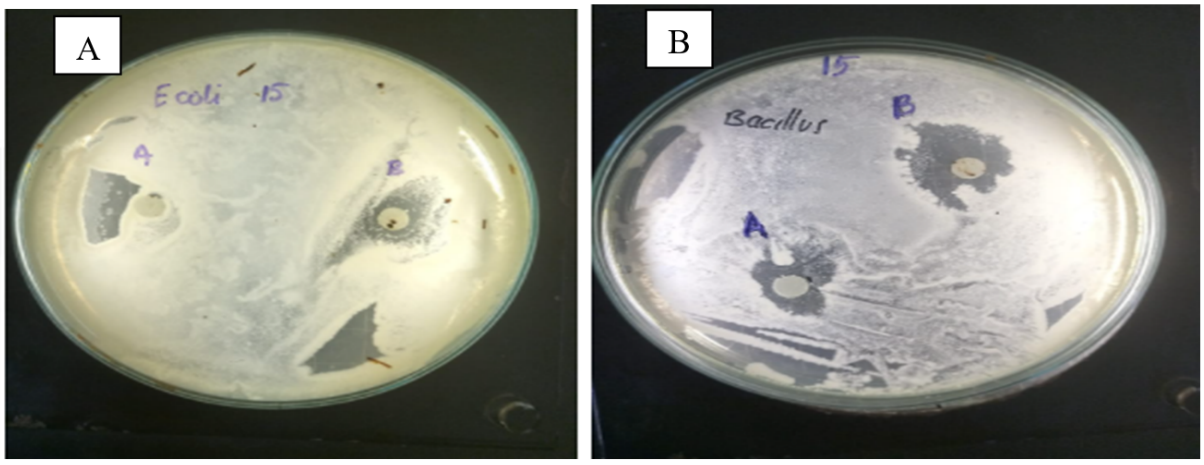


**Figure 2:** The diameter of the inhibition zone produced by the ethanolic extract of RnLK flowers on *E. coli* (A) and *B. subtilis* (B).

**Table 1: Reduced levels of uric acid (percent) following treatment with allopurinol and floral extract of RnKL.**

| Sample | Uric Acid Concentration (mg/L) | | A Decrease in the Level of Uric Acid (%) |
|---|---|---|---|
| Standard Uric Acid | 0.5000 | | |
| | interacting with the sample | the remaining portion of reaction | |
| Allopurinol | 0.0112±0.004 | 0.4888±0.004 | 2.25±0.8 |
| Flower extract | 0.4097±0.0006 | 0.0903±0.0006 | 81.95±0.1 |

**Table 2: The findings of the antibacterial test conducted on RnLK flower ethanol extract against E. coli and B. subtilis.**

| Bacteria | Average (mm) | The activity of the antimicrobial inhibition zone |
|---|---|---|
| *E. coli* | 0.90 | Weak |
| *B. subtilis* | 2.70 | Weak |

ethanolic extract of RnLK flower may reduce the levels of uric acid in the body. The uricase enzyme was necessary for the conversion of uric acid into the molecules of allantoin and peroxide that were used as the foundation for this particular test. In addition, the resulting peroxidase molecule reacts with the TBHBA to generate a quinonemin product, the presence of which may be measured with a spectrophotometer set to 546 nm.[25] Table 1 displays the percentage reduction in uric acid that was achieved following incubation with allopurinol and flower extract of RnKL.

According to Table 1, the amounts of lowering uric acid in RnLK flower ethanol extract and allupurinol were, respectively, 2.25±0.8% and 81.95±0.1%. According to these findings, the ethanolic extract of RnLK flowers has the potential to be used as an alternate method for reducing the amount of uric acid found in the blood. These findings were linked to the presence of a potent antioxidant activity. By inhibiting xanthine oxidase production, antioxidants reduce blood creatine uric acid. This was discovered through research that was conducted.[26] It has been stated in the past that RnLK flower extract possesses phenolic chemicals.[3] Due to the fact that xanthine oxidase prefers to oxidize poly phenolic compounds rather than xanthine, the presence of this substance can cause a reduction in the generation of uric acid.[27,28]

### Antibacterial activity

Table 2 presents the findings of an antibacterial activity test conducted on an ethanol extract of RnLK flower. The test was conducted on the growth of *E. coli* and *B. subtilis* bacteria. Antibacterial activity could be seen by looking at the zone of inhibition that surrounded the paper disc. The agar diffusion method was used to carry out the observation of the zone of inhibition. Figure 2 depicts the findings of an activity test conducted with an extract of the test substance at a concentration of 0.11 mg/L against bacteria belonging to the species *E. coli* and *B. subtilis*.

According to Figure 2 and Table 2, the diameters of the inhibition zones on the paper discs produced by E. coli bacteria and Bacillus subtilis bacteria have diameters of 0.9 mm and 2.70 mm, respectively. Based on these findings, it can be seen that these activities fall into the fairly weak category.[29] The degree of sensitivity possessed by the bacteria can have an impact on the disparity that exists between the diameters of the inhibition zones produced by the various bacteria.

The level of antibacterial activity exhibited by *B. Subtilis* is higher than that of *E. coli*. This is because the cell architectures of its basic components are not identical to each other. *B. subtilis* is a gram-positive bacterium while *E. coli* is a gram-negative bacterium. Gram-positive bacteria have a composition that is 90 percent peptidoglycan and have a thin layer of teichoic and teicuronic acids that are negatively charged. The outer layer of the cell wall in gram-negative bacteria has been found to contain 5 to 20% peptidoglycan in its composition. This layer, which is the second lipid layer and is known as the lipopolysaccharide layer, is in the middle. Phospholipids, polysaccharides, and proteins are the components that make up this layer.[30]

## CONCLUSION

Using the FRAP method, RnLK ethanol floral extract's $IC_{50}$ value for the antioxidant activity test was determined to be 8.62±0.006 mg/L. The uric acid level decreased by 27.950.08% as a result of the antigout activity. This outcome surpassed allupurinol. Therefore, the flower of RnLK has the potential to be an alternative chemical that can lower uric acid levels in the blood. The antibacterial activity tests revealed that it had a weak effect on *E. coli* and *B. subtilis*.

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## CONFLICTS OF INTEREST

The authors report that there are no potential conflicts of interest associated with this work.

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## GRAPHICAL ABSTRACT

***Rhinachantus nasutus* (L.) Kurz Flower (RnLK)**

**Simplicia Set Up and Extraction with UAE Methods**

**The yield of RnLK flower ethanol extract obtained in this study was 14.30%**

**FRAP Test**

The $IC_{50}$ values for gallic acid and the ethanol extract of the RnLK flower, according to this equation, were 0.74±0.004 mg/L and 8.62±0.006 mg/L, respectively.

**Uric Acid Test**

The amounts of lowering uric acid in RnLK flower ethanol extract and allupurinol were, respectively, 2.25±0.8% and 81.95±0.1%. According to these findings, the ethanolic extract of RnLK flowers has the potential to be used as an alternate method for reducing the amount of uric acid found in the blood.

**Antibacterial Test**

The diameters of the inhibition zones on the paper discs produced by E. coli bacteria and Bacillus subtilis bacteria have diameters of 0.9 mm and 2.70 mm, respectively. Based on these findings, it can be seen that these activities fall into the fairly weak category

Using the FRAP method, RnLK ethanol floral extract's $IC_{50}$ value for the antioxidant activity test was determined to be 8.62±0.006 mg/L. The uric acid level decreased by 27.950.08% as a result of the antigout activity. This outcome surpassed allupurinol. Therefore, the flower of RnLK has the potential to be an alternative chemical that can lower uric acid levels in the blood. The antibacterial activity tests revealed that it had a weak effect on E. coli and B. subtilis.

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