



Full Length Article

Evaluation of Cytotoxic, Anti-Inflammatory and Antibacterial Activities of Ethanol Extract of *Rhinachantus nasutus* Leaves

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Abstract

Ultrasonication produced an ethanolic extract of *Rhinachantus nasutus* (L.) Kurz (RX) leaves with high total phenolic content, strong antioxidant activity and was active in inhibiting alpha-glucosidase activity. However, its cytotoxic, anti-inflammatory, and antibacterial properties are still unknown. The objectives of this research were to evaluate the ethanol extract of RX leaves for its cytotoxicity using the Brine Shrimp Lethality Test (BSLT) with *Artemia salina* larvae, to evaluate its anti-inflammatory activity using the protein denaturation inhibition method, and to evaluate its antibacterial activity against *Escherichia coli* and *Bacillus subtilis*. Based on the results of this study, the ethanolic extract of RX leaves was found to have a very strong cytotoxic effect, with an LC₅₀ value of 10.93 ppm. In addition, this leaf extract's anti-inflammatory action is highly effective, with an IC₅₀ value of 7.55 ± 0.2 mg/L. In contrast, *E. coli* and *B. subtilis* bacteria do not appear to be significantly affected by the leaf extract. These findings suggest that the leaves of the RX have the potential to function as agents that both reduce inflammation and prevent cancer. © 2023 Friends Science Publishers

Keywords: Antibacterial; Anti-inflammatory; Cytotoxicity; *Rhinachantus nasutus*

Introduction

A plant known as *Rhinachantus nasutus* (L.) Kurz (RK) is a type of shrub that is frequently discovered growing in the shade by the side of roadways. This plant can be found growing wild throughout much of Southeast Asia, India and China (Farnsworth and Bunyapraphatsara 1992; Maarisit *et al.* 2017). The community makes frequent use of RK's leaves and roots as a treatment for a variety of conditions, including diabetes, eczema, pulmonary tuberculosis, herpes, hypertension, leprosy and scabies, as indicated by empirical data (Siripong *et al.* 2006). According to findings from earlier studies, a leaf extract that was obtained using ultrasonic waves possesses a significant level of antioxidant activity (Irawan *et al.* 2022a). Antioxidants found in plants are an essential component in the fight against degenerative conditions brought on by oxidative stress (Akinmoladun *et al.* 2010; Özen *et al.* 2010). Such as cancer and inflammatory diseases (Joon and Takayuki 2009; Badarinath *et al.* 2010).

Inflammation is a complex biological response that is triggered when vascular tissue is exposed to noxious stimuli such as pathogens, damaged body cells, or irritants (Egesie *et al.* 2011). Chronic sickness, which is characterized by

discomfort, organ and tissue dysfunction, edema, and redness, is also associated with inflammation as a marker of the condition (Manson *et al.* 2009; Ehlers and Kaufmann 2010). There are many different treatments available to alleviate the discomfort brought on by inflammation, but traditional medicine has not proven to be effective enough or safe enough to be considered an acceptable option. Because of this, there is a pressing need for a powerful and efficient anti-inflammatory medication, in particular for the treatment of chronic disorders (Cruz *et al.* 2016).

Cancer is a major killer in developed countries and is one of the leading causes of mortality worldwide. Cytotoxicity test carried out utilizing the Brine Shrimp Lethality Test (BSLT) protocol with *Artemia salina* as the test organism. The use of leach larvae as a preliminary test for determining anti-tumor and anti-cancer efficacy is common practice (Janakiraman and Johnson 2016). A connection between bacteria and particular cancers has been demonstrated by a number of research (Cummins and Tangney 2013). Antibiotics are the medications that are prescribed for the treatment of bacterial illnesses. The increased use of antibiotics as a treatment for disease, the administration of doses that are lower than the prescribed amount of medication and the consequences of

discontinuing antibiotic treatment before bacteria have been completely eradicated by the medication all contribute to the development of antibiotic resistance in bacteria (Dwidjoesepuro 2003; Mardiasuti 2007). Because of this, there is a pressing need for alternative therapeutic compounds that can not only combat the issue of bacterial resistance but also have properties that make them effective against inflammation or cancer.

The BSLT approach was utilized in the present work to carry out an analysis of the cytotoxic potential of an ethanolic extract of RK leaves. In addition to this, the possible anti-inflammatory and antibacterial activities of an ethanolic extract of RK leaves were studied. Ultrasonic extraction with 70% ethanol was used to get the RK extract that was used in this study.

Materials and Methods

Preparation and extraction of simplicia

In this study, the RK leaves samples used were prepared by ultrasonic extraction using 70% ethanol as the solvent, as reported in previous studies (Irawan *et al.* 2022a).

Cytotoxicity test

Cytotoxicity test carried out utilizing the Brine Shrimp Lethality Test (BSLT) protocol with *Artemia salina* as the test organism (Janakiraman and Johnson 2016).

The eggs of the brine shrimp *Artemia salina*, amounting to 30 mg, are added to the saltwater in a sealed container. The hatching process is aided by inserting an air hose into the container's base. After 24 h, *A. salina* eggs will hatch and become larvae. Next, 10 larvae of each species were collected and placed into a container containing a sample solution with a concentration of 0; 10; 100 and 250 mg/L. After 24 hours, it was clear that the *A. salina* larvae in the samples and controls had died. The criteria for determining whether or not *A. salina* larvae have passed away is when the larvae do not display movement for a few seconds while being observed. Once the percentage of *A. salina* larvae that died was determined, the probit value was found by searching through the probit table, and the data was then linearly regressed.

Anti-inflammatory test

The method of protein denaturation inhibition was used for the testing of anti-inflammatory properties, and bovine serum albumin (BSA) served as the substance that was put to the test (Irawan *et al.* 2022b).

After pipetting samples of an extract solution containing 1000 mg/L into 80, 120 and 160 μ L, each sample was placed in a volumetric flask containing 5 mL. After that, it was dissolved using a 0.2% BSA solution that was in tris buffered saline (TBS). After incubating the solution at room

temperature for 30 min, it was heated in a water bath to 72°C for 5 min. For 25 min, the solution sat at room temperature. Absorption was determined using a visible spectrophotometer with a 660 nm setting. The experiment was run five times with blank controls and three times with sodium diclofenac (0.5, 0.75 and 1.0 mg/L) as the positive control.

Antibacterial test

The antibacterial activity of *Escherichia coli* and *Bacillus subtilis* was tested using the disc diffusion method, with paper discs having a diameter of about 6 mm. The testing of the antibacterial agents was carried out twice. After being submerged in samples with a concentration of 100 mg/L, the paper discs were then placed on a culture medium (Mueller Hinton Agar) that had already been injected with a pathogen suspension. Two cycles of 24 h each were conducted at a temperature of 37°C during the incubation process. On the paper disc, the diameter of the inhibitory zone was seen and measured (Banjara *et al.* 2012; Chusri *et al.* 2012).

Results

Cytotoxicity activity

The BSLT method was used to assess the cytotoxicity of RX leaves ethanol extract at concentrations of 0; 10; 100 and 500 mg/L. Table 1 displays the number and percentage of *A. salina* larvae that perished at each test concentration. According to Table 1, all *A. salina* larvae perished at a concentration of 10 mg/L. After obtaining these data, the statistical probit analysis is carried on, which results in a graph depicting the relationship between the probit value and the concentration log (Fig. 1). The probit analysis also yielded the line equation $y = 2.7675x + 2.1246$ from which the LC_{50} value of the RK leaves ethanol extract was calculated to be 10.93 ppm.

Anti-inflammatory activity

Inhibition of protein denaturation was utilized as an anti-inflammatory test in this investigation. Percent inhibition was found to be 10.20 ± 0.5 , 40.82 ± 1.8 and 62.24 ± 1.4 when 4, 6 and 8 mg/L of ethanol extract of RX leaves were applied, respectively. When compared, the percent inhibition provided by diclofenac sodium solution at dosages of 0.5, 0.75 and 1 mg/L was respectively 30.61 ± 1.4 , 40.82 ± 1.8 and 57.14 ± 1.3 (Table 2). The linear association between sodium diclofenac or leaves extract concentration and percent inhibition is shown graphically in Fig. 2 ($y = 53.061x + 3.0612$ and $y = 10.969x - 32.823$, respectively). The 50% effective dose (IC_{50}) of sodium diclofenac was calculated to be 0.88 ± 0.02 mg/L, while that of the leaf extract was calculated to be 7.55 ± 0.2 mg/L.

Table 1: RX leaves ethanol extract cytotoxicity test results

| Concentration (mg/L) | Log Concentration | Total Larva Test | Larva Mortality | % Mortality | Probit Value |
|----------------------|-------------------|------------------|-----------------|-------------|--------------|
| 0 | 0 | 10 | 0 | 0% | 0 |
| 10 | 1 | 10 | 10 | 100% | 8,09 |
| 100 | 2 | 10 | 10 | 100% | 8,09 |
| 500 | 2,699 | 10 | 10 | 100% | 8,09 |

Table 2: Observations from anti-inflammatory drug trials

| 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 | |
| Ethanol Extract | 4 | 10.20 ± 0.5 | 7.55 ± 0.2 |
| | 6 | 31.63 ± 3.0 | |
| | 8 | 57.14 ± 2.4 | |
| | | | |

Table 3: The findings of the antibacterial test conducted on RnLK leaves ethanol extract against *E. coli* and *B. subtilis*

| Bacteria | Average (mm) | The activity of the antimicrobial inhibition zone |
|--------------------|--------------|---|
| <i>E. coli</i> | 1.30 | Weak |
| <i>B. subtilis</i> | 4.70 | Weak |

Antibacterial activity

The results of an antibacterial activity test performed on an ethanol extract of the RnLK leaves are shown in Table 3. The bacteria strains *E. coli* and *B. subtilis* were used in the experiment. By observing the zone of inhibition around the paper disc, antibacterial activity may be gauged. In order to observe the zone of inhibition, the agar diffusion technique was performed. The test material extract was tested for its activity against *E. coli* and *B. subtilis* at a concentration of 0.11 mg/L and the results are shown in Fig. 3. The resulting inhibition zones were 1.30 and 4.70 mm, respectively.

Discussion

In this investigation, the ethanol extract of RX leaves exhibited a cytotoxicity level of 10.93 ppm. This indicates that the extract is poisonous, as it caused the death of 50 percent of the test animals at concentrations below 1000 ppm (Meyer *et al.* 1982). It has been found that there is a positive link between the cytotoxicity of the extract and the antiproliferative power of the extract against cancer cells. Cytotoxicity is the ability of a substance to kill cancer cells. Antiproliferative power stops the growth of cancer cells. According to the BSLT test, the ethanol extract from the leaves of the RX plant, which is poisonous, has the potential to have an antiproliferative effect on cancer cells (Elsyana *et al.* 2016; Bouothmany *et al.* 2022).

Previous research has demonstrated that the ethanolic extract of nasustus leaves contains a significant quantity of total phenol (Irawan *et al.* 2022a). The category of chemicals known as polyphenols is the one that is found in

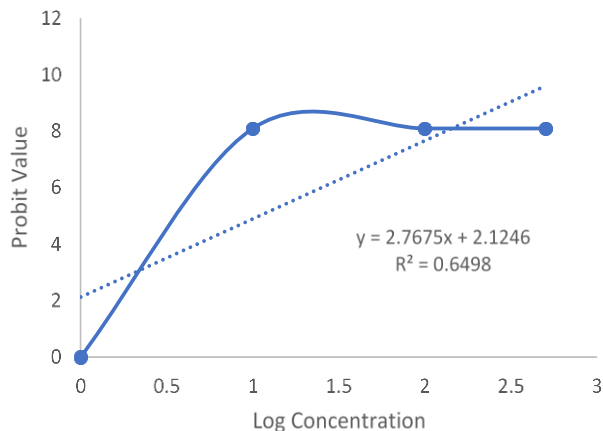


Fig. 1: Probit chart for RX leaves ethanol extract

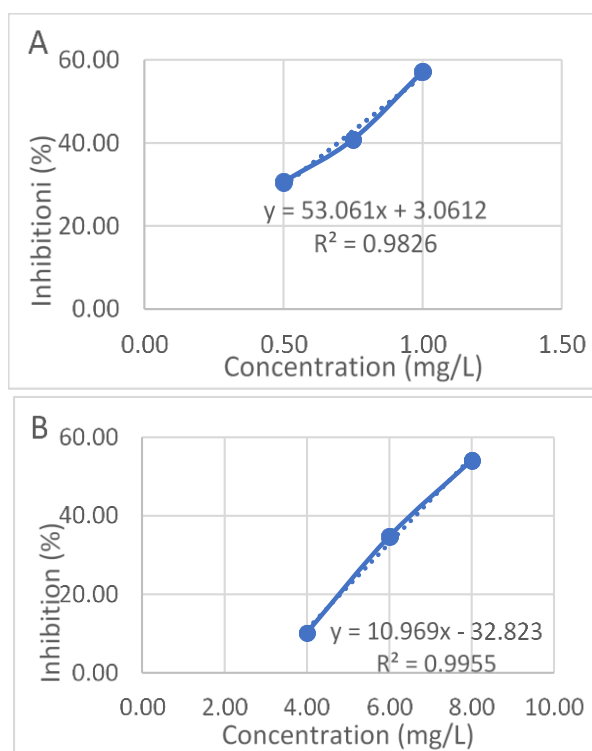


Fig. 2: A graph that shows how the IC₅₀ values for sodium diclofenac (A) and ethanol leaves extract RX (B) were found

the widest variety of plant species. Polyphenolic substances can be subdivided into groups of phenolic acids, flavonoids, polyphenol amides and other polyphenols based on the structure of the aglycoside. Each of these classes of polyphenols possesses their own unique features (Tsao 2010). Research has been done on phenolic compounds to investigate the antioxidant, anti-carcinogenic, alpha-glucosidase activity inhibiting, anti-inflammatory, and free radical scavenging characteristics they possess (Perez *et al.* 1989; Yin *et al.* 2014; Khan *et al.* 2019).

As seen in Williams' research, blocking BSA protein

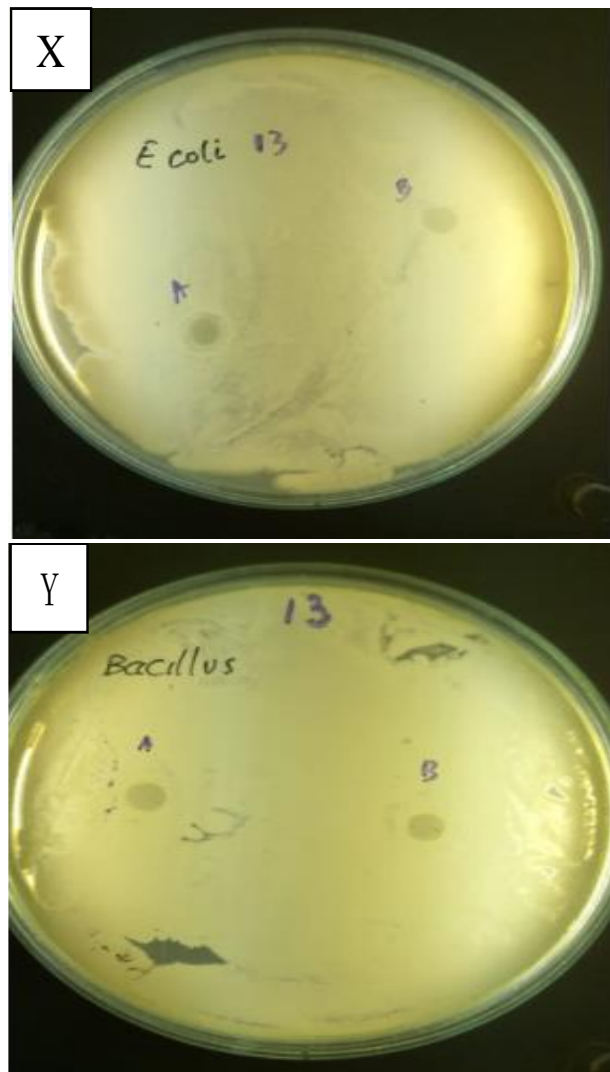


Fig. 3: The diameter of the inhibitory zone created by the ethanolic extract of RK leaves against *E. coli* (X) and *B. subtilis* (Y)

denaturation can serve as a preliminary screening for anti-inflammatory action before anti-inflammatory tests are conducted on experimental animals (Williams *et al.* 2008). BSA is denatured when heated so that the secondary and tertiary structures change. This suggests that heat damages albumin, leading the organism to react negatively to itself. Because of this, the body has trouble controlling its inflammatory responses (Leelaprakash and Mohan 2010; Ingle and Patel 2011). The body's proteins are vulnerable to denaturation due to the production of free radicals, which results in the release of inflammatory mediators and subsequently causes inflammation (Chick and Martin 1990).

The fact that RnK leaf extract has a high anti-inflammatory effect is in line with the fact that it has a high total phenolic content and a high antioxidant effect (Irawan *et al.* 2022a). Natural ingredients with antioxidant properties have phenolic chemicals in them. Denatured albumin acts as

an antigen in immune reactions like type III hypersensitivity, serum sickness and glomerulonephritis, which is an inflammatory-based autoimmune disease. Then, an agent that stops albumin from breaking down or stabilizes it by more than 20% can be thought to have anti-inflammatory properties and may be put through more anti-inflammatory testing (Williams *et al.* 2008; Tatti *et al.* 2012; Bailey-Shaw *et al.* 2017).

The findings of the antibacterial tests indicate that its action is fairly weak against *E. coli* and *B. subtilis* germs. The level of sensitivity of these bacteria can influence the size of the gap between the inhibition zones they produce (Pan *et al.* 2009). *B. Subtilis* displays more potent antibacterial action than *E. coli*. This is because its fundamental components all have slightly different cell layouts. Gram-positive bacteria, such as *B. subtilis*, are distinguished from gram-negative bacteria, such as *E. coli*, by their surface morphology. Gram-positive bacteria have a structure that is made up of peptidoglycan for the most part (90 percent) and they also contain a thin layer of negatively charged teichoic and teicuronic acids on their surface. It has been discovered that the outermost layer of the cell wall in gram-negative bacteria contains between 5 and 20% peptidoglycan as part of its makeup. The centre of the structure contains this layer, which is the second lipid layer and is also known as the lipopolysaccharide layer. This layer is made up of phospholipids, polysaccharides, and proteins, which are the three components that make it up (Madigan *et al.* 2000).

Conclusion

In this investigation, the cytotoxic activity of the ethanolic extract of RX leaves was shown to be extremely high, with an LC₅₀ value of 10.93 ppm. In addition, the leaf extract possesses extremely significant anti-inflammatory activity with an IC₅₀ value of 7.55 ± 0.2 mg/L, although its antibacterial activity against *E. coli* and *B. subtilis* is quite poor. According to these findings, the leaves of the *R. nasutus* have the potential to act as agents that both reduce inflammation and prevent cancer.

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Author Contributions

CI designed the experiments, conducted the experiments, compiled the findings, interpreted the results, and statistically analyzed the results; BE designed the experiments, compiled the findings, and interpreted the results and MH and FCS compiled the findings, statistically analyzed and interpreted the results.

Conflict of Interest

The authors have stated that they have no competing interests related to this study.

Data Availability

On reasonable request, the corresponding author will provide access to the data given in this work.

Ethics Approval

Outside the scope of this paper.

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