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Research Article

EFFECTS OF ETHANOLIC EXTRACT OF *RHINACANTHUS NASUTUS* (L) KURZ AS AN ANTIMICROBIAL AGAINST *ESCHERICHIA COLI* BACTERIA USING *IN VITRO* METHOD

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ABSTRACT

Objective: The objective of this study was to discover of the ethanolic extract of *Rhinacanthus nasutus* (L) Kurz in inhibiting *Escherichia coli* bacteria using an *in vitro* method.

Methods: This is an experimental study using a laboratory test with Kirby-Bauer or paper disc method by observing and measuring the inhibition zone of the ethanolic extract of *R. nasutus* against *E. coli* bacteria with extract concentrations of 15%, 30%, and 60% consisting of control groups and treatment group. The positive control group used chloramphenicol antibiotics and negative control groups used Aquadest. *E. coli* was incubated at 37°C for 24 h. Then, the plates were incubated for 24 h at 37°C and the diameter of the inhibition zone was observed until the 3rd day with three repetitions.

Results: The results of the study showed that the mean inhibition zone of *E. coli* bacteria was 10.93 mm, 12.09 mm, and 18.90 mm. The results of the Shapiro–Wilk test were p=0.199. The results of the one-way analysis of variance test were p<0.05 and that of the *post hoc* test indicated a significant value of p<0.05. Based on the results of the research, there were significant differences in the inhibition zone between the control group and the treatment group at a concentration of 15%, 30%, and 60%.

Conclusion: *R. nasutus* extract was effective to inhibit the growth of *E. coli* bacteria at concentrations of 15%, 30%, and 60%, so *R. nasutus* is effective as an antimicrobial.

Keywords: *Rhinacanthus nasutus*, *Escherichia coli*, Inhibition zone.

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INTRODUCTION

The various antibiotic resistance to microorganisms increases, which is caused by the use of uncontrolled free antibiotics for the treatment and disease prevention. It was an important to find alternative natural medicine as a safe antimicrobial agent. Therefore, there is a high interest in the study for the identification and development of natural antimicrobial compounds that are effective non-toxic and side effects [1].

Rhinacanthus nasutus is a natural antimicrobial spread out in several countries such as India, Southeast Asian countries, and China as traditional medicine [2]. Extracts of *R. nasutus* which have been identified to have very important secondary metabolites such as flavonoids, anthraquinones, triterpenes, and steroids, but there are the most active compounds, naphthoquinones. In naphthoquinone, the main compound is Rhinacantin C which is an active substance as an antimicrobial [3]. The use of *R. nasutus* leaves is more effective than its flowers, stems, and roots because the leaves of *R. nasutus* contain more secondary metabolites such as flavonoids, benzenoid, coumarin, anthraquinones, quinones, glycosides, triterpenes, sterols, and lupeol which are used as natural medicines. This plant is very effective as an antimicrobial [4].

Escherichia coli bacteria are bacteria found in human intestines; some strains of these bacteria may cause infectious diseases such as diarrhea, meningitis, septicemia, and urinary tract infection [5]. Diarrhea is a public health problem and a major cause of morbidity and mortality in infants and children [6]. Infectious diseases caused by *E. coli* increases because of resistance to antibiotic drugs [7]. It is found 6.9% of the 14 types of antibiotic drugs could inhibit bacterial growth [8]. Increased resistance of microorganisms to drugs was a global threat in therapy. This can be caused by the use of synthetic drugs that ware irregular and incompatible with the pathogens of bacteria, viruses, and fungi and the presence of several chemicals inhibiting the action of antimicrobial drugs leading to changes in the target and ability of drugs to penetrate across bacterial cell wall [9].

Extract of *R. nasutus* had maximum bio-efficacy compared to other solvents because it had more compounds such as saponins, steroids, tannins, phenolic, triterpenoids, more alkaloids, and flavonoids that were produced using *in vitro* method and were able to inhibit pathogenic bacteria [10]. *R. nasutus* was able to reduce the activity of viruses – causing herpes, namely, herpesvirus (HVS)-1 and HVS-2 [11]. *R. nasutus* root extract had quinonoid chemical content as an antimicrobial in Gram-positive bacteria [12]. The importance of other alternatives to eradicate microbes resulted from increasing drug resistance; the researcher is interested to conduct this study. This study has a novelty to find out the antimicrobial activity of the ethanol extract of *R. nasutus* against Gram-negative bacteria (*E. coli*) *in vitro* at concentrations of 15, 30, and 60%.

METHODS

Research design

This study was a true experiment using Kirby-Bauer or paper disc laboratory test method with observations and assessments of inhibition zones of the ethanolic extract of *R. nasutus* consisting of the control group and treatment group. The treatment group consisted of extract provision at concentrations of 15%, 30%, and 60%, positive control groups provided chloramphenicol, and negative control group provided Aquadest. The study was conducted in the Laboratory of Pharmacy and the Laboratory of Molecular Biology, Medical Faculty in University of Prima Indonesia.

Plant material

The *R. nasutus* material comes from Pasar Gunung Village, North Sumatra Indonesia, then it was identified in the laboratory of the Herbarium Medanese Faculty of Biology in Universitas Sumatera Utara with letter number 1964/MEDA/2019.

Preparation of *R. nasutus* extract

R. nasutus leaves 2000 g were washed and then dried. After that, it is dried in a drying oven at 60°C. After drying, it becomes simplicia weighing 520 g then crushed in an electric blender. The simplicia homogenate powder was macerated in 96% ethanol with a comparison of 1:10 in maceration jars for 72 h (3×24 h) occasionally stirred. Then, the filtrate is filtered with Whatman filter paper (0.45 µ). Then, the extract is evaporated using a rotary evaporator at a temperature range of 40–60°C until it thickens. The result is then applied with a porcelain dish until a thick extract is obtained [13].

Phytochemical screening

The results of the extract were evaluated to investigate the phytochemical tests such as alkaloids, steroids, and triterpenes, saponins, flavonoids, tannins, and glycosides [14].

Preparation of *E. coli* bacteria and experimental procedures

The *E. coli* bacteria were obtained from the Laboratory of Microbiology at the University of Sumatera Utara. The materials used in this study were sterilized. Petri dishes, test tubes, Erlenmeyer flask, measuring cups, use needles, and stirring rods were washed, dried, and wrapped in clean clothes. Then, these tools were sterilized in a sterilizer (Hot air oven) at 160°C. Materials to be used such as Mueller-Hilton Agar, nutrient agar, and Aquadest were sterilized by autoclaving at 121°C for 15 min. The other tools were sterilized in alcohol with a concentration of 70% and fire spirits [15]. Bacterial suspensions were made by taking *E. coli* bacteria colonies using a test tube containing 0.9% of NaCl (physiological) and osemata. They were then mixed in a vortex to make them homogeneous. Inoculum is made in accordance with McFarland standard, i.e. 0.5 to obtain 1.5×108 cell/ml of bacteria. Next, the suspension was put into brain heart infusion plates in a test tube and incubated at 37°C for 6–10 h. Then, the suspension was planted in natrium plates by rubbing the *E. coli* culture using a sterile cotton stick. The disc paper (diameter of 6 mm) of the treatment group was infused with extracts of *R. nasutus* at a concentration of 15%, 30%, and 60%. The disc papers of positive control and negative control groups were infused with chloramphenicol and Aquadest, respectively. They were placed on agar plates which had been inoculated with *E. coli* bacteria at 4°C for 2 h, and the bacteria were then incubated at 37°C for the next 24 h to measure the diameter of its inhibition zone using the calipers [16]. All tests were carried out in three repetitions and tested by Kirby-Bauer agar diffusion method using a paper disc with a diameter of 6 mm [17].

Statistical analysis

The data were presented in the mean and deviation standard. The research used primary data that were tested with SPSS 25.0 for Windows. The data were tested for normality with Shapiro–Wilk. If they were normally distributed, they would then be tested using one-way ANOVA (analysis of variance) and a *post hoc* test at $\alpha<0.05$.

Ethical clearance

This study was conducted pursuant to the ethical clearance governed by the Animal Ethics Committee of Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, Medan, under a letter numbered: 0292/KEPK-FMIPA/2019.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of the ethanol extract of *R. nasutus* discovered alkaloid, saponin, tannin, flavonoid, steroid, and triterpene; while glycoside was not found. These results are illustrated, as shown in Table 1.

Inhibition zone of *E. coli* bacteria

The inhibition zones of *E. coli* that was formed around the disc are presented (Fig. 1), and it is tabulated in Table 2. Mean zones of inhibition of the ethanolic extract *R. nasutus* against *E. coli* were calculated and tabulated in Table 3.

The results of the normality test with the Shapiro–Wilk test showed p=0.199. The results of one-way ANOVA test showed significant differences in the inhibition zones of *E. coli* with p=0.000. To discover which groups experienced differences from the results of this assessment, the *post hoc* test was conducted. The test results were indicated by differences in the notation of each group with a significance value of $\alpha<0.05$. The results of the *post hoc* test demonstrated that there were significant differences between the control group and the treatment group using the extract at a concentration of 15%, 30%, and 60% with the values of each p=0.000, as shown in Table 4. This indicated that there was a significant increase in the inhibition zone of the ethanolic extract of *R. nasutus* against Gram-negative bacteria (*E. coli*).

E. coli is a bacterium that causes diarrhea. Diarrhea is a condition in which there is an increased frequency of defecation and decreased consistency. Diarrhea can also be chronic and even cause death due to dehydration when not treated properly, especially if there is a loss of

Table 1: Phytochemical screening of the ethanol extract of *Rhinacanthus nasutus*

Phytochemical test	Extract test
Alkaloids	+
Saponin	+
Tannins	+
Flavonoids	+
Steroids	+
Triterpenes	+
Glycosides	–

(+): Present, (–): Absent

Table 2: Inhibition zone of *Escherichia coli* bacteria

Treatment group	Group I	Group II	Group III
K0	0	0	0
K1	20.30 mm	23.00 mm	25.90 mm
P1	10.60 mm	11.85 mm	10.35 mm
P2	12.70 mm	13.05 mm	10.55 mm
P3	18.65 mm	20.25 mm	17.80 mm

Table 3: Mean of inhibition zone values in *Escherichia coli* bacteria

Treatment group *Escherichia coli*	Inhibition zone (Mean±SD)
K0	0.00±0.00
K1	23.07±2.80
P1	10.93±0.80
P2	12.09±1.35
P3	18.90±1.24

SD: Standard deviation

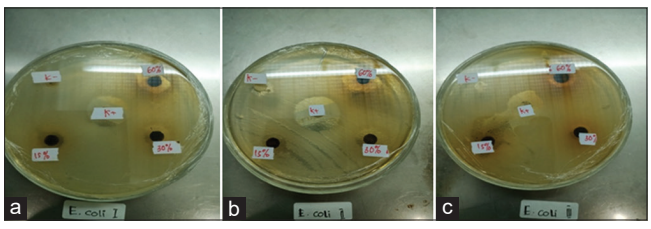

Fig. 1: Description of inhibition zone of *Escherichia coli* bacteria

Table 4: Antimicrobial activity of ethanolic extract of *Rhinacanthus nasutus*

Treatment group	Inhibition zone (p-value)
K0-K1	0.000*
K0-P1	0.000*
K0-P2	0.000*
K0-P3	0.000*

post hoc test *p<0.05

body fluids and salt, namely, sodium and potassium, especially in the elderly and infants [18].

The resistance of various antimicrobial drugs occurs due to the free and widespread use of antimicrobials over many years so that it can cause changes in the profile of pathogenic microbial isolates cause of diarrhea. Besides, those synthetic drugs can cause various side effects. Hence, it is necessary to develop antimicrobials from natural compounds for the production of new drugs with a minimal side effect [19]. The development of antibacterial drugs based on natural products has been developed at this time; this can be caused by the use of synthetic antibacterial drugs in the long term which can cause many side effects also become resistant to antibiotics [20].

The results of this study indicate that the ethanolic extract of *R. nasutus* contains secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroids, and triterpenes. This study is consistent with previous studies, which suggested that the main naphthoquinone, i.e. Rhinacanthins-C, -N, and –Q of *R. nasutus* had antibacterial potential by showing strong antibacterial activity against β-hemolytic streptococci, enterococci, and staphylococci, with its potential comparable to antibiotic gentamicin drugs [21]. Some of the important biological activities in *R. nasutus* are anticancer, antibacterial, and anti-inflammatory, which are associated with the presence of various functional compounds such as carotenoids, flavonoids, phenolic acids, chlorophyll, and naphthoquinones [22].

The results of the study found that *R. nasutus* can be used as a natural antibacterial, supported by another study that found that *R. nasutus* was a strong antibacterial against *Staphylococcus epidermidis* with a dose of 1000 µg/ml and 500 µg/ml [23]. Based on the results of the study, on inhibition zones, the control group (K0) using Aquadest did not show any inhibition zones in *E. coli* because the Aquadest did not have any antibacterial effect. This group was then compared to *R. nasutus* extract at concentrations of 15%, 30%, and 60% which showed significant results, i.e., it increased the inhibition zone in *E. coli* bacteria. The previous study also found that the active substance of Rhinacantin A of *R. nasutus* plant could increase the inhibition zone of *Staphylococcus aureus* with inhibition zones of 16 mm and 20 mm at 25 µg/disc and *Mycobacterium smegmatis* compounds (20 mm at 25 µg/disc) [24].

CONCLUSION

The results of the study showed a significant increase in the inhibition zone of ethanol extract *Rhinacanthus nasutus* against Escherichia coli bacteria at concentrations of 15, 30 and 60%. So that the ethanol extract of *Rhinacanthus nasutus* is effective as an antimicrobial.

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AUTHORS' CONTRIBUTIONS

Debora Paninsari contributed to the collection of the plant sample and sample bacteria; Sunarti has carried out extraction and phytochemical screening, Preparation of *E. coli* bacteria and measure the diameter of inhibition zone, contributed to the study guide and coordinated the manuscript writing, editing, and finalization.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest, the research subject, and others.

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