Eduvest – Journal of Universal Studies
Volume 6 Number 9, September, 2026, pp. 9764-9774
E-ISSN 2775-3727
DOI: 10.59188/eduvest.v6i9.53374

A Study on the Effectiveness of Cinnamon Bark Extract (Cinnamomum burmannii) in Inhibiting the Growth of Klebsiella pneumoniae in Vitro

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Keywords
Papaya Seed Extract; Hydrogel; Chitosan; Incision Wound.

ABSTRACT
Infections caused by Klebsiella pneumoniae represent a significant health concern due to the increasing prevalence of antibiotic resistance. One potential natural antibacterial agent is cinnamon bark (Cinnamomum burmannii), which contains various bioactive compounds. This study aimed to identify the secondary metabolites present in cinnamon bark extract and evaluate the effectiveness of cinnamon bark ethanol extract in inhibiting the growth of Klebsiella pneumoniae in vitro. The study employed a true experimental method with a post-test-only control group design. Antibacterial activity was evaluated using the disc diffusion method on Mueller–Hinton Agar (MHA) with five treatment groups: a negative control (sterile distilled water), a positive control (ciprofloxacin), and cinnamon bark ethanol extract at concentrations of 25%, 50%, and 75%, with three repetitions for each treatment. Data were analyzed using the Shapiro–Wilk test, Levene's test, and one-way ANOVA. Phytochemical screening showed that cinnamon bark extract contained alkaloids, flavonoids, saponins, phenols, and terpenoids. The mean inhibition zone diameters at concentrations of 25%, 50%, and 75% were 8.93 mm, 10.57 mm, and 10.99 mm, respectively. Based on the Davis and Stout criteria, the 25% concentration was categorized as moderate, while the 50% and 75% concentrations were categorized as strong. However, the one-way ANOVA results showed a significance value of 0.296 ($p > 0.05$), indicating no statistically significant differences among concentrations. Therefore, cinnamon bark ethanol extract (Cinnamomum burmannii) demonstrated antibacterial activity against Klebsiella pneumoniae; however, increasing the extract concentration from 25% to 75% did not produce a statistically significant improvement in antibacterial effectiveness.

INTRODUCTION

Infectious diseases remain a major global health concern, contributing substantially to morbidity and mortality at local, national, and international levels. They occur when pathogenic microorganisms enter the human body through various routes, including air, food, water, and biological vectors. These microorganisms can proliferate and trigger immune responses that may not always effectively eliminate the infection. Infectious diseases are caused by various pathogens, including viruses, fungi, parasites, and bacteria. Among these pathogens, bacterial infections account for approximately 7.7 million deaths or 13.6% of global deaths attributed to bacterial infections. These findings indicate that bacterial infections remain a significant global health burden, not only due to their impact on mortality but also because of increasing healthcare costs and pressure on healthcare systems (Gu et al., 2025).

One of the pathogenic bacteria associated with severe human infections is Klebsiella pneumoniae. This bacterium is an opportunistic pathogen that commonly affects individuals

with compromised immune systems. Globally, Klebsiella pneumoniae has been reported to cause hundreds of thousands of infections annually. In 2019, infections caused by this bacterium were estimated to reach approximately 790,000 cases, with a high mortality rate, particularly among neonates. This age group reportedly experienced the highest mortality burden, with approximately 124,000 deaths, indicating the severe impact of Klebsiella pneumoniae infections among newborns. The high morbidity and mortality associated with this pathogen make it a significant public health concern, particularly in the context of healthcare-associated infections (Chen et al., 2024).

Klebsiella pneumoniae is a Gram-negative bacterium belonging to the family Enterobacteriaceae. It is characterized by a short, non-motile rod-shaped structure and a prominent polysaccharide capsule that contributes to its virulence by reducing susceptibility to phagocytosis and promoting the formation of mucoid colonies. These bacteria are widely distributed in the environment and may exist as part of the normal human microbiota; however, under certain conditions, they can become opportunistic pathogens capable of causing severe infections (Djarot et al., 2023).

Klebsiella pneumoniae is associated with various healthcare-associated infections, including urinary tract infections, pneumonia, sepsis, and bacteremia. These infections frequently occur among patients receiving long-term care, undergoing mechanical ventilation, or experiencing immunosuppressive conditions. The increasing virulence of this bacterium contributes to the severity and complexity of treatment (Pintavirooj et al., 2022).

The clinical management of Klebsiella pneumoniae infections has become increasingly challenging due to the emergence of antibiotic resistance, particularly through the production of Extended-Spectrum β-Lactamases (ESBLs), which reduce the effectiveness of β-lactam antibiotics (Kocsis, 2023). This condition limits therapeutic options, prolongs treatment duration, and increases healthcare costs, making it an urgent global health issue (Hu et al., 2021). The growing antibiotic resistance crisis has encouraged the exploration of safer and more effective therapeutic alternatives, including natural products. Medicinal plants contain various bioactive compounds with potential antibacterial properties (Inayati et al., 2025).

Cinnamon is a medicinal plant containing various bioactive compounds, including cinnamaldehyde, eugenol, flavonoids, phenolic compounds, and saponins, which may contribute to antibacterial activity through mechanisms such as disruption of bacterial cell walls and membranes (Ekasinta & -, 2025; Nurul, 2021). Previous studies have demonstrated that cinnamon extracts possess antibacterial and antibiofilm activities against Gram-negative bacteria, including Klebsiella pneumoniae. However, studies specifically evaluating the antibacterial effectiveness of cinnamon bark extract (Cinnamomum burmannii) against Klebsiella pneumoniae remain limited and have reported varying results. Therefore, this study was conducted to identify the phytochemical compounds present in cinnamon bark extract, evaluate its antibacterial potential against Klebsiella pneumoniae, compare bacterial growth inhibition between cinnamon bark extract, negative controls, and standard antibiotics as positive controls, and determine the effectiveness of different extract concentrations based on inhibition zone diameter. The findings are expected to contribute to microbiological research, support future investigations into plant-based antibacterial agents, and enhance understanding of the potential application of cinnamon bark extract as an alternative antibacterial agent against Klebsiella pneumoniae.

The novelty of this study lies in its specific evaluation of cinnamon bark extract (Cinnamomum burmannii) and its antibacterial activity against Klebsiella pneumoniae across multiple concentrations. Unlike previous studies that have examined cinnamon extracts from different species or plant parts, this study focused on the bark of Cinnamomum burmannii, a species commonly found in Indonesia, and assessed its potential as a natural antibacterial agent. Therefore, this study aimed to identify the bioactive compounds contained in cinnamon bark extract, evaluate its antibacterial activity against Klebsiella pneumoniae, compare bacterial growth inhibition between extract treatments, negative controls, and standard antibiotics (in vitro), and determine the concentration producing the highest antibacterial activity.

METHODS

Types of Research

This study employed a true experimental design to evaluate the effectiveness of cinnamon ethanol extract (Cinnamomum burmannii) in inhibiting the growth of Klebsiella pneumoniae in vitro. The research used a post-test-only control group design, in which observations were conducted after treatment administration to evaluate antibacterial activity based on differences in inhibition zone diameters among treatment groups.

The study was conducted at the Basic Science Laboratory of Universitas Prima Indonesia, which provided facilities for microbiological research and in vitro antibacterial activity testing. The research was conducted in March 2026. The extraction process was performed using the maceration method for seven days, while bacterial incubation on Mueller–Hinton Agar (MHA) media was carried out for 24 hours at 37°C, which is suitable for the growth of Klebsiella pneumoniae.

Research Sample

In the sample in this study, the bacterium Klebsiella pneumoniae was used. The determination of the number of samples in this study was determined based on the number of replications commonly used in laboratory experimental research in vitro. Each treatment group was carried out three times or triples to improve accuracy, consistency, and validity. Repetition is performed to minimize errors that can occur during the research process and obtain a more representative average value of the antibacterial activity of the extracts tested.

This study consisted of five groups, namely negative control with sterile aquadest, positive control with antibiotic ciprofloxacin, cinnamon bark ethanol extract (Cinnamomum burmannii), which was 25%, 50%, 75% concentration. Thus, each group will be tested three times so that the number of samples is calculated as follows (Balouiri et al., 2016), Remarks:

n = Number of samples

5 = Number of groups

3 = Number of repetitions (triple)

Number of samples = Number of groups x Number of repetitions (triple)

n = 5 x 3

n = 15 samples

Based on the total calculation, the research group consisted of:

Group 1: Negative control (sterile aquadest) - 3 samples

Group 2: Positive control (siprofloxacin) - 3 samples

Group 3: Cinnamon bark ethanol extract concentration 25% - 3 samples

Group 4: Cinnamon bark ethanol extract concentration 50% - 3 samples

Group 5: Cinnamon bark ethanol extract 75% concentration - 3 samples

The use of triple repetition in each group is based on common practices in microbiology research to obtain more valid, reliable, and reproducible results on in vitro measurements of antibacterial activity (Armengol, n.d.; Masota et al., 2021)

Research Tools and Materials

The tools used in this study include maceration, knives, blenders, filters, test tubes, test tube racks, measuring cups, rotary vacuum evaporators, hot plates, magnetic stirrers, laminar air flow (LAF), autoclaves, micropipettes, ose needles, petri dishes (petri dishes), incubators, and calipers. These tools are used to prepare cinnamon bark simplicia into powder, carry out extraction, thickening, mixing, sterilization, bacterial inoculation, incubation, and accurate measurement of the diameter of the barrier zone. The research materials used included cinnamon bark simplicia (Cinnamomum burmannii), 96% ethanol, sterile aqueducts, Mueller Hinton Agar (MHA) media, pure cultures of Klebsiella pneumoniae bacteria, antibiotics ciprofloxacin as positive control, paper discs, and various phytochemical reagents, such as Mayer, Wagner, Dragendorff reagents, 1% $FeCl_3$ solution, 50% methanol, magnesium metal, chloroform, anhydrous acetic acid, and concentrated sulfuric acid. All of these ingredients are used according to their functions, namely for the extraction process, testing antibacterial activity in vitro, and identification of secondary metabolite compound content in cinnamon bark extract.

Research Procedure

The research procedure begins with the manufacture of cinnamon bark extract (Cinnamomum burmannii) through the stages of wet sorting, washing, cutting, drying using an oven, smoothing into powder, and filtering until a uniform particle size is obtained. The simplicia powder is then extracted using the maceration method with 96% ethanol solvent for three days accompanied by periodic stirring, then the macerated filtrate is filtered and evaporated using a rotary vacuum evaporator at a temperature of about 60°C until a thick extract is obtained. Next, phytochemical tests were carried out to identify the content of secondary metabolites, including flavonoids, alkaloids, saponins, phenols, terpenoids, and steroids, using appropriate reagents to determine the active compounds that are suspected to act as antibacterials against Klebsiella pneumoniae. After that, Mueller Hinton Agar (MHA) media is prepared by dissolving 3.8 grams of MHA powder in 100 mL of aqueducts, heated to homogeneous, sterilized using an autoclave at 121°C for 20 minutes, then aseptically poured into a sterile petri dish and cooled until solid.

The next stage involves the rejuvenation of Klebsiella pneumoniae cultures in MHA media incubated at 37°C for 24 hours to obtain bacteria in the optimal growth phase. The bacterial colonies were then made into suspensions with a turbidity equivalent to the McFarland standard of 0.5 ($\pm 1.5 \times 10^8$ CFU/mL) so that the number of bacteria in each treatment was uniform. Antibacterial effectiveness testing was carried out using the disc diffusion method, namely by applying a bacterial suspension on the surface of the MHA media, then placing disc paper that had been given cinnamon bark extract with concentrations of 25%, 50%, and 75%, accompanied by sterile aqueducts as negative control and syprofloxacin antibiotics as positive control. All treatments were carried out three times and incubated at 37°C for 24 hours. Furthermore, the diameter of the barrier zone formed was measured using a caliper in

millimeters, then the results were classified based on the criteria of David and Stout (1971) for the extract and Greenwood (1995) for the antibiotic cyprofloxacin to determine the level of antibacterial activity of cinnamon bark extract against the growth of Klebsiella pneumoniae.

Data Analysis

The data from the measurement of the diameter of the barrier zone was analyzed using Statistical Product and Service Solutions (SPSS) software. Before statistical testing was carried out, the data were first tested for normality using the Shapiro-Wilk test and the homogeneity of variance was tested using the Levene test. If the data is distributed normally and homogeneously (p> value 0.05), then the analysis is continued using the One-Way ANOVA test to determine the difference in the average diameter of the barrier zone between the treatment groups. If the results of the menun test show a statistically significant difference ($p < 0.05$), then a further test (Post Hoc) is carried out to find out the treatment group that has significant differences. On the other hand, if the data is not normally distributed, the Kruskal-Wallis non-parametic test is used followed by the Mann-Whitney test as a Post Hoc test. All statistical tests were carried out with a confidence level of 95% (Dewi Ratih Handayani et al., 2023).

RESULT AND DISCUSSION

Research Results

Table 1 Results of Phytochemical Test of Cinnamon Bark Extract

Golongan Senyawa	Reagents	Observation Results	Results
Alkaloid	Dragendrof	Brown deposits are formed	Positive (+)
Flavonoid	Mg + HCl concentrate (Shinoda Test)	Brown color	Positive (+)
Saponins	Foam Test	Formed stable foam > 1 cm	Positive (+)
Phenol	$FeCl_3$ 1%	Blackish green color	Positive (+)
Terpenoid	Liebermann-Burchard	Red-brown color	Positive (+)

Table 2 Measurement Results of Inhibition Zone Diameter

Extract Concentration	Repetition 1 (mm)	Repeatability 2 (mm)	Repetition 3 (mm)
25%	9,40	8,77	8,63
50%	9,68	12,55	9,49
75%	11,58	12,63	8,77

Based on the results of testing the antibacterial activity of *Klebsiella pneumoniae*, it can be seen that the extracts tested are able to inhibit the growth of bacteria at all concentrations used. The average inhibition zone diameters at concentrations of 25%, 50%, and 75% were 8.93 mm, 10.57 mm, and 10.99 mm, respectively. These results show a tendency to increase antibacterial activity along with increasing extract concentration. A concentration of 75% results in the largest average inhibition zone, which is 10.99 mm, while a concentration of 25% results in the smallest inhibition zone, which is 8.93 mm. This shows that the higher the concentration of the extract, the more active compounds it contains, so that the ability to inhibit the growth of bacteria becomes greater.

According to the criteria of Davis and Stout (1971), the diameter of the inhibition zone of 5–10 mm belongs to the medium category, while 10–20 mm belongs to the strong category. Based on these criteria, the concentration of 25% has moderate antibacterial activity, while the concentrations of 50% and 75% are in the strong category. This antibacterial activity is thought to come from the content of secondary metabolites such as flavonoids, alkaloids, tannins, saponins, or phenolic compounds that can damage bacterial cell membranes, disrupt cell wall permeability, and inhibit the activity of enzymes necessary for bacterial growth.

Data Analysis

1. Normality Test

Table 3 Shapiro-Wilk Normality Test Results

Concentration	Statistics	df	Say.	Remarks
25%	0,981	3	0,328	Normal
50%	0,796	3	0,106	Normal
75%	0,935	3	0,508	Normal

The normality test was performed using Shapiro-Wilk because the number of samples in each group was less than 50. The results of the normality test showed a significance value at a concentration of 25% of 0.328, a concentration of 50% of 0.106, and a concentration of 75% of 0.508. The entire significance value is greater than 0.05. This means that the data in each concentration group is normally distributed. Thus, the data meets one of the requirements for the ANOVA One-Way parametric test.

2. Homogeneity Test

The homogeneity test was carried out to find out whether the variance of data in each concentration group was homogeneous. Based on the results of the Levene Test, a significance value based on mean of 0.100 was obtained.

Table 4 Variance Homogeneity Test Results

Variabel	Levene Statistic	df1	df2	Say.	Remarks
Obstruction zone	3,461	2	6	0,100	Homogeneous

The significance value is greater than 0.05. This means that the data has a homogeneous variance. Since the data is normally distributed and homogeneous, the analysis can be continued using the One-Way ANOVA test.

3. Uji One-Way ANOVA

The One-Way ANOVA test was conducted to determine if there was an average difference in the diameter of the inhibition zone between the concentrations of 25%, 50%, and 75% cinnamon bark extract.

Table 5 ANOVA One-Way Test Results

Source of Variation	Sum of Squares	df	Mean Square	F	Say.
Between Groups	7,110	2	3,555	1,504	0,296
Within Groups	14,181	6	2,364		
Total	21,291	8			

The results of the ANOVA One-Way test showed an F value of 1.504 with a significance value of 0.296. The significance value is greater than 0.05. Thus, H0 is accepted and H1 is rejected. These results showed that there was no significant difference in the average diameter of the inhibition zone of Klebsiella pneumoniae bacteria between the concentrations of cinnamon bark extract of 25%, 50%, and 75%.

4. Tukey HSD Advanced Test

Table 6 Tukey HSD Advanced Test Results

Concentration	N	Average Buffer Zone (mm)	Subset
25%	3	8,9333	1
50%	3	10,5733	1
75%	3	10,9933	1
Say.		0,301	

Tukey HSD's follow-up test showed that the concentrations of 25%, 50%, and 75% were in the same subset with a significance value of 0.301. This shows that there is no significant difference between the concentration groups. Although the average diameter of the barrier zone increased from a concentration of 25% to 75%, the difference was not statistically strong enough to be statistically significant.

The results of the study showed that cinnamon bark extract (*Cinnamomum burmannii*) was able to inhibit the growth of Klebsiella pneumoniae bacteria in vitro. This is indicated by the formation of inhibition zones in all concentrations of the extracts tested. The average inhibition zone at a concentration of 25% is 8.93 mm. A concentration of 50% results in an average inhibition zone of 10.57 mm. A concentration of 75% results in an average inhibition zone of 10.99 mm. Based on these results, the 75% concentration has the greatest descriptive inhibition.

An increase in the diameter of the buffer zone is in line with an increase in the concentration of the extract. This can happen because the higher the concentration of the extract, the greater the amount of active compounds it contains. These active compounds can work in inhibiting the growth of bacteria. The antibacterial activity of cinnamon bark extract is thought to be related to the content of secondary metabolite compounds such as alkaloids, flavonoids, saponins, phenols, and terpenoids. Flavonoids and phenols can disrupt the structure of the cell wall and cell membrane of bacteria. Saponins can increase the permeability of cell membranes. Alkaloids can interfere with the metabolic processes of bacterial cells. Terpenoids can also damage the structure of cell membranes so that bacterial growth is disrupted.

Although descriptively the concentration of 75% results in the largest inhibition zone, the results of the One-Way ANOVA test show a significance value of 0.296. This value is greater than 0.05. This means that the difference in the mean of the inhibition zone between the concentrations of 25%, 50%, and 75% is not statistically significant. The results of the Tukey HSD test also showed that the three concentrations were in the same subset. This means that there is no noticeable difference between the concentrations of 25%, 50%, and 75% in inhibiting the growth of Klebsiella pneumoniae.

Thus, cinnamon bark extract can be declared to have antibacterial activity against Klebsiella pneumoniae. However, an increase in extract concentration from 25% to 75% has not shown a statistically significant difference in effectiveness.

CONCLUSION

Based on the results of the study on the effectiveness of cinnamon bark extract (Cinnamomum burmannii) in inhibiting the growth of Klebsiella pneumoniae in vitro, it was concluded that cinnamon bark extract exhibited antibacterial activity against Klebsiella pneumoniae. This activity was indicated by the formation of inhibition zones at all tested extract concentrations. The mean inhibition zone diameters were 8.93 mm at a 25%

concentration, 10.57 mm at a 50% concentration, and 10.99 mm at a 75% concentration. The 75% concentration produced the largest mean inhibition zone diameter compared with the 25% and 50% concentrations. However, the one-way ANOVA test showed a significance value of 0.296 ($p > 0.05$), indicating that there was no statistically significant difference in the mean inhibition zone diameters among the 25%, 50%, and 75% concentrations of cinnamon bark extract against Klebsiella pneumoniae. Therefore, cinnamon bark extract has potential as a natural antibacterial agent; however, increasing the concentration from 25% to 75% did not result in a statistically significant increase in antibacterial effectiveness.

Based on the findings of this study, several recommendations are proposed. Future studies should evaluate a broader range of extract concentrations, such as 10%, 25%, 50%, 75%, and 100%, to better characterize the concentration–response pattern. Further research should include appropriate positive and negative controls in statistical analyses to allow comparison between extract activity, standard antibacterial agents, and solvents. Increasing the number of experimental repetitions is also recommended to improve the reliability of results and statistical accuracy. Additional investigations using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays are needed to determine the lowest extract concentrations capable of inhibiting and eliminating Klebsiella pneumoniae. Future studies may also compare cinnamon bark extract activity against other bacterial species to further evaluate its antibacterial potential. Researchers are encouraged to investigate other parts of the cinnamon plant, such as cinnamon leaves (Cinnamomum burmannii), which may contain different levels of essential oils and bioactive compounds, to determine their potential antibacterial effects. Furthermore, standardized extraction procedures should be applied to ensure consistency in the concentration of active compounds obtained.

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