

***Efektivitas Antibakteri Ekstrak Bunga Lawang (*Illicium verum*)
Terhadap Pertumbuhan *Klebsiella pneumoniae* ESBL***
***Antibacterial Effectiveness of Star Anise Extract (*Illicium verum*) Against
the Growth of *Klebsiella pneumoniae* ESBL***
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Abstract

Klebsiella pneumoniae is an opportunistic pathogen with high antibiotic resistance, posing significant treatment challenges. Star anise (*Illicium verum*) contains bioactive compounds like shikimic acid and anethole, which exhibit antibacterial potential. However, its effectiveness against *Klebsiella pneumoniae* remains underexplored. This study aimed to evaluate the antibacterial efficacy of star anise extract against *Klebsiella pneumoniae* and compare it with meropenem as a positive control. This in vitro experimental study employed the Kirby–Bauer disc method with a post-test-only control group design. Star anise extract was prepared via maceration and tested at concentrations of 20%, 40%, 60%, 80%, and 100%. Phytochemical tests identified active compounds, while *Klebsiella pneumoniae* was confirmed through biochemical tests and Gram staining. Antibacterial effectiveness was assessed based on inhibition zone diameters on Mueller Hinton agar. Data were analyzed using one-way ANOVA at a 95% significance level. The extract contained saponins, alkaloids, flavonoids, tannins, and trans-anethole. While increasing extract concentration significantly enhanced the inhibition zone diameter ($p < 0.05$), all concentrations were categorized as weak against *Klebsiella pneumoniae*. Based on the diameter of the inhibition zones, the antibacterial activity of the star anise extract against *Klebsiella pneumoniae* can be classified as weak, particularly against resistant strains.

Keywords: antibacterial activity, *Illicium verum*, *Klebsiella pneumoniae*

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Abstrak

Klebsiella pneumoniae adalah patogen oportunistik dengan resistensi antibiotik yang tinggi, menimbulkan tantangan pengobatan yang signifikan. Bunga lawang (*Illicium verum*) mengandung senyawa bioaktif seperti asam shikimat dan anetol, yang menunjukkan potensi antibakteri. Namun, efektivitasnya terhadap *Klebsiella pneumoniae* masih belum banyak dieksplorasi. Penelitian ini bertujuan untuk mengevaluasi efektivitas antibakteri ekstrak star anise terhadap *Klebsiella pneumoniae* dan membandingkannya dengan meropenem sebagai kontrol positif. Studi eksperimental *in vitro* ini menggunakan metode Kirby–Bauer disc dengan desain kelompok kontrol pasca-tes. Ekstrak star anise disiapkan melalui maserasi dan diuji pada konsentrasi 20%, 40%, 60%, 80%, dan 100%. Uji fitokimia mengidentifikasi senyawa aktif, sementara *Klebsiella pneumoniae* dikonfirmasi melalui uji biokimia dan pewarnaan Gram. Efektivitas antibakteri dievaluasi berdasarkan diameter zona penghambatan pada agar Mueller Hinton. Data dianalisis menggunakan one-way ANOVA pada tingkat signifikansi 95%. Ekstrak mengandung saponin, alkaloid, flavonoid, tanin, dan trans-anetol. Meskipun peningkatan konsentrasi ekstrak secara signifikan meningkatkan diameter zona penghambatan ($p < 0,05$), semua konsentrasi dikategorikan sebagai lemah terhadap *Klebsiella pneumoniae*. Berdasarkan diameter zona penghambatan, aktivitas antibakteri ekstrak bunga lawang terhadap *Klebsiella pneumoniae* dapat diklasifikasikan sebagai lemah, terutama terhadap strain resisten.

Kata Kunci: aktivitas antibakteri, *Illicium verum*, *Klebsiella pneumoniae*

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Highlight:

- Phytochemical screening showed that the star anise (*Illicium verum*) extract contains various bioactive compounds, including saponins, alkaloids, flavonoids, tannins, and trans-anethole, which possess natural antibacterial potential.
- Although higher concentrations of the extract significantly increased the diameter of the bacterial inhibition zone, the overall antibacterial activity against ESBL-producing *Klebsiella pneumoniae* was still classified as weak across all tested concentrations (ranging from a 5.7 mm zone at 20% concentration to 8.1 mm at 100% concentration).
- The extract's inhibition zones were considerably smaller than those produced by the positive control, meropenem (which reached 31.6 mm). This indicates that star anise extract has limited capability and is ineffective as a standalone treatment against resistant ESBL-producing bacterial strains.

INTRODUCTION

Star anise (*Illicium verum*) is a spice plant originating from China and now widely cultivated in tropical regions, including Indonesia (Intan et al., 2023). This plant is known for its star-shaped, reddish-brown fruit and sharp aroma. In addition to its culinary uses, *Illicium verum* has long been utilized in traditional medicine thanks to its bioactive components such as flavonoids, saponins, tannins, alkaloids, and trans-anetol (Idris et al., 2024). Trans-anetol, the main component of star anise essential oil, is known to have

antibacterial activity against various bacteria, including Gram-negative bacteria such as *Escherichia coli* (Wu et al., 2022). Several previous studies have also shown that *Illicium verum* extract has antibacterial potential against various pathogens, including *Salmonella typhi* and *Streptococcus viridans*, with the effect increasing with increasing extract concentration (Hayati and Lestari, 2020; Yang et al., 2021). These findings indicate that *Illicium verum* contains bioactive compounds that have the ability to inhibit the growth of Gram-positive and Gram-negative bacteria.

However, there is a lack of research examining the antibacterial potential of *Illicium verum* extract against *Klebsiella pneumoniae*, particularly extended-spectrum β -lactamase (ESBL)-producing strains. *Klebsiella pneumoniae* is a clinically significant opportunistic pathogen, causing serious infections such as pneumonia, urinary tract infections, and sepsis. One of the main challenges in treating infections caused by *Klebsiella pneumoniae* is the high prevalence of antibiotic resistance, primarily due to ESBL production. ESBL-producing strains can inactivate β -lactam antibiotics, such as penicillin and third-generation cephalosporins, limiting available therapeutic options (Gajdacs et al., 2019; Hafiz et al., 2023). In Indonesia and Southeast Asia, the prevalence of antibiotic-resistant *Klebsiella pneumoniae* is very high, with resistance reaching 21.4% in some strains (Husna et al., 2023). The increasing resistance to antibiotics makes *Klebsiella pneumoniae* one of the greatest threats to public health, necessitating efforts to find effective alternative therapies, one of which is through the use of plant extracts with proven antibacterial components.

Although various plant extracts, including *Illicium verum*, have been studied for their antibacterial activity, no studies have specifically tested the potential of *Illicium verum* extract against ESBL-producing *Klebsiella pneumoniae*. This study aims to fill this gap by evaluating the antibacterial efficacy of *Illicium verum* extract against the growth of ESBL-producing *Klebsiella pneumoniae* strains. This study will use a disc diffusion method to measure the antibacterial activity of *Illicium verum* extract and compare it with the standard antibiotic, meropenem, as a positive control.

This study aims to evaluate the antibacterial efficacy of *Illicium verum* extract against the growth of ESBL-producing *Klebsiella pneumoniae*, with the hypothesis that the extract has a significant inhibitory effect on the growth of this bacterium compared to the positive control, meropenem. This research is expected to make an important contribution to the search for natural-based antibacterial agents capable of addressing the problem of antibiotic resistance, particularly in Southeast Asia, which faces significant challenges related to multiresistant infections.

METHODS

This study was an in vitro experimental study using a post-test control group design to evaluate the antibacterial effectiveness of star anise (*Illicium verum*) extract against an extended-spectrum β -lactamase (ESBL)-producing strain of *Klebsiella pneumoniae* (ATCC 700603 PK/5) (Agnesia et al., 2023; Liberty, 2024).

Bacterial identification was conducted using a selective subculturing method. The isolate was first grown on Nutrient Agar (NA) and subsequently subcultured onto MacConkey agar to obtain isolated colonies. MacConkey agar was selected because it is selective for Gram-negative bacteria and inhibits the growth of Gram-positive organisms. Gram staining was performed to observe bacterial morphology, followed by biochemical identification using Sulfur-Indole-Motility (SIM) tests to confirm characteristic features of *Klebsiella pneumoniae* (Jung and Hoilat, 2024).

Star anise extraction was performed using the maceration method. A total of 150 g of dried star anise was soaked in ethanol for 72 hours at room temperature. The filtrate was then concentrated using a rotary evaporator to obtain a viscous crude extract. Phytochemical screening was conducted to detect bioactive compounds, including saponins, alkaloids, tannins, flavonoids, and trans-anethole, which are known to possess antibacterial properties (Nguyen et al., 2021).

The crude extract was diluted using dimethyl sulfoxide (DMSO) to obtain concentrations of 20%, 40%, 60%, 80%, and 100%. Antibacterial activity was evaluated using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (MHA) plates inoculated with ESBL-producing *Klebsiella pneumoniae*. Sterile paper discs impregnated with each concentration of star anise extract were placed on the agar surface. A 10 µg meropenem disc was used as the positive control, while a DMSO-impregnated disc served as the negative control. Following incubation, the diameter of the inhibition zones was measured to assess antibacterial activity.

The inclusion of a 100% extract concentration may appear unusually high. However, this concentration was intentionally used to determine the maximum inhibitory potential of the crude extract under experimental conditions. While such a concentration may not directly reflect clinically applicable dosing, it provides important preliminary data regarding the extract's intrinsic antibacterial capability. In clinical or pharmaceutical applications, standardized or lower concentrations would be more relevant.

It should also be noted that this study employed only the disc diffusion method, which provides preliminary qualitative and semi-quantitative data. Further investigations, including determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), are recommended to obtain more comprehensive and quantitative evidence of antibacterial efficacy.

RESULTS AND DISCUSSIONS

Identification of *Klebsiella pneumoniae*

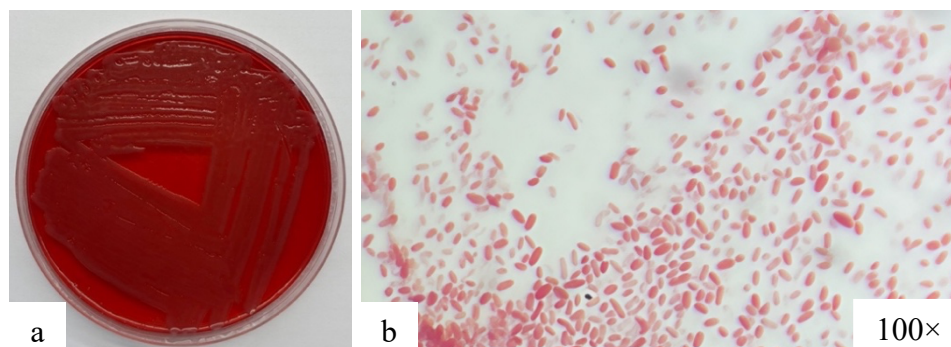


Figure 1. Identification results of *Klebsiella pneumoniae* on MacConkey agar media and Gram staining (a) Growth of *Klebsiella pneumoniae* on MacConkey agar showing mucoid lactose-fermenting colonies. (b) Gram staining of *Klebsiella pneumoniae* observed under 100× magnification showing Gram-negative bacilli

Klebsiella pneumoniae growth on MacConkey agar showed pink, mucoid colonies, which are characteristic of this bacterium. These colonies indicate lactose fermentation, evidenced by the color change to pink on the selective medium (Figure 1.a). Furthermore,

Gram staining revealed red-colored, rod-shaped (*bacilli*) bacteria, confirming that *Klebsiella pneumoniae* is a Gram-negative bacterium (Figure 1.b) (Lenchenko et al., 2020).



Figure 2. Identification results of *Klebsiella pneumoniae* by biochemical test

Table 1. Identification results of *Klebsiella pneumoniae* by biochemical test

Test Name	Result	Interpretation
Sulfur	(-)	No black deposits form on the medium
Motility	(-)	Bacterial growth only along the inoculation line
Indole	(-)	No red rings form on the surface of the medium

Table 1 presents the findings of *Klebsiella pneumoniae* identification using biochemical tests to characterise their qualities. The sulfur test yielded a negative result, with no black deposits accumulating in the media, showing that this bacterium is incapable of making H₂S from sulphur compounds. The *Motility* test similarly produced negative findings, with bacterial growth apparent solely along the inoculation line, showing that *Klebsiella pneumoniae* was non-motile or incapable of moving actively. Furthermore, the *Indole* test yielded a negative result, as evidenced by the absence of a red ring on the medium's surface, confirming that *Klebsiella pneumoniae* was incapable of producing indole from the breakdown of the amino acid tryptophan (Thakur et al., 2021).

Identification of active compounds of star anise (*Illicium verum*) extract

Phytochemical screening of star anise (*Illicium verum*) extract revealed the presence of various bioactive components with potential antibacterial activity. The saponin test showed a positive result with the formation of stable foam that persisted for 10 minutes (Figure 3.a), although no significant foam formation was observed in this study. This result contradicts several previous studies, such as those reported by Nguyen et al. (2021), which showed clear foam formation in star anise extract. This could be due to differences in extraction methods or concentrations of active ingredients used. The alkaloid test also yielded a positive result with a brown precipitate, indicating the presence of alkaloids in the star anise extract (Figure 3.b).

This finding aligns with research by Idris et al. (2024), which also reported the presence of alkaloids in *Illicium verum* extract that contribute to its biological activity. Furthermore, the flavonoid test showed a positive result with the extract solution turning red-orange (Figure 3.c), indicating the presence of flavonoids, compounds known to have various biological activities, including antibacterial activity (Wu et al., 2022). The tannin test also yielded a positive result, indicated by the appearance of a bluish color in the extract solution (Figure 3.d). This finding is similar to a report by Hayati and Lestari (2020), who noted the presence of tannins in *Illicium verum* extract, which have

antimicrobial activity. Furthermore, the trans-anethole test yielded a positive result, with the extract solution turning yellow (Figure 3.e). Trans-anethole, a major component of star anise essential oil, is known to have significant antibacterial potential against Gram-negative bacteria (Wu et al., 2022).

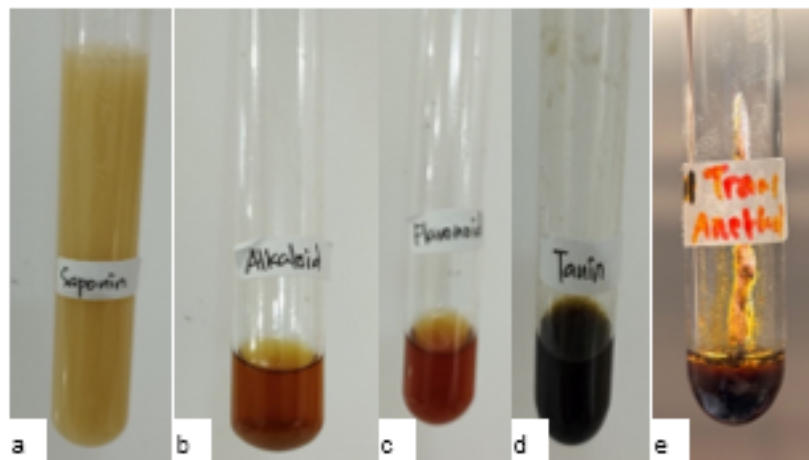
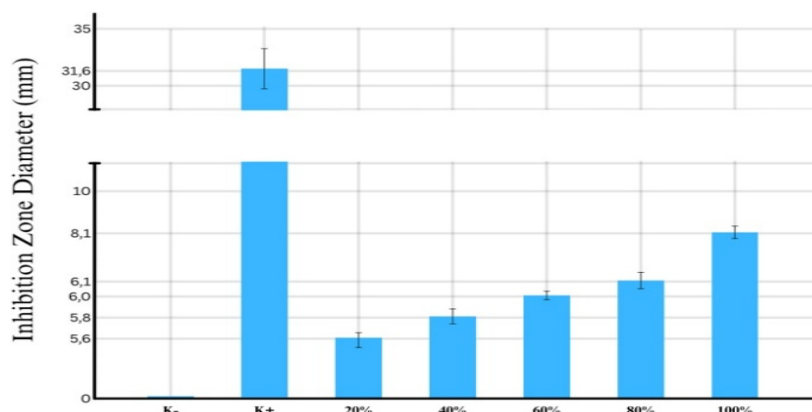


Figure 3. Results of identification of active compounds of Star anise (*Illicium verum*) extract (a) Saponin test of Star anise (*Illicium verum*) extract (b) Alkaloid test of Star anise (*Illicium verum*) extract (c) Flavonoid test of Star anise (*Illicium verum*) extract (d) Tannin test of Star anise (*Illicium verum*) extract (e) trans-anethole test of Star anise (*Illicium verum*) extract

Phytochemical analysis of star anise (*Illicium verum*) extract revealed the existence of many bioactive components with potential antibacterial activity. A positive saponin test resulted in the production of a stable foam lasting 10 minutes (Figure 3.a). The alkaloid test revealed a positive result in the form of brownish deposits (Figure 3.b). The flavonoid test yielded positive findings with the extract solution turning red-orange (Figure 3.c). A positive tannin test result is indicated by the appearance of a bluish colour in the extract solution (Figure 3.d). Meanwhile, the trans-anethole test yielded a positive result, with the extract solution turning yellow (Figure 3.e). These findings suggest that star anise extract contains active components such as saponins, alkaloids, flavonoids, tannins, and trans-anethole, all of which have biological activity, including antibacterial properties.

Test results of antibacterial effectiveness of star anise (*Illicium verum*) extract against *Klebsiella pneumoniae*



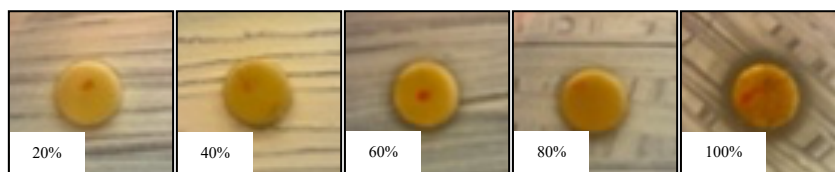


Figure 4. Inhibition zone diameter of star anise (*Illicium verum*) extract against *Klebsiella pneumoniae* (a) Inhibition zone diameter of star anise (*Illicium verum*) extract against *Klebsiella pneumoniae* graphic (b) Antibacterial effectiveness of star anise (*Illicium verum*) extract against *Klebsiella pneumoniae* on Mueller Hinton agar media

The antibacterial effectiveness test of star anise seed extract (*Illicium verum*) against *Klebsiella pneumoniae*, as shown in Figure 4, demonstrated the formation of an inhibition zone from star anise extract at concentrations of 20%, 40%, 60%, 80%, and 100% against the growth of *Klebsiella pneumoniae* bacteria, indicating that star anise extract has antibacterial properties (Kačániová et al., 2024).

Data analysis

Data analysis was performed using a one-way ANOVA test to compare the effects of star anise (*Illicium verum*) extract concentrations at 20%, 40%, 60%, 80%, and 100% on inhibiting the growth of *Klebsiella pneumoniae*. The results of the one-way ANOVA test showed significant differences between extract concentration groups, with a significance level of $p < 0.05$, confirming that star anise (*Illicium verum*) extract has an inhibitory effect on the growth of *Klebsiella pneumoniae* (Darma, 2021; Hardani et al., 2020; Sugiyono, 2022). These findings indicate that higher extract concentrations correlate with greater bacterial growth inhibition, as reflected by an increase in the diameter of the inhibition zone on Mueller Hinton Agar. For the one-way ANOVA analysis, the experiment was conducted with three biological replicates ($n=3$) for each extract concentration. Prior to conducting the ANOVA, the data were tested to ensure that the assumptions of normality and homogeneity of variance were met. Normality was tested using the Shapiro-Wilk test, and homogeneity of variance was tested using Levene's test. Both assumptions were met, as indicated by p-values greater than 0.05 in both tests. For further analysis, a Tukey HSD post-hoc test was performed to identify which groups differed significantly.

Characteristic differences between ESBL and Non-ESBL strains of *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative bacterium with a thick capsule, which plays a key role in its virulence. The main difference between ESBL and non-ESBL strains lies in their ability to produce extended-spectrum β -lactamase (ESBL) enzymes, which can degrade a variety of β -lactam antibiotics, including penicillins and cephalosporins. Non-ESBL strains of *Klebsiella pneumoniae* do not produce this enzyme, have a lower biofilm-forming ability, and a less active efflux pump system for releasing antimicrobial compounds (Ashurst and Dawson, 2023). In contrast, ESBL strains produce this enzyme, enhancing their ability to form biofilms that protect the bacteria from antibiotic penetration and increasing the activity of efflux pumps, which contribute to antibiotic resistance (Karampatakis et al., 2023; Ndlovu et al., 2023).

Effectiveness of *Illicium verum* extract against ESBL strains of *Klebsiella pneumoniae*

This study evaluated the antibacterial potential of star anise (*Illicium verum*) extract against ESBL strains of *Klebsiella pneumoniae*. The results showed that star anise extract has antibacterial potential, with an increase in the zone of inhibition with increasing extract concentration. At a concentration of 20%, the extract produced a zone of inhibition of 5.7 mm, while at a concentration of 100%, this increased to 8.1 mm. However, the resulting zone of inhibition was still significantly smaller than that of the positive control (meropenem, 31.6 mm), indicating that although this extract has antibacterial properties, its ability to target ESBL strains is limited.

The reduced effectiveness of star anise extract against ESBL strains may be related to the structure of the outer membrane of *Klebsiella pneumoniae* bacteria, which is rich in hydrophilic lipopolysaccharides (LPS). This structure inhibits the penetration of lipophilic compounds such as alkaloids and trans-anetol contained in star anise extract. Furthermore, mutations in efflux pumps in ESBL strains increase the bacteria's ability to actively secrete antimicrobial compounds, such as flavonoids and tannins, which reduces the antibacterial effectiveness of star anise extract (Muhsinah et al., 2022; Salem et al., 2021).

Correlation between phytochemical content and antibacterial activity

Phytochemical screening of star anise extract revealed the presence of active compounds such as saponins, alkaloids, flavonoids, tannins, and trans-anethole, all of which are known to possess antibacterial potential. However, although star anise extract contains these compounds, its effectiveness against ESBL strains of *Klebsiella pneumoniae* is still limited. The antibacterial activity of saponins, flavonoids, and tannins is related to their ability to disrupt bacterial membranes, but their effectiveness is reduced by the complex structure of the LPS-rich outer membrane of Gram-negative bacteria. Furthermore, trans-anethole, known to have antibacterial activity against Gram-negative bacteria, may have difficulty penetrating these bacterial membranes. Therefore, there is a clear relationship between the phytochemical components in star anise extract and its antibacterial activity, although the inhibition is still limited.

Comparison with previous research

The results of this study align with previous studies showing that essential oils from star anise extract exhibit variable antibacterial activity against various microorganisms, including *Klebsiella pneumoniae*. For example, a study by Nguyen et al. (2021) found an MIC value for *Klebsiella pneumoniae* of 100 mg/mL, indicating that star anise extract requires a relatively high concentration to inhibit the growth of this bacterium. This supports our finding that although star anise extract showed an increase in the zone of inhibition with increasing concentration, the resulting zone of inhibition was still weak compared to the positive control.

Limitations and directions for further research

While this study provides important insights into the potential of star anise extract as an antibacterial agent against ESBL strains of *Klebsiella pneumoniae*, several limitations should be noted. First, this study was conducted only under in vitro conditions, meaning the results may not fully reflect the extract's effectiveness under in vivo conditions. Factors such as metabolism, immune system interactions, and bioavailability of the active compound in the human body could not be evaluated in this study.

Furthermore, this study did not investigate the specific mechanisms underlying the antibacterial activity of star anise extract, such as the interaction between the active compounds and bacterial cell structure.

To strengthen these findings, MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) tests should be conducted to provide a more comprehensive picture of the antibacterial effectiveness of star anise extract. Further research is also needed to explore the potential for developing complementary therapies based on star anise extract, which can be combined with conventional antibiotic therapy to treat resistant bacterial infections. Furthermore, *in vivo* studies are essential to evaluate the bioavailability and interactions of the active compounds in the human body, which will be a crucial step in developing star anise extract as an alternative antibacterial therapy.

CONCLUSIONS

The star anise extract (*Illicium verum*) investigated in this study contains a variety of bioactive compounds, including antimicrobial saponins, alkaloids, flavonoids, tannins, and trans-anethole. While the extract demonstrated some antibacterial activity against *Klebsiella pneumoniae* in a concentration-dependent manner, the resulting inhibition zones were considerably smaller than those produced by the positive control, meropenem. This indicates that the extract possesses minimal antibacterial efficacy relative to a standard potent antibiotic, suggesting limited potential as a standalone treatment for *K. pneumoniae* infections, particularly those involving extended-spectrum beta-lactamase (ESBL)-producing strains. The study is primarily limited by its lack of in-depth analysis regarding the extract's mechanisms of action. The specific active compounds responsible for the observed antibacterial effect were not isolated or tested for their direct impact on *K. pneumoniae*, nor was their effect on key resistance mechanisms, such as ESBL production, explored. Furthermore, the *in vitro* nature of the research restricts the direct applicability of its findings to clinical settings. Factors like bioavailability, metabolism, and interactions with the host immune system, which would significantly influence the extract's efficacy *in vivo*, were not considered. Finally, the study did not determine critical quantitative metrics such as the Minimum Inhibitory Concentration (MIC) or Minimum Bactericidal Concentration (MBC), which are essential for accurately assessing and comparing the extract's antibacterial potency. To address these limitations, further research is essential. Future studies should prioritize determining the MIC and MBC to establish a baseline for the extract's effectiveness. Investigating the potential synergistic effects of combining star anise extract with conventional antimicrobials could reveal strategies to overcome resistance mechanisms, particularly in ESBL-producing strains. Additionally, optimizing the extraction process and developing novel formulations could enhance the bioavailability and stability of its active components. A deeper investigation into the interactions between star anise's purified active chemicals and *K. pneumoniae*'s cellular structure and resistance pathways is also crucial for developing more effective therapeutic strategies.

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CONFLICT OF INTEREST

The author(s) declare that they have no conflict of interest.

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