THE POTENTIAL OF BLACK CUMIN SEED OIL (Nigella sativa L.) AS A HALAL ANTIBACTERIAL AGENT AGAINST EXTENDED SPECTRUM Β-LACTAMASE (ESBL)-PRODUCING ESCHERICHIA COLI

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Submitted: January 24, 2025 Revised: February 22, 2025 Accepted: March 3, 2025

ABSTRACT

Infectious diseases remain a major issue in tropical countries such as Indonesia. Infectious diseases can be caused by pathogenic microorganisms such as bacteria, viruses, parasites, or fungi. Development of antibacterial agents from natural sources is essential to combat the growing issue of antibiotic resistance. One potential plant is black cumin seed (Nigella sativa L.) contains thymoquinone, a compound known for its antibacterial properties. In addition to thymoquinone, the essential oil of black cumin contains thymol, carvacrol, and p-cymene, along with metabolites such as alkaloids, saponins, and tannins. This study aimed to investigate the antibacterial activity of black cumin seed oil (Nigella sativa L.) against Extended Spectrum β-lactamase-producing Escherichia coli (E. coli ESBL). The antibacterial test in this study used the disc diffusion method at various concentrations, including 100%, 75%, 50%, and 25%. The extract was obtained by distillation, using pure water as the solvent. The test groups included a negative control, positive control using chloramphenicol, and negative control using pure water. The results of the antibacterial activity test showed that the positive control produced an average inhibition zone of 25 mm, classified as susceptible, whereas the negative control showed no inhibition zone (0 mm). At 100% concentration, the inhibition zone averaged 18,67 mm, at 75% it averaged 16 mm, at 50% it averaged 11,67 mm, and at 25%, the inhibition zone averaged 6,67 mm. The three lower concentrations were categorized as resistant.

***Keywords**: Black Cumin Seed, Essential Oil, Extended Spectrum β-Lactamase Producing Escherichia coli (E. coli ESBL), Nigella sativa L.*

INTRODUCTION

Indonesia, a developing country, faces significant challenges with infectious diseases, which remain a leading cause of high mortality rates. Every year, infections are responsible for the deaths of 3.5 million people, the majority of whom are poor children living in low-income and middle-income countries (Nurrosyidah et al., 2023). The incidence of infections caused by extended-spectrum β-lactamase (ESBL)-producing bacteria in the United States ranges from 0 to 25%, while in Europe, excluding the Netherlands, the incidence is less than 1%. The beta-lactam ring functions by binding to beta-lactamase enzymes, thereby inhibiting bacterial cell wall synthesis (Forbes et al., 2007; Nurjanah et al., 2020). However, there is an increasing misuse or irrational use of antibiotics for nonessential conditions, often with antibiotics being purchased over the counter or without a prescription, which has led to the development of antibiotic-resistant bacteria (Desrini, 2015).

In other Asian countries, the prevalence of ESBL-producing Escherichia coli and Klebsiella pneumoniae varies, with rates of 8.5% in Taiwan, 12% in Hong Kong, and 4.8% in Korea. Escherichia coli and Klebsiella pneumoniae are the most common ESBL-producing bacteria. ESBL-producing bacteria can also infect the skin, particularly in open wounds, in addition to causing infections in the urinary tract and intestine. The symptoms include redness and discharge in the infected area. These infections not only cause physical and financial harm but also negatively impact national productivity (Nurrosyidah et al., 2021).

The spread of infectious diseases in Indonesia is a significant public health concern, with bacteria like Escherichia coli (E. coli) and Klebsiella pneumoniae (K. pneumoniae) being major contributors (Angelina, et al.,2015). These bacteria, particularly those producing extended-spectrum β-lactamases (ESBL), exhibit resistance to β-lactam antibiotics, making treatment challenging (Novard et al., 2019). Studies have indicated that ESBL-producing bacteria are frequently found in the Enterobacteriaceae family and exhibit resistance to β-lactam antibiotics (Pajariu, 2010). ESBL-producing bacteria can spread through contaminated food, direct contact, and poor hygiene, causing infections with symptoms, such as diarrhea, nausea, and stomach pain. The increasing misuse of antibiotics further exacerbates bacterial resistance, limits treatment options, and poses a global health threat (Nurrosyidah et al., 2022).

Indonesia's rich biodiversity makes it a promising source of natural antibacterial agents. Black cumin (*Nigella sativa* L.), traditionally used in herbal medicine, has demonstrated antibacterial properties attributed to active compounds, such as thymoquinone, flavonoids, and tannins (Sulistiawati et al., 2014; Balkrishna et al., 2024). Previous studies have shown its effectiveness against various bacteria, including *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Despite its traditional use in treating infections, further scientific validation is necessary to establish its antibacterial efficacy against ESBL-producing bacteria (Mulyono, 2013; Callixte et al., 2021).

This study aims to evaluate the antibacterial activity of black cumin seed oil in inhibiting bacterial growth, particularly against ESBL-producing E. coli. By assessing its effectiveness, this study seeks to explore the potential of black cumin as a natural antibacterial alternative, addressing the urgent need for new strategies to combat antibiotic-resistant infections.

METHOD

Plant Material and Preparation

Plant Source: Black cumin seeds (*Nigella sativa* L.) used in this study were sourced from UPT Materia Medika Batu Malang, East Java with ID number 067/ 082/ 102.20/ 2023. The extraction of black cumin (Nigella sativa L.) seed oil using steam distillation requires a boiling flask (1000–5000 mL), stainless steel or glass distillation chamber, water-cooled or air-cooled condenser, and collection vessel for oil separation. A grinding machine was used to finely crush the dried seeds, and a digital scale ensured precise measurement. A heating source, such as an electric mantle or gas burner, provides controlled steam generation, which is monitored by a thermometer (0–100°C). A water circulation system maintains condenser efficiency, and filter paper with a funnel removes impurities. The extracted oil was stored in amber glass bottles to protect the bioactive compounds. Essential materials include dried black cumin seeds, distilled water for steam production, and ethanol for equipment sterilization.

Extraction: The powdered seeds were subjected to extraction using the The black cumin seed powder was subjected to extraction using steam distillation with pure water. Extraction of black cumin (*Nigella sativa* L.) seed oil using steam distillation begins with cleaning, drying, and grinding the seeds into powder. The steam is then passed through the powder, causing the volatile oil components to evaporate and condense into a liquid mixture. The essential oil was separated from the aqueous distillate (hydrosol) and filtered to remove impurities. It was then stored in amber-colored containers to maintain stability. This method preserves bioactive compounds, ensuring the effectiveness of the oil for medicinal and pharmaceutical applications.

Antibacterial Activity Testing

Bacterial Strains: The Escherichia coli (*E. coli*) producing Extended Spectrum β-lactamases (ESBL) was used as the test organism. The bacterial strain was procured from a reliable culture collection or laboratory and was confirmed for ESBL *production*. The ESBL-producing strains were obtained from Airlangga Hospital.

Disc Diffusion Method: The antibacterial activity of black cumin seed extract was evaluated using the disc diffusion method. This method involves the following steps:

1. Preparation of Inoculum: A bacterial suspension of E. coli was prepared in Mueller-Hinton broth and adjusted to a McFarland turbidity standard of 0.5 to achieve a concentration of approximately 1 × 10^8 CFU/mL (Nurrosyidah & Mertaniasih, 2020).
2. Agar Plate Inoculation: Mueller-Hinton agar plates were inoculated with the bacterial suspension using a sterile swab to ensure even distribution of bacteria on the agar surface.
3. Disc Application: Sterile paper discs (6 mm in diameter) were impregnated with different concentrations of black cumin seed extract (100, 75, 50, and 25%). The discs were placed on inoculated agar plates. Control discs containing pure water and chloramphenicol (antibiotic control) were also used.
4. Incubation: Plates were incubated at 37°C for 24 hours.
5. Measurement of Inhibition Zones: After incubation, the zones of inhibition around the discs were measured in millimeters using calipers. The sizes of the inhibition zones were compared with those of the control discs to determine their antibacterial activity.

Quality Control

Negative Control: Pure water was used as the negative control to ensure that any observed inhibition was due to the extract and not the solvent.

Positive Control: Chloramphenicol was used as the positive control to confirm the susceptibility of E. coli to a known antibiotic.

RESULT AND DISCUSSION

Antibacterial activity

In this study, six treatment groups were used: a positive control, a negative control, and concentrations of 100%, 75%, 50%, and 25%. The results are shown in the table below:

Table I. Antibacterial activity Black Cumin Seed Essential Oil (*Nigella sativa* L.) Against Extended Spectrum β-Lactamase Producing *Escherichia coli* (E. coli ESBL)

Sample group	Replication	Inhibition zone (mm)	Avarage	Category
Kloramfenikol (+) Control	1	25	25	*Susceptibel*
	2	25		
	3	25		
Pure water	1	0	0	-

(-) Control	2	0		
	3	0		
100%	1	19	18,67	*Suscebtible*
	2	19		
	3	18		
75%	1	16	16	*Suscebtible*
	2	16		
	3	16		
50%	1	11	11,67	*Intermediate*
	2	10		
	3	11		
25%	1	5	6,67	*Resistant*
	2	6		
	3	6		

According to CLSI (Clinical and Laboratory Standards Institute) guidelines, antibacterial categories based on the zone of inhibition measurements are as follows:

1. Susceptibility: Bacteria are considered susceptible if the zone of inhibition is greater than the threshold set by CLSI, indicating that the bacteria can be effectively controlled by the antibacterial agent at clinically relevant concentrations (16-20 mm).
2. Intermediate: Bacteria fall into the intermediate category if the zone of inhibition falls within the range specified by the CLSI, suggesting that the bacteria may be controlled at higher concentrations of the agent or under specific conditions (15-10 mm).
3. Resistance: Bacteria were classified as resistant if the zone of inhibition was smaller than the threshold set by CLSI, indicating that the antibacterial agent was ineffective at standard concentrations (less than 10 mm) (https://clsi.org/).

Based on the antibacterial activity results for black cumin oil at concentrations of 100% and 75%, the inhibition zone was classified as strong or susceptible. This indicates that black cumin oil demonstrates significant antibacterial activity at these concentrations, depending on the size of the inhibition zones, compared to the standards provided by relevant guidelines, such as those from CLSI or other authoritative sources. If the inhibition zones were large, the oil would be highly effective at these concentrations. Thymoquinone, a major bioactive compound found in black cumin seeds (Nigella sativa), has been studied for its antibacterial activity. Thymoquinone acts by disrupting the bacterial cell membrane, leading to the leakage of intracellular components and cell death. This disruption is attributed to the ability of the compound to interact with lipid bilayers and alter the membrane permeability (Elnawasany, 2022; Amit Baran, 2016).

Black cumin seeds (Nigella sativa) are composed of the following components:

1. Fixed Oil (32-40%): This oil contains unsaturated fatty acids, such as arachidonic, eicosadienoic, linoleic, linolenic, oleic, almitoleic, palmitic, stearic, and myristic acids. Additionally, it contains beta-sitosterol, cycloeucalenol, cycloartenol, sterol esters, and sterol glucosides (Tembhurne et al., 2014).
2. Volatile Oil (0.4-0.45%): The volatile oil contains saturated fatty acids and several active compounds, including nigellone, which is the sole component of the oil's carbonyl fraction, along with Thymoquinone (TQ), thymohydroquinone (THQ), dithymoquinone, thymol, carvacrol, α and β-pinene, d-limonene, d-citronellol, p-cymene, carvacrol, t-anethole, 4-terpineol, and longifoline(Tembhurne et al., 2014).

3. Alkaloids: Black cumin seeds contain two types of alkaloids: isoquinoline alkaloids, such as nigellicimine and nigellicimine n-oxide, and pyrazol alkaloids, such as nigellidine and nigellicine (Tembhurne et al., 2014).
4. Nutritional Components: The seeds are rich in vitamins, carbohydrates, minerals, fats, and proteins, including eight or nine essential amino acids.
5. Additional Compounds: Black cumin seeds also contain saponins and alpha hederine, with trace amounts of carvone, limonene, and citronellol. They provide substantial amounts of vitamins and minerals such as iron (Fe), calcium (Ca), potassium (K), zinc (Zn), phosphorus (P), and copper (Cu) (Forouzanfar et al., 2014).
6. The pharmacological effects of black cumin seeds are largely attributed to thymoquinone (TQ), the predominant quinone component (Tembhurne et al., 2014)..

CONCLUSION

Based on the research conducted, it can be concluded that black cumin seed oil (*Nigella sativa* L.) exhibits antibacterial activity against Escherichia coli extended-spectrum β-lactamases (ESBL), showing strong inhibition at concentrations of 100% and 75%, with an inhibition zone of 16–19 mm. Therefore, black cumin seed oil holds significant potential for development as an antibacterial agent, particularly against resistant bacteria such as ESBL

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