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ARTICLE

TESTING THE ANTIBACTERIAL EFFECTIVENESS OF BLACK CUMIN EXTRACT ON THE GROWTH OF BACTERIA *Bacillus cereus* Disk Diffusion Method

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ABSTRACT

Indonesia is known to have many medicinal plants and spices that can be used as traditional medicines, which have long helped cure various diseases because they do not cause side effects. One of these conventional medicines is black cumin (*Nigella sativa*), which contains thymoquinone, thymohydroquinone, and tannin, the main chemical substances that function as antibacterials. This research was conducted to determine the antibacterial effectiveness of black cumin extract against the growth of *Bacillus cereus* bacteria. This research is an experimental laboratory research using the disc diffusion method. Results: Black cumin extract was proven to weakly inhibit the growth of *Bacillus cereus* bacteria at concentrations of 10%, 25%, 50%, 75%, and 100% with an average diameter of 8.67 mm, 10.67 mm, 12 mm, 16.33mm, and 17.67mm. Conclusions from research testing the antibacterial effectiveness of black cumin extract at concentrations of 10%, 25%, 50%, 75%, and 100%, based on the Greenwood classification at concentrations of 10%, 25%, and 50%, have weak inhibitory power. In comparison, 75% and 100% concentrations have moderate inhibitory power against the growth of *Bacillus cereus* bacteria.

Keywords: Black Cumin Extract; *Bacillus cereus*; Diffusion method

АБСТРАКТ

В Индонезии произрастает множество лекарственных растений и специй, которые можно использовать в качестве традиционных лекарств, издавна помогающих лечить различные заболевания, поскольку они не вызывают побочных эффектов. Одним из таких традиционных лекарств является черный тмин (Nigella sativa), который содержит тимохинон, тимогидрохинон и танин - основные химические вещества, выполняющие функцию антибактериальных средств. Данное исследование проводилось с целью определения антибактериальной эффективности экстракта черного тмина против роста бактерий Bacillus cereus. Данное исследование является экспериментальным лабораторным исследованием с использованием метода диффузии дисков. Результаты: Было доказано, что экстракт черного тмина слабо подавляет рост бактерий Bacillus cereus в концентрациях 10%, 25%, 50%, 75% и 100% со средним диаметром 8,67 мм, 10,67 мм, 12 мм, 16,33 мм и 17,67 мм. Выводы по результатам исследования антибактериальной эффективности экстракта черного тмина в концентрациях 10%, 25%, 50%, 75% и 100%, основанные на классификации Гринвуда, при концентрациях 10%, 25% и 50%, обладают слабой ингибирующей способностью. Для сравнения, 75%-ная и 100%-ная концентрации обладают умеренной ингибирующей способностью в отношении роста бактерий Bacillus cereus.

Ключевые слова: Экстракт черного тмина; Bacillus cereus; диффузионный метод

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INTRODUCTION

Black cumin (Nigella sativa) is popular in the Middle East, Africa, Asia, and Indonesia. Health benefits from this plant include protection and treatment. Muslims think black cumin can cure everything except aging and death. In vitro and in vivo black cumin seed extract and oil research has advanced during the past two decades. Black cumin seed extract with thymoquinone, fixed oil, and its derivatives was immunopotentiating, anti-histamine, anti-diabetic, anti-hypertension, anti-inflammatory, and antimicrobial.[1,2]

Black cumin (*Nigella sativa*) is a plant belonging to the Ranunculaceae family, native to Mediterranean countries and exported to various countries worldwide. Research has shown that black cumin plant extract has antibacterial solid activity.[3] The seeds of black cumin contain chemical compounds such as thymoquinone, thymohydroquinone, and thymol. Additionally, ethanol extract from black cumin has been proven to contain alkaloids, saponins, and steroids.[4] Several research have shown black cumin's antimicrobial properties. Aisniyah used disc diffusion to show that black cumin seed extract inhibits E. coli growth at 50%, 75%, and 100%. Toppozada found phenolic components in Nigella sativa oil, while Mahony et al. found that hot-water-extracted Nigella was 100% bactericidal against Helicobacter pylori. Zaidi et al. discovered that Nigella sativa extracted with 70% alcohol suppressed Helicobacter pylori growth at 500µg/ml, with the minimum bactericidal concentration (MBC) at 62.5µg/ml. Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Salmonella typhimurium, Salmonella enteritidis, and S. aureus have been shown to be significantly affected by Nigella sativa in various solvents and concentrations.[2]

Bacteria enter and multiply in the body, causing infection7. Bacillus cereus and Shigella sonnei cause infections. Bacillus cereus bacteria metabolize carbohydrates, fats, and proteins, spoiling and poisoning food.[8] Between 2000 and 2015, there were 175 outbreak reports in Indonesia, 19.4% of which were caused by *Bacillus cereus.*[9]

Food poisoning and gastrointestinal illnesses are caused by Bacillus cereus, which causes diarrhea. Enterotoxins cause food poisoning. Food enterotoxins are caused by endospores. Cooking does not kill food endospores.[10]

Based on the description above, research was carried out to directly test the antibacterial effectiveness of black cumin extract (*Nigella sativa*) on the growth of *Bacillus cereus* using the disc diffusion method.

MATERIAL AND METHODS

The disc diffusion test for Bacillus cereus antibacterial activities of Black Cumin Extract. Microbiology Laboratory, Faculty of Medicine, UIN Syarif Hidayatullah Jakarta, will undertake this research from July 2022 to June 2023. FK UI microbiology lab and BALITRO black cumin harvest provided Bacillus cereus. Tweezers, incubator, beaker, measuring cup, Erlenmeyer, LAF, vortex, oven, autoclave, magnetic hot plate stirr, Mc Farland standard 0.5 CFU (1.5 x 108), refrigerator, test tube rack, test tubes, spoons, micropipettes, Petri plates, spreader glass, round tube needle
In this study, black cumin extract (Nigella sativa), 96% ethanol, 70% alcohol, Bacillus cereus bacteria, aluminum foil, distilled water, NA/MHA media, NaCl, tips, blank disk, ten µg ampicillin disk, and spiritus were Create stocks of black cumin extract at 0% (control), 10%, 25%, 50%, 75%, and 100% in triplicate using Amoxicillin as a positive control and distilled water as a negative control.
Three blank paper disks in each concentration vial should dissolve in the diluted black cumin extract after 15 minutes. Mix one dose of nutritious agar Bacillus cereus germs with 10 ml of sterile NaCl in a test tube and vortex. Standardizing bacterial turbidity at 0.5 Mc Farland. To inoculate Mueller Hinton agar, a sterile swab is dipped into the NaCl bacterial sample. MHA plate germ-injected media Use tweezers and MHA Petri dishes with bacterial suspensions to assess antibacterial action. Per

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petri plate, write 10%, 25%, 50%, 75%, and 100% black cumin extract. Select each petri dish-soaked paper disk at 10%, 25%, 50%, 75%, and 100%. Lay the paper disk in the middle of the MHA petri plate at the same concentration as the diluted black cumin extract disk. Repeat the amoxicillin disk positive control test. We measured the clear zone diameter with a caliper or ruler the day after incubating the MHA with the paper disk at 37°C for 24 hours.

RESULT

Gram-positive This study used B. cereus. Antibacterial testing is easy with disk diffusion. Black cumin inhibited Bacillus cereus at 10%, 25%, 50%, 75%, and 100%. Concentration expands the inhibitory zone. This image illustrates inhibitory zone results:

Table 1. Results of measuring the activity of black cumin extract (*Nigella sativa*)on the growth of *Bacillus cereus* bacteria

Test	Black Cumin Extract Inhibition Zone (In Millimeters)						
	10%	25%	50%	75%	100%	Positive Control	Negative Control
1	9	10	11	17	17	12	0
2	10	11	11	16	18	13	0
3	7	11	14	16	18	15	0
Total	26	32	36	49	53	40	0
Mean	8,67	10,67	12	16,33	17,67	13,33	0

The results of the table demonstrate that Bacillus cereus is inhibited by 100% black cumin. There is a significant difference between the positive control and the zone that was formed. Amoxicillin played the role of the positive control. B. cereus could be lowered by 10% black cumin. MDA levels are reduced when rambutan honey is administered at doses of 250, 500, and 1000 mg/kgBW compared to both negative and positive controls. Honey made from rambutan boosted the number of normal hepatocyte cells at doses of 500 and 1000 mg/kgBW, which is indicative of regeneration. Honey containing 500 and 1000 mg/kg body weight of rambutan was found to have antioxidant and hepatoprotective activities in rats. It was found to increase the number of normal hepatocyte cells and lower MDA levels. It is difficult to beat 250 mg/kgBW. Vitamin C and a catalase-like superoxide dismutase are both produced by the flavonoids found in rambutan honey. when there is less MDA, there are more hepatocytes.

Table 2. MIC test results for black cumin extract against *Bacillus cereus* Extract concentration. Antibacterial activity test results against

Concentration of Black cumin extract	Antibacterial activity test results against *Bacillus cereus*
2%	-
4%	-
6%	-
8%	-
9%	-
10%	+
Positive Control	+
Negative Control	-

Note: (-) = There is no inhibition zone
(+) = There is an inhibition zone

The minimum inhibitory concentration of black cumin extract is determined by determining the lowest level of the extracted sample that still provides antibacterial activity against the test bacteria, namely *Bacillus cereus*. Based on the table above, it can be seen that the level of black cumin extract in ethanol can inhibit the growth of *Bacillus cereus* at a concentration as low as 10%.

DISCUSSION

Fera and Cita also tested black cumin extract (methanol) against Bacillus cereus

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bacteria at 2, 4, 6, and 8 mg/disk, finding an inhibitory zone of 9.3, 13.3, 18.5, and 19.5 mm. The ethyl acetate component of black cumin extract at 6 mg/disk inhibited Bacillus cereus bacteria with a 9.7-mm zone.[11]

The results of this study were compared with the results of research by Fera and Cita[11], showing that the inhibition zone was more petite because the concentration used was also a different method.

Recent investigations indicate that ethanol extract of black cumin seeds at 2.5 mg/disk inhibits Bacillus subtilis bacteria with a zone of 1.328 ± 0.028 cm and Escherichia coli with 1.187 ± 0.103 cm.[5] Previous research has proven that black cumin can inhibit the growth of *Escherichia coli* bacteria at concentrations of 10, 7.5, and 50 mg/disk. In research conducted by Salma.[6] *Nigella sativa* extract had antibacterial activity against *Shigella dysenteriae* at a concentration of 0.175 mg/disk with an inhibition zone of 35.66 mm. Black cumin inhibited the growth of several bacteria, including *Escherichia coli, Salmonella typhii, and Staphylococcus aureus.*[5]

Nevi Selvita researched the effectiveness of black seed oil on the growth of Staphylococcus aureus, concluding that black seed oil at a concentration of 10% based on the Greenwood classification did not have an inhibitory effect on the development of *Staphylococcus aureus* bacteria. Meanwhile, at concentrations of 30%, 40%, and 50%, each has an inhibition zone of 26.5 mm, 29 mm, and 36 mm. According to the Greenwood classification, the inhibition zone includes potent inhibition of the growth of *Staphylococcus aureus.*[12]

Guntur Satrio researched the potential inhibitory power test of black cumin seed methanol extract against *Streptococcus mutans* bacteria. The test results showed that there was an activity that could inhibit the growth of *Streptococcus mutans* bacteria from low concentrations of 3%, 4%, 5%, 6%, and 7%, providing the highest inhibitory power at a concentration of 6%, namely 0.83 mm, and the lowest concentration at a concentration of 3%, namely 0.69 mm, against *Streptococcus mutans* bacteria.[13]

Salma again tested Nigella sativa against Shigella dysenteriae germs using black cumin seed extract. This study examined black cumin seed extract at 1%, 1.25%, 1.5%, and 1.75%. The study found substantial Shigella bacteria suppression at all doses. Black cumin seed extract inhibited Streptococcus pyogenes, Pseudomonas aeruginosae, Klebsiella pneumoniae, and Proteus vulgaris bacteria in distilled water, according to Aishah. The concentrations are 100, 50, 20, 10, 5, and 1 mg/ml. A 15-mm inhibitory zone was observed for Klebsiella pneumonae and Proteus vulgaris at 100 mg/ml. Four bacteria showed no inhibitory zone at 1mg/ml, 5mg/ml, and 10mg/ml.[6]

In another Musdalifah trial, black cumin oil suppressed Salmonella typhi growth at 80% and 100% with a small diameter, less effectively than chloramphenicol. This differs from Noorhamdani's research at Brawijaya University's Faculty of Medicine, which revealed that black cumin seed extract had a 45% MIC and 47.5% KBM against Salmonella typhi. Black seed oil's antibacterial qualities derive from thymoquinone, dithymoquinone, hydroquinone, thymol, and tannin. Thymoquinone's irreversible reaction with nucleophilic amino acids may inactivate bacterial proteins. Tannins create hydrophobic protein molecules that inactivate adhesins, enzymes, and cell wall transport proteins, preventing microorganism growth.[6,14] The description above proves black cumin seed extract is antibacterial against Staphylococcus aureus. Thymoquinone in black seed oil synergistically outperforms ceftriaxone as a positive control.

Fatima found that black cumin seed extract inhibited gram-positive bacteria more than gram-negative bacteria. Due to the cell wall's outer membrane, which acts as a permeability barrier and inhibits polyphenol absorption, phenolic chemicals were more effective against gram-positive bacteria than gram-negative bacteria.[15] Fatima found that black cumin seed extract inhibited gram-positive bacteria more than gram-negative bacteria. Due to the cell wall's outer membrane,

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phenolic chemicals were more effective against gram-positive bacteria than gram-negative bacteria. which acts as a permeability barrier and reduces the absorption of polyphenols.[15] Several investigations have found that black cumin extract, also known as Black Seed, contains bactericidal saponins, thymohydroquinone, phenylpropanoids, and aminoglycosides. Thymohydroquinone is a quinone, while saponin is an aminoglycoside glycoside. Aminoglycosides hinder protein production.[16]

Inoculum concentration, incubation period, disk attachment time, agar medium thickness, and media composition affect Kirby-Bauer inhibition zone size. Suspension turbidity impacts inhibition zone diameter. Too much turbidity reduces the inhibitory zone's diameter, while too little turbidity increases it. Around 4 mm is the ideal agar medium thickness. Thin agar media accelerates extract diffusion. A thick agar media slows extract diffusion.[17]

Dissolved components in black cumin essential oil alter its bacterial growth inhibitory activity. Lots of parts make up essential oils. According to several studies, Cyemene, thymoquinone, pinene, and carvacrol prevent bacteria growth.[18] Traditional medicine uses black cumin seeds for numerous ailments. By soaking alcohol with ethanol, cumin seed compounds become antimicrobial. These have 96% alkaloids, flavonoids, saponins, and proteins. Alkaloids interact with bacterial DNA and cell wall amino acids. Some amino acids react with alkaloid base groups, affecting bacterium structure and amino acid composition. The genetic balance of DNA amino acids in the bacterial cell nucleus is altered by DNA sequence changes, favoring lysis. The bacteria will inactivate and dissociate.[6]

This study employed ethanol to dissolve black cumin extract since it is inert and won't react with other components. Low boiling point ethanol separates oil from solvent readily during distillation. A 96 percent solvent, ethanol attracts polar, non-polar, and semi-polar molecules. This attracts several active chemicals to the plant. Ethanol is a solvent because it is less harmful than methanol.[16]

Amoxicillin, a broad-spectrum antibiotic, was employed as a positive control in this investigation. Amoxicillin inhibits cell wall mucopeptide production, namely the peptidyl transferase enzyme, which catalyzes peptide bonds during bacterial protein synthesis.[16]

This study's inhibition zone results are affected by many things, stated Anggita. Treatment, test microorganisms, and medium can cause these traits. Media factors include agar depth, pH, and storage temp. Kind, sample response, and origin (cultured or specimen) are bacteria parameters. Ventilation, bacterial contamination testing, injection-disc time, and inoculation-incubation conditions affect treatment.[19]

CONCLUSION

This study concludes: Bacillus cereus was inhibited by black cumin. A 10% (8.67 mm), 25% (10.67 mm), 50% (12 mm), 75% (16.33), and 100% (17.67 mm) inhibition of Bacillus cereus growth by black cumin Under the Green Wood classification, black cumin extract can inhibit Bacillus cereus growth at 10%, 25%, 50%, 75%, and 100%.

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DECLARATIONS

Conflict of interest . The author declares no conflict of interest.

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