BioLink
Jurnal Biologi Lingkungan, Industri, Kesehatan

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EFFECTIVENESS TEST OF BLACK CUMIN SEEDS (NIGELLA SATIVA) EXTRACT ON THE GROWTH OF KLEBSIELLA PNEUMONIAE AND PSEUDOMONAS AERUGINOSA BACTERIA

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Submitted : 24-04-2020; Reviewed :06-10-2020; Accepted : 18-12-2020

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Abstract

Black cumin seeds are known to have beneficial contents as antibacterial. This study aimed to determine the effectiveness of black cumin seed extract (Nigella sativa) on the growth of Klebsiella pneumoniae and Pseudomonas aeruginosa. The method used was the disc diffusion method. The ethanol extract of black cumin seeds gain by maceration method and was made into various concentration (5, 7.5, 10, 12.5, and 15)%. For positive control, ciprofloxacin disc were used and negative control used distilled water. The research model used was in vitro experimental research. The results showed that ethanol extract of black cumin seeds have antibacterial activity against Klebsiella pneumoniae with inhibition zone diameters (4.5, 6.7, 10.1, 11, 12.8) mm, Pseudomonas aeruginosa with inhibition zone diameters (7.5, 12, 12.6, 13.4, 15.6) mm, positive controls respectively (24.3 and 20.7) mm and negative controls (0). It can be concluded that black cumin seed extract has an inhibiting effect on the growth of Klebsiella pneumoniae and Pseudomonas aeruginosa.

Keywords: Black Cumin Seed; Disc Diffusion; Ethanol Extract; Klebsiella pneumoniae; Pseudomonas aeruginosa

How to Cite: Putri, N.N., Chiuman, L., Ginting, C.N., & Girsang, E. (2021). Effectiveness Test Of Black Cumin Seeds (Nigella sativa) Extract On The Growth Of Klebsiella pneumoniae and Pseudomonas aeruginosa Bacteria, BioLink: Jurnal Biologi Lingkungan, Industri dan Kesehatan, Vol.7 (2): Hal. 130- 138

INTRODUCTION

Hospital-acquired pneumonia (HAP) or respiratory infection that occurs in health facilities is a health problem that we often encounter in the scope of work of medical personnel in the world. Most cases of nosocomial infections in hospitals are caused due to the use of medical devices such as ventilators, catheters, use of intravenous lines, or postoperative infections. HAP cases are most often caused by gram-negative bacilli such as *Pseudomonas aeruginosa*, *Eschericia coli*, *Klebsiella pneumoniae*, and *Acinetobacter* sp. Patients who are hospitalized in the hospital usually have weak immunity so that are getting infection easily (Bennett, 2018).

Klebsiella pneumoniae and *Pseudomonas aeruginosa* are pathogenic bacteria that often attack patients with weak immunity. These two bacteria often cause the high morbidity and mortality in HAP due to antibiotic resistance (Craven *et al.*, 2010).

The high incidence of resistance has made a lot of research on plants to be used as antibacterial agents, one of them is black cumin seeds (*Nigella sativa*). According to Al-Jassir (2008), black cumin seeds have the ability to be anti-histamine, anti-diabetes, anti-hypertensive, anti-inflammatory, anti-bacterial, anti-fungal,

anti-cancer, and enhancing immunity (Forouzanfar *et al.*, 2014). The antibacterial effect of black cumin seeds is caused by the content of thymohydroquinone, dithymoquinone, alkaloids, triterpene, saponins, thymol, and others which denature proteins in the bacterial cell wall and damage the bacterial cell cytoplasm (Sultana *et al.*, 2015; Silvani NS., 2018; Tacakkoli *et al.*, 2017).

The type of black cumin seeds itself can affect the antibacterial activity produced, such as a study (Bakathir *et al.*, 2011) where the researchers compared the antibacterial activity between types of black cumin seeds obtained in Ethiopia and Hadramout against *Staphylococcus aureus*. The results showed that the black cumin seeds obtained from Hadramout were stronger than those obtained in Ethiopia. This could be due to geography and the season when the cumin seeds were harvested.

Black cumin seeds have antibacterial benefits which have been proven by (Foroughi *et al.*, 2016) that black cumin seeds inhibit the growth of *Eschericia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* by the disc and well diffusion method at concentrations of 0.3%, 0.7%, 1.5 %, 3%, 6%, and 12.5% and the minimum inhibitory levels were obtained at a concentration of 3% against the growth of

Escherichia coli and *Staphylococcus aureus* while the minimum inhibitory levels on the growth of *Bacillus subtilis* occurred at a concentration of 1.5%. Other studies have also shown that black cumin had strong bacterial inhibition against gram-positive bacteria but weak against gram-negative bacteria (Bakathir *et al.*, 2011; Butar-Butar, 2018; Hannan *et al.*, 2008).

Based on the description above, it is necessary to conduct a research to determine the effectiveness of black cumin seed extract (*Nigella sativa*) on the growth of the bacteria *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Microbiology, Faculty of Medicine, Prima Indonesia University, Medan and the process of extracting black cumin seeds was carried out at the USU Mathematics and Natural Sciences Laboratory. This research was conducted from May to June 2019. This type of research used a pure laboratory experimental method with a post-test only group design approach. 1000gr of black cumin obtained from the Indian market, Medan was used as the research sample. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* bacteria were isolated and incubated in NA medium at 37 ° C for 24 hours.

The tools in this study were petri dishes, autoclave, aluminum foil, measuring cup, erlenmeyer, filter funnel, filter paper, disc paper, analytical balance, ose needle, test tube, tweezers, object glass, dropper pipette, camera, cotton pad, caliper. , and stationery.

The materials used were black cumin, distilled water, Nutrient Agar, Mc Farland solution, sterile NaCl, *Klebsiella pneumoniae* culture, *Pseudomonas aeruginosa* culture, ciprofloxacin antibiotic disc 5µ / L, and 96% ethanol.

Making Black Cumin Seed Extract. 1000gr of black cumin, mashed and soaked in 5000 ml of 96% ethanol. Macerated for one week at room temperature. The filtrate results were then in a rotary evaporator.

Concentration Making. The concentrations used were 5%, 7.5%, 10%, 12.5%, and 15%. Aquadest as a negative control and ciprofloxacin as a positive control. The calculation for the concentration of black cumin seed extract used the following dilution formula:

$$V1 \times M1 = V2 \times M2$$

Making NA Media and Bacterial Culture. NA powder was weighed as much as 20gr in 1 liter of distilled water and stirred until homogeneous and autoclaved. NA was poured into a petri dish and stored in the refrigerator until hardened. NA medium was then given a pure culture smear of *Klebsiella pneumoniae*. The same

thing was done for pure cultures of *Pseudomonas aeruginosa* (Wadud, 2014).

Testing stage. Empty test discs soaked in black cumin seed extract of various concentrations were placed on the agar surface with the *Klebsiella pneumoniae* bacteria culture. The media was then incubated at 37 ° C for 24 hours and then the diameter of the clear area was measured using a caliper. The same thing was done with the *Pseudomonas aeruginosa* bacteria.

Data processing. Data processing which includes editing, tabulation and grouping was done manually using Microsoft Office Word 2019 and the SPSS

2018 for Windows program which started with the normality test and homogeneity test to find out which test to use and continues with post hoc analysis.

RESULTS AND DISCUSSION

Antibacterial Test of Black Cummin Seed Ethanol Extract

This study used the disc diffusion method test with three repetitions with extract concentrations of 5%, 7.5%, 10%, 12.5%, and 15%. Below is a picture of the antibacterial test of ethanol extract of black cumin seeds against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* on agar media as follows:

Figure 1. Ethanol Extract of Black Cumin Seed Against *Klebsiella pneumoniae* Bacteria

Figure 2. Ethanol Extract of Black Cumin Seed Against *Pseudomonas aeruginosa* Bacteria

From Figure 1 and 2, the broadest inhibition zone was produced by positive control for both types of bacteria compared to black cumin seed extract. The results of the inhibition zone measurement are presented in the following table:

Table 1. Results of Inhibition Zone Diameter Measurement

Test Bacteria	Treatment	Zone of resistance (mm)			
		R1	R2	R3	Average
<i>Klebsiella pneumoniae</i>	Control +	22.1	25.2	25.5	24.3
	Control -	0	0	0	0
	5%	6.7	6.7	0	4.5
	7.5%	6.8	6.7	6.7	6.7
	10%	10.8	9.6	9.8	10.1
	12.5%	11.6	10.9	10.5	11
	15%	12.8	12.9	12.8	12.8
<i>Pseudomonas aeruginosa</i>	Control +	20.6	20.7	20.8	20.7
	Control -	0	0	0	0
	5%	12.1	10.3	0	7.5
	7.5%	12.5	10.7	12.9	12
	10%	13.4	11.1	13.3	12.6
	12.5%	14.0	12.1	14.2	13.4
	15%	16.1	14.6	16.1	15.6

From table 1 above, the results showed that the highest inhibition zone in black cumin seed extract against *Klebsiella pneumoniae* was found at a concentration of 15% with an average inhibition zone of 12.8 mm and in *Pseudomonas aeruginosa* the highest inhibition zone was also found at a concentration of 15% with an average inhibition zone diameter 15.6mm.

Figure 3. The Average Diameter of the Inhibition Zone of Black Cumin Extract against *Klebsiella pneumoniae*

Figure 4. Average Diameter of the Inhibition Zone of Black Cumin Extract against *Pseudomonas aeruginosa*

Figures 3 and 4 above show the results of the calculation of the average inhibition zone in the form of a graph that indicate the largest extract inhibition zone diameter produced by a concentration of 15% with a value of 12.8mm for *Klebsiella pneumoniae* and 15.6mm for *Pseudomonas aeruginosa*.

Based on the bacterial inhibition criteria according to David and Stout in Rita (2010), it was found that if the diameter of the inhibition zone formed was ≤ 5 mm, the extract had weak inhibition, 5-10 mm had moderate

inhibition, 10-20 mm had strong inhibition, and ≥ 20 mm have very strong inhibition. From the criteria for the inhibition of black cumin seed extract against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* varied between weak and strong depending on the concentration of the extract.

Statistical Test of Antibacterial Significance of Black Cumin Seed Extract

The results of the post hoc test analysis used the Mann-Whitney test with the following results:

Table 2. Results of Post Hoc Analysis of Black Cumin Seed Extract on the Growth of *Klebsiella pneumoniae* Bacteria

Treatment	5%	7.5%	10%	12.5%	15%	Control +	Control -
5%		0.197	0.046	0.046	0.043	0.046	0.114
7.5%			0.046	0.046	0.043	0.046	0.034
10%				0.127	0.046	0.050	0.037
12.5%					0.046	0.050	0.037
15%						0.046	0.034
Control +							0.037
Control -							

From table 2 above, there were significant differences ($p < 0.05$) in all concentrations against negative controls

except at 5% concentration. This indicates that the black cumin seed extract inhibits the growth of the *Klebsiella pneumoniae* bacteria.

Table 3. Results of Post Hoc Analysis of Black Cumin Seed Extract on the Growth of *Pseudomonas aeruginosa* Bacteria

Treatment	5%	7.5%	10%	12.5%	15%	Control +	Control -
5%		0.127	0.127	0.077	0.046	0.050	0.121
7.5%			0.275	0.275	0.046	0.050	0.034
10%				0.275	0.046	0.050	0.037
12.5%					0.046	0.050	0.037
15%						0.046	0.034
Control +							0.037
Control -							

From table 3 above, there was a significant difference ($p < 0.05$) at a concentration of 15% against negative controls. It showed that there was an inhibitory power at a concentration of 15% black cumin seed extract against the growth of the bacteria *Pseudomonas aeruginosa*.

In this study, the sample was generated by the ethanol 96% maceration method so that there were less active compounds in the extract of this study. The inhibition of growth of the bacteria *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was greater in line with the high concentration of black cumin seed extract used.

The results showed that the black cumin seed extract had an effect in inhibiting the growth of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* at concentrations of 5%, 7.5%, 10%, 12.5%, and 15%.

The data were analyzed using the

Kruskal-Wallis nonparametric test with a result of $p < 0.05$ which proved the effect of black cumin seed extract in inhibiting the growth of the bacteria *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. It is in accordance with the research by (Hassan *et al.*, 2008) regarding the activity test of *Nigella sativa* as an antibacterial against the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Eschericia coli*, and *Bacillus cereus* bacteria. In his research, the process of extracting black cumin seeds used methanol solvent and with concentrations of 25mg / 20 μ L, 50mg / 20 μ L, and 100mg / 20 μ L. The result of the inhibition of bacterial growth was weak.

Likewise, the results of other studies that tested the effects of black cumin seeds with the Soxhlet extraction method and methanol maceration against 21 *Pseudomonas aeruginosa* strains that were resistant to several types of antibiotics with the results of methanol extract had better

inhibition than the essential oil extract with inhibitory results against *Pseudomonas aeruginosa*. 12 and 11 strains were resistant to antibiotics, respectively (Salman *et al.*, 2008).

The differences between this study and the two studies conducted were the type of black cumin seeds, the concentration used, and the type of solvent in extracting the black cumin seeds.

CONCLUSION

From the results of the study, it can be concluded that the ethanol extract of black cumin seeds using the disc diffusion method had been proved to have an effect in inhibiting bacterial growth by obtaining inhibition zone in *Klebsiella pneumoniae* with extract concentrations of 5%, 7.5%, 10%, 12.5%, and 15%, respectively. were 4.5, 6.7, 10.1, 11, and 12.8 mm and in *Pseudomonas aeruginosa* 7.5, 12, 12.6, 13.4, and 15.6 mm. The higher the concentration of black cumin seed extract, the stronger the antibacterial effect against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

REFERENCES

- Al-Jassir, MS. (2008). Chemical Composition and Microflora of Black Cumin (*Nigella sativa* L.) and Aleppo Pine (*Pinus halpensis* Mill.) Seed Oils. *J Food Comp Anal.* 21(2): 162-168
- Bakathir, HA., & Abbas NA. (2011). Detection of the Antibacterial Effect of *Nigella sativa* Ground Seeds with Water. *Afr J Tradit Complement Altern Med.* 8(2): 159-164
- Butar-Butar, RR. (2018). Uji Efektivitas Ekstrak Jintan Hitam Terhadap Pertumbuhan *Staphilokokus aureus* Isolat Pus Infeksi Odontogenik. Skripsi. Medan: Universitas Sumatera Utara.
- Craven DE. & Chroneou A. Nosocomial Pneumonia. dalam: Mandell G.L., Bennett J. E., dan Dolin R (Eds). (2010). *Principles and Practice of Infectious Disease.* 2(7): 3717-24. Philadelphia: Elsevier.
- Bennett J. (2018). BMJ Best Practice: Hospital-Acquired Pneumonia. Diunduh di <https://bestpractice.bmj.com/topics/en-gb/720> tanggal 1 Juni 2019.
- Foroughi A., Pournaghi P., Tahvilian R., Zangeneh MM., Zangeneh A., & Moradi R. (2016). Ethnomedicinal Plants: Study on the Chemical Composition and Antibacterial Activity of the *Nigella sativa* (Black Seed) Oil's. *International Journal of Pharmaceutical and Clinical Research.* 8(11): 1528-1532.
- Forouzanfar F., Bazzaz B.S.F., & Hosseinzadeh H. (2014). Black Cumin (*Nigella sativa*) and Its Constituent (Thymoquinone): A Review on Antimicrobial Effects. *Iran J Basic Med Sci:* 929-938
- Hannan A., Saleem S., Chaudhary S., Barkaat M., & Arshad MU. (2008). Antibacterial Activity of *Nigella sativa* Against Clinical Isolates of Methicillin Resistant *Staphylococcus aureus*. *J Ayub Med Coll Abbottabad.* 20(3):72-74
- Hassan Z., Fairuz A.R.M., Zakri A.H.Z., & Rahim M.N.A. (2008). In vitro Antibacterial Activity of *Nigella sativa* Against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Eschericia coli* and *Bacillus cereus*. *Asian Journal of Plant Sciences.* 7(3):331-333
- Rita, WS. (2010). Isolasi Identifikasi dan Uji Aktivitas Antibakteri Senyawa Golongan Triterpenoid pada Rimpang Temu Putih (*Curcuma zedoaria* (Berg) Roscoe). *Jurnal Kimia.* 4:20-26
- Salman, MT., Khan RA., & Shukla I. (2008). Antimicrobial activity of *Nigella sativa* Linn.

- Seed Oil Against Multi-Drug Resistant Bacteria From Clinical Isolates. *Natural Product Radiance*. 7(1): 10-14
- Silvani, NS. (2018). Kajian Aktivitas Antimikroba Ekstrak Jintan Putih (*Cuminum cyminum*) Terhadap Mikroba Patogen dan Perusak Pangan. Skripsi. Bogor: Institut Pertanian Bogor.
- Sultana S., Muhammad H., Akhtar N., & Iqbal A. (2015). *Nigella sativa*: Monograph. *Journal of Pharmacognosy and Phytochemistry*. 4(4): 103-106.
- Tacakkoli A., & Mahdian V. (2017). Review on Clinical Trials of Black Seed (*Nigella sativa*) and Its Active Constituent, Thymoquinone. *Journal of Pharmacopuncture*. 20(3): 179-193
- Wadud, SA. (2014). Uji Efektivitas Ekstrak Biji Jintan Hitam (*Nigella sativa*) Terhadap Pertumbuhan Bakteri *Shigella dysenteriae*. Skripsi. Jakarta: UIN Syarif Hidayatullah Jakarta.