



ANTIFUNGAL ACTIVITY OF METHANOL EXTRACT OF BLACK CUMIN SEEDS ON *Aspergillus* sp.

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ABSTRACT

Aspergillus sp. is a type of pathogenic fungus and can cause *pulmonary aspergillosis*. This research aims to determine the concentration of methanol extract from black cumin (*Nigella sativa*) seeds which effective to inhibit the growth of *Aspergillus* sp. (RMPRB₂). This research used poisoned food method with Completely Randomized Design consisting of six treatments with four replications. The results showed that the methanol extract of *Nigella sativa* seeds could inhibit the growth of fungal isolates from *Aspergillus* sp. (RMPRB₂). The Methanol extract of *Nigella sativa* with a concentration of 2% and the positive control gave a strong inhibitory activity to the growth of *Aspergillus* sp. (RMPRB₂) with a percentage of 100%. Concentration of 1% is the most effective result because gave a very strong inhibitory activity to the growth of fungal isolates of *Aspergillus* sp. (RMPRB₂).

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1. Introduction

Fungi are one of the microorganisms that often contaminate the environment. Fungi produce mycotoxins and allergen compounds that are harmful to health^[1]. *Aspergillus* is a fungus belonging to the *Ascomycetes* class that can be found everywhere in nature. It grows as a saprophyte in decaying vegetation and is present in soil, organic dust, food and is a contaminant commonly found in hospitals and laboratories. *Aspergillus* forms a long branched filamentary filament, and in the culture medium, it forms as mycelia and conidiospores. *Aspergillus* breeds with the formation of hyphae or buds and produces spore-forming conidophores. The spores are spread freely in the open air so that inhalation can not be avoided and enter through the respiratory tract into the lungs and can cause *pulmonary aspergillosis* disease^[2]. The member genus of *Aspergillus* is the most dominant type of pathogenic fungus in the air^{[3],[4]}.

Treatment of diseases caused by fungi has been widely practiced using synthetic antifungals such as imidazole derivatives, triazoles, nystatin and amphotericin B^[6]. The use of synthetic antibiotics in the health field too often will have an impact on human health such as, anemia, leukopenia, and thrombocytopenia, especially in patients with haematological disorders. One alternative that can be done is the use of medicinal plants as antifungal^[6]. One of the medicinal plant that can be used as antifungal is black cumin plant.

Black cumin plant is an herbal medicine that has been used since 2000 years ago^[7]. Black cumin plant contains flavonoid, alkaloid and thymoquinone which act as antibacterial, antifungal,

antihelmintik and anti-inflamasi^[8]. In addition, black cumin also has a major content that is considered to have various pharmacological activities of the nigellon. Nigellon can decrease the histamine blood produced by mast cells through decreased levels of calcium (Ca²⁺) intracellular^[8].

The black cumin seeds contain essential oils, saponins, tannins and quinones that can inhibit the growth of fungal species *Malassezia furfur*^[9]. There is no information states that the plant part of black cumin seeds especially for antifungal drugs against members of the genus *Aspergillus*. The research aims to determine the concentration of black cumin seeds extract (*Nigella sativa*) that is effective to inhibit *Aspergillus* sp. (RMPRB₂).

2. Materials and Methods

2.1. Collection of sample

The materials used in this research were black cumin seeds (*Nigella sativa*), and *Aspergillus* sp. (RMPRB₂). The isolate is a collection of Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Tanjungpura. It was isolated from air by Rahmawati (2018)^[3].

2.2. Extraction

The production of methanol extract from black cumin (*Nigella sativa*) seeds used maceration method. *N. sativa* powder (200 g) was immersed in 1000 mL of methanol at room temperature and protected from direct sunlight. The maceration process was carried out for ± three days until the solvent was clear. The mixture was stirred for 1x24 hours^[10].

The solution was filtered by using a filter cloth to obtain a macerate. All the macerates from the filtrate were collected together and evaporated with a rotary evaporator. A thick methanol extract obtained was stored in a sterile container, then stored in a silica gel desiccator^[10].

2.3. Isolation

Pure cultured fungi *Aspergillus* sp. (RMPRB₂) was inoculated on the slant PDA in the test tube aseptically, then incubated for 48 hours at 37°C. Determination of antifungal activity was done by poisoned food technique^[11].

The extract solution of black cumin was taken according to a predetermined concentration, then poured into petri dish contained 20 mL of PDA media. Colonies of *Aspergillus* sp. (RMPRB₂) that have been grown previously was inoculated by direct planting method using sterile ose in the middle of the PDA media. Inoculated PDA media were subsequently incubated at 25 °C for 7 days.

The parameters measured were the diameter of the fungal colony and the percentage of inhibitory power. According to^[12] the percentage of inhibition activity of fungal growth is calculated by using the formula:

$$p = \frac{D1 - D2}{D1} \times 100\%$$

P : Percentage of inhibition of fungal growth of species members

D1 : Diameter of fungal colonies of species that grow on treatment

D2 : Diameter of fungal colony of member species that grow on treatment of *N.sativa* methanol extract

Table 1. The Classification of Fungal Inhibition Activities (Mori et al., 1997)

Antifungal activity (AFA)	Activity level
$P > 75\%$	Very strong (++++)
$50\% < P \leq 75\%$	Strong (+++)
$25\% < P \leq 50\%$	Moderate (++)
$0\% < P \leq 25\%$	Weak (+)
0	Not active (-)

The activity of each concentration of black cumin extracts could be determined by calculating the amount of inhibitory value. The data were analyzed with Analysis of Variance (ANOVA) using SPSS 18. If the results of the analyzed data showed a significant difference, the test was continued with Duncan Test at 95% confidence^{[13],[14]}.

3. Results and Discussion

3.1. Results

Fungal colonies of isolates of *Aspergillus* sp. (RMPRB₂) grown in each treatment showed a marked difference from the mean diameter of the fungus after incubation for 7 days. Based on the results of the study showed that the higher concentration of extract given the greater the influence of inhibition (Figure 1).

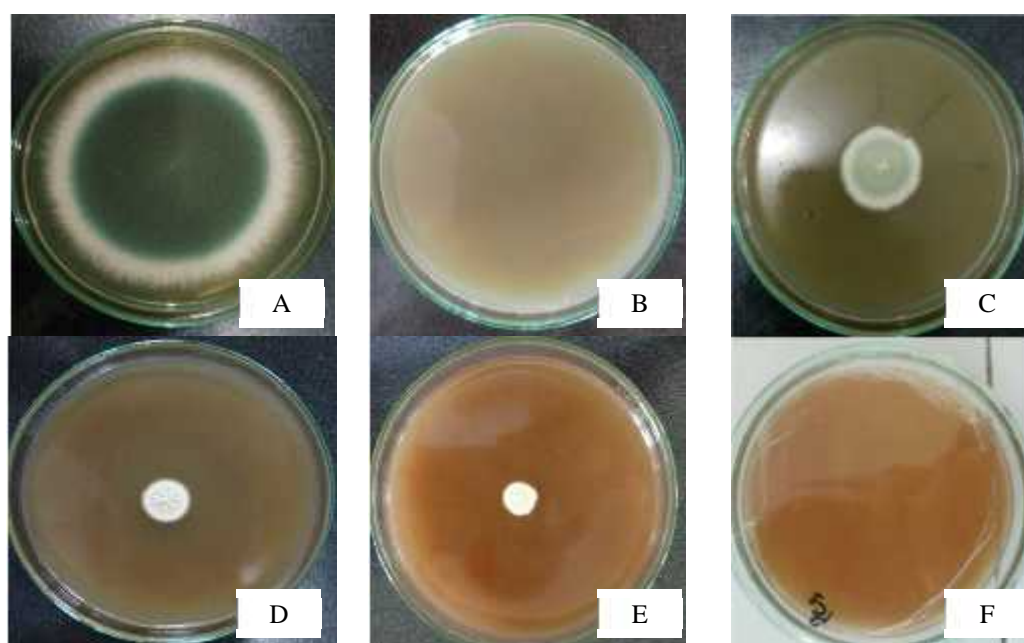


Figure 1. The Growth of *Aspergillus* sp. (RMPRB₂) After 7 Days of Treatment. A. Negative Control (Just with PDA); B. Positive Control (added ketoconazole of 2%); C, D, E and F Black cumin seed with concentration 0.5%, 1%; 1.5%; and 2% respectively.

The mean value of growth diameter of isolate of *Aspergillus* sp. (RMPRB₂) ($F_{3,12} = 1345,025$, $p = 0,0000$; ANOVA) and analysis result of percentage value of inhibition of growth of isolate of *Aspergillus* sp. (RMPRB₂) ($F_{4,15} = 3432,929$, $p = 0,000$; ANOVA) showed that giving of black cumin seed methanol extract significantly influenced inhibition of growth of isolate of member of species *Aspergillus* sp. (RMPRB₂) (Table 2)

Table 2. The inhibitory activity of black cumin seeds methanol extract on the growth of *Aspergillus* sp. (RMPRB₂).

Treatment cumin seed extract (%)	Mean of Colony Diameter fungus (mm)	Percentage of inhibitory (%)	Activity level
Positive control (ketoconazole of 2%)	0,00±0,00 ^b	100±0,00 ^e	Very strong
Negative control (Just with PDA)	67,1±2,97 ^e	0,00±0,00 ^a	Not active
Concentration of 0,5%	24,3±0,87 ^d	63,7±2,30 ^b	Strong
Concentration of 1%	15,8±1,06 ^c	76,3±1,40 ^c	Very strong
Concentration of 1,5%	8,26±0,80 ^a	87,6±1,25 ^d	Very strong
Concentration of 2%	0,00±0,00 ^b	100±0,00 ^e	Very strong

Information : The numbers in the columns followed by unequal letters show a 0.5% effect, significantly different according to the Duncan test on a significant 5%. Negative control, positive control, Concentration of 1%; Concentration of 1.5%; and Concentration of 2%.

By the research showed that methanol extract of *N. sativa* in average high anti fungal activity (AFA). Duncan's test showed that methanol extract of *N. sativa* exhibited the highest inhibition on positive control and black cumin extract 2% (AFA 100%, respectively), and it can be said that the most effective treatment results are black cumin seeds methanol extract with the concentration of 1% because it is the smallest concentration with very strong inhibitory activity (Table 2).

3.2. Discussion

Based on the research that has been done, the methanol extract of black cumin seed has inhibitory effect on the growth of fungi of isolate of *Aspergillus* sp. (RMPRB₂). Inhibitory ability can be known from the average value of growth diameter of the fungal colony and the percentage of inhibition in the treatment (Table 2). Examination of black cumin seed methanol extract to *Aspergillus* sp. (RMPRB₂) showed the lowest percentage of inhibition on the growth of *Aspergillus* sp. (RMPRB₂) was found in the concentration treatment of black cumin seed extract 0.5% that is equal to 63,7% with strong activity. The concentration 2% is the highest percentage of inhibition of *Aspergillus* sp. (RMPRB₂) that is equal to 100% with very strong activity. Concentration 2% and positive control treatment added ketoconazole 2% showed significant differences (Table 2). So both treatments can be said to be effective in killing fungi (fungistatic). Ketoconazole is one of the anti-synthetic azole classic fungi that is an imidazole derivative with a broad spectrum and high effectiveness that works inhibit ergosterol synthesis is an important component for fungal integrity. Ketoconazole has a fungistatic effect but can have fungicidal effects at high levels after long incubation or against highly susceptible organisms^[15].

Treatment of extracted PDA media resulted in the diameter of *Aspergillus* sp. (RMPRB₂) is smaller than the negative control treatment. This is due to the presence of secondary metabolite compounds in methanolic extract of black cumin seeds that have activity as antifungal. According to Waluyo (2008) antifungal agents can denature the bonding of functional proteins that can damage fungal cells^[16]. In accordance with the statement Djunaedy (2008), antifungal compounds have a working mechanism by neutralizing the enzyme or toxin associated in the fungus invasion, destroys the mushroom cell membrane, inhibits the fungus enzyme system so as to disrupt the formation of apresorium and haustorium and affect the synthesis of nucleic acids and proteins^[17]. In addition, an increasingly high concentration is thought to have an effect on inhibiting fungal growth. The greater the concentration of extract contained in the media, the amount of extract that diffuses into the fungal cells is increasing, causing disruption of fungal growth can even cause fungal death^[18]. High concentrations of extracts contain higher secondary metabolite compounds that can provide greater inhibitory power^[17].

According to Paraakh (2010), black cumin contains alkaloid, flavonoid, essential oils, saponin, tannins⁷. Each secondary metabolite compounds has a different way of working. The alkaloid group

of compounds can inactivate the function of the genetic material, by interfering with the formation of DNA and RNA in the fungal cells¹⁹. The working mechanisms of alkaloids and flavonoids affect the fungal cell components by destroying cell membranes and denaturing proteins. Flavonoids contain phenol compounds. The phenol compounds contained in the extract has a working mechanism to destroy cell membranes of fungi^[20].

Phenol compounds will bind to ergosterol which is the constituent of fungal membrane cells that produce the formation of cell membranes^[21]. The formation of these pores causes cellular components such as amino acids, carboxylic acids, inorganic phosphates and phosphate esters out of cells causing the death of fungal cells^[21]. The phenol compounds in low concentrations can damage the cytoplasmic membrane and cause leakage of cell membranes, but in high concentrations will cause protein coagulation and cell membrane damage^[22].

The mechanism of action of saponin compounds as antifungal is to work by lowering the surface tension of the sterol membrane from the cell wall of the fungus^[21]. The reduced surface tension of the sterol membrane results in an increase in cell permeability^[23]. Increased cell permeability can lead to disruption of the absorption of necessary fungi for growth^[23]. While the mechanism of tannin work is its ability to inhibit chitin synthesis used for the formation of cell walls in the fungus and damage the cell membrane so that the growth of the fungus is inhibited^[24].

4. Conclusion

Methanol extract of *Nigella sativa* seeds with 1% concentration is the most effective result because gave a very strong inhibitory activity to the growth of fungal isolates of *Aspergillus* sp. (RMPRB₂).

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