

Antifungal Activity Test on α -guaiene Patchouli Oil Against *Aspergillus niger* and *Candida albicans*

Uji Aktivitas Antijamur pada α -guaiene Minyak Nilam terhadap Jamur *Aspergillus niger* dan *Candida albicans*

Zhaqqu Ilham Ahafidz^{1*}, Sarifah Nurjanah¹, Tita Rialita²

¹Department of Agricultural and Biosystem Engineering, Faculty of Agricultural Industrial Technology, Universitas Padjadjaran, Highway Bandung-Sumedang KM 21, Jatinangor, 40600, Indonesia

²Department of Food Industry Technology, Faculty of Agricultural Industrial Technology, Universitas Padjadjaran, Highway Bandung-Sumedang KM 21, Jatinangor, 40600, Indonesia

*E-mail: zhaqqu17001@mail.unpad.ac.id

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ABSTRAK

Minyak nilam tersusun atas beberapa fraksi komponen dengan beragam manfaat. α -guaiene adalah salah satu fraksi komponen minyak nilam yang diduga memiliki aktivitas antijamur. Penelitian ini bertujuan untuk mengetahui aktivitas antijamur pada fraksi I minyak nilam dengan kandungan α -guaiene 38% terhadap jamur *Aspergillus niger* dan *Candida albicans* ATCC 7102 yang memengaruhi kesehatan manusia. Penelitian ini berjenis penelitian eksperimental dengan metode difusi sumuran untuk melihat diameter daya hambat yang terbentuk. Perlakuan yang diterapkan yaitu perbedaan konsentrasi zat uji (20%; 40%, 60%; 80% dan 100%) serta kontrol positif ketokonazol 2% dan kontrol negatif n-heksana. Hasil penelitian ini menunjukkan bahwa fraksi I minyak nilam memiliki aktivitas antijamur. Nilai diameter daya hambat yang terbentuk berbanding lurus dengan peningkatan konsentrasi zat uji. Rata-rata diameter daya hambat terbesar didapatkan dari perlakuan konsentrasi 100% untuk kedua jamur. Rata-rata diameter daya hambat pada *A. niger* sebesar 2,82 mm sementara *C. albicans* yaitu 7,69 mm. Ketokonazol 2% membentuk diameter rata-rata sebesar 20,78 mm untuk *A. niger* dan 32,28 mm untuk *C. albicans*, sementara n-heksana tidak membentuk zona hambat.

Kata kunci: α -guaiene; *A. niger*; antijamur; *C. albicans*; minyak nilam

ABSTRACT

Patchouli oil is composed by several component fractions with various benefits. The α -guaiene is one of the components of patchouli oil, which is thought to have antifungal activity. This study aimed to determine the antifungal activity of fraction I from patchouli oil fractionation containing 38,8% α -guaiene against *Aspergillus niger* and *Candida albicans* ATCC 7102 that affects human health. This research was an experimental study using the well diffusion method to determine the diameter of the inhibition. The treatment applied was the differences in the concentration level of fraction 1 patchouli oil (20%; 40%, 60%; 80% and 100%) and a positive control of ketoconazole 2% and a negative control of n-hexane. This study indicated that fraction I patchouli oil has antifungal activity. The diameter of the inhibition formed was directly proportional to the increase in the concentration of the test substance. The average diameter of the greatest inhibitory was obtained from the 100% concentration treatment for both fungi. The average diameter of inhibition in *A. niger* is 2.82 mm, while *C. albicans* is 7.69 mm. Ketoconazole 2% produced an average inhibitory diameter of 20.78 mm for *C. albicans* and 32.28 mm for *A. niger*, while n-hexane did not produce an inhibition zone.

Keywords: α -guaiene; *A. niger*; antifungal; *C. albicans*; patchouli oil

INTRODUCTION

Patchouli oil is one of the largest essential export commodities in Indonesia. Currently, the number of patchouli oil exports ranges from 1,200-1,500 tons/year and dominates about 85% of Indonesia's total exports of essential oils (Ditjenbun, 2020). Patchouli oil is composed of several component fractions that can be separated through a fractionation process. Patchouli alcohol (PA) is the most widely used patchouli oil fraction, one of which is as a fixative in the perfume industry. Besides PA, other compounds such as α -guaiene that can be utilized. The α -guaiene component fraction in patchouli oil can reach 17,89% (Arpima *et al.*, 2020). α -guaiene is a sesquiterpene compound. Terpene compounds in plants can damage cell membranes through an adsorption process that results in protein denaturation (Parwata *et al.*, 2008).

The use of α -guaiene as an antifungal agent can be developed because there is a possibility that this substance can damage fungal cell membranes. Fungi are one of the causes of various diseases for humans. Fungi can negatively impact animals, plants, and the environment as a whole. *C. albicans* is a type of fungi that causes health problems for humans. Bindusari & Suyoso, (2001) stated that *C. albicans* is the main cause of vaginal candidiasis infection. *A. niger* is another fungus that can cause adverse effects on humans. This fungus can cause aspergillosis and cause various diseases such as pneumonia (Hasanah, 2017). Prevention of fungal infections and treatment of fungal diseases can be done by using antifungal agents. The addition of antifungal substances can form a passive immune system to avoid pathogenic fungal infections (Entjang, 2001). Ketoconazole is one of the antifungal substances commonly used today, ketoconazole has good

antifungal activity but can cause side effects such as nausea and vomiting (Bahry & Setiabudy, 2011).

Antifungal substances can be developed by utilizing natural ingredients, such as patchouli oil. Research on the antifungal activity of patchouli oil conducted by Wahyunita (2017) showed that the increase in inhibition (clear zone) was directly proportional to the increase in essential oil concentrate. The antimicrobial activity of patchouli oil, especially α -guaiene, has also been investigated by Widyaningrum *et al.* (2020) where α -guaiene with a concentration of 80% can inhibit the growth of *E. coli* bacteria with an inhibitory diameter of 3.22 mm and α -guaiene oil with a concentration of 100% can inhibit the growth of *P. aeruginosa* bacteria.

Research by Risnawati *et al.* (2017) showed that testing the antifungal activity of patchouli oil against the fungi *C. albicans* showed a significant difference from variations in concentrations of 12.5%, 25%, 50% and 100%. The average diameter of the highest clear zone of patchouli oil is 18.8 mm. A similar study conducted by Setyaningrum *et al.* (2017) showed that fraction 8 patchouli oil with a PA content of 54.59% produced an inhibition zone of 9.24 mm on the fungi *C. albicans*. Wulandari (2016) stated that the antifungal mechanism of patchouli oil occurs due to the synergistic effect of several compounds found in patchouli oil so that it can inhibit the growth of fungi.

Fractionation of patchouli oil contains different compounds, each compound has its own function. The antifungal activity of patchouli oil fractions such as α -guaiene compounds remains unclear, especially against the pathogenic fungi *A. niger* and *C. albicans*. This study aimed to determine the antifungal activity of α -guaiene patchouli oil against *A. niger* and *C. albicans*. The results of this study will enrich the literature on the antimicrobial activity of patchouli oil and can be used as a comparison for similar studies.

METHODOLOGY

This research was carried out from June 2021 to November 2021 at Food Microbiology Laboratory and Postharvest Laboratory and Process Technology, Faculty of Agricultural Industrial Technology, Universitas Padjadjaran. The materials used in this study were *A. niger* fungi and *C. albicans* ATCC 7102 obtained from the Microbiology-Biotechnology Laboratory of Faculty of Pharmacy, Universitas Padjadjaran, and α -guaiene patchouli oil obtained from the fractionation of Subang patchouli oil at a temperature of 249°C-254°C with pressure of 10 mmHg (Arpima *et al.*, 2020). The culture media used were Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB). Besides, several chemicals were also used chemicals used include 1% BaCl₂, 1% H₂SO₄, sterile distilled water, 70% alcohol, n-hexane, 0.85% NaCl and 2% ketoconazole. The tools used in this research include B/R Instrument Spinning Band Distillation System Model 36-100, autoclave, beaker glass, scotch bottle, Bunsen, petri dish, cotton, cuvette, finpipet, ose needle, Erlenmeyer flask, laminar airflow, micropipette, microplate, microscope, oven, ruler, spatula, spectrophotometer, and vortex.

The research method used was an experimental method with a completely randomized design (CRD). The treatment applied in this study was the difference in concentration of fraction 1 patchouli oil containing 38.8% α -guaiene with levels of 20%, 40%, 60%, 80% 100%. There was a positive control of 2% ketoconazole and a negative control of n-hexane in the two test fungi. The parameter observed was the diameter of the inhibition (DDH) formed. This study

consisted of three stages: preparation of test fungi, antifungal testing, and data analysis.

Preparation of test fungi was done by identifying the fungi. Fungal identification was carried out to determine and ensure the accuracy of the test fungi following the characteristics of fungi based on existing theories. The identification of the test fungi was carried out using macroscopic and microscopic methods. Macroscopic identification was carried out by planting the fungi on the media and was continued by observing the length of time for growth, colony morphology, color, and shape. Microscopic identification is done by observing the results of a microscope magnification.

Tests for the antifungal activity of α -guaiene patchouli oil were carried out on fungi *A. niger* and *C. albicans*. The fungi *A. niger* was incubated at 27°C for 72 hours while the fungi *C. albicans* was incubated at 27°C for 48 hours. Preparation of various concentrations of α -guaiene patchouli oil was carried out by diluting fraction 1 of patchouli oil using n-hexane. DDH test parameters were observed using the well diffusion method. The data obtained were analyzed using the single factor analysis of variance (ANOVA) method and continued with Duncan's test if the results obtained from the treatments were significantly different. The flow chart of the DDH testing process is presented in Figure 1.

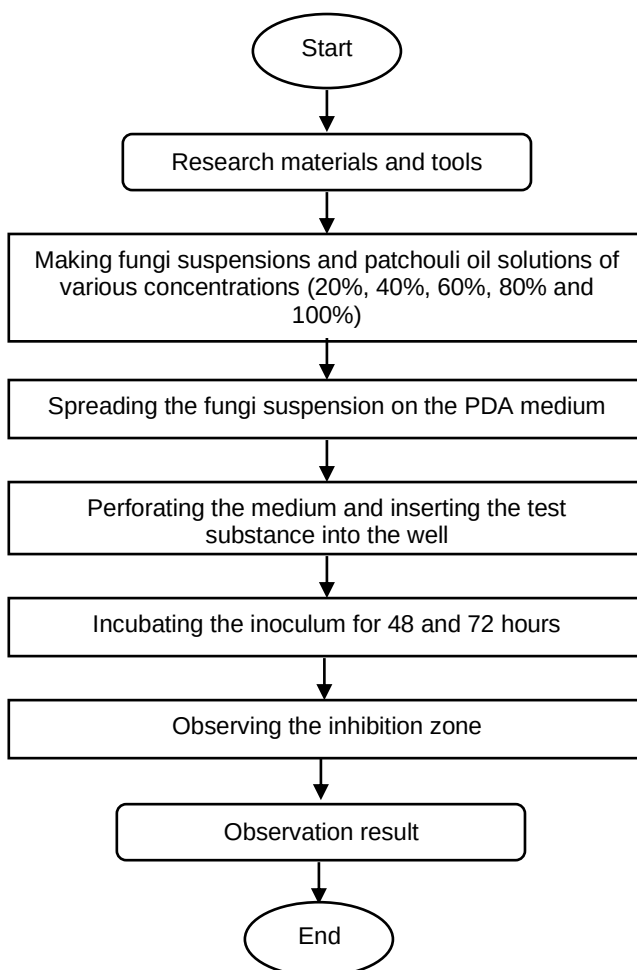


Figure 1. Flow chart of the DDH testing process

RESULTS AND DISCUSSION

GC-MS Test Results Patchouli Oil Fraction 1

The Gas Chromatography - Mass Spectroscopy (GC-MS) test showed that α -guaiene was the largest component of patchouli oil fraction 1. The content of α -guaiene in this fraction reached 38.8%. Although α -guaiene is the largest constituent of this fraction, the purity level is still relatively low. Fraction 1 of patchouli oil also contains other compounds such as caryophyllene, seychellene, azulene, ledene oxide and menthol.

The α -guaiene compound was identified starting at 20.004 minutes to 22.108 minutes with a peak at 21.837 minutes during the testing process. These results were obtained after fraction 1 of patchouli oil was converted into the vapor phase and separated into a single compound according to the specific time and differences in the chemical properties of each constituent compound. Identification of single compounds occurs by comparing the results of the ionized molecular fragmentation pattern with the standard compound fragmentation pattern based on the percentage of similarity index (BTBRD, 2014). The image of the fragmentation pattern of α -guaiene compound fraction 1 patchouli oil is presented in Figure 2.

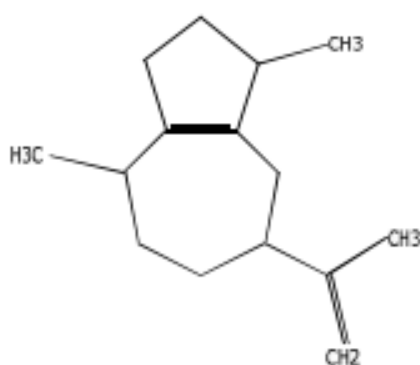


Figure 2. Fragmentation pattern of α -guaiene compound fraction 1 patchouli oil

Test Fungi Identification

The identification results showed that the two fungi had very different color, shape, size and number of colonies in general. The results of the macroscopic identification are presented in Figure 3. Figure A is a culture of the fungi *C. albicans* grown on PDA medium while Figure B is a culture of the fungi *A. niger* on the same medium. The culture of *A. niger* has cotton-like characteristics with brownish white color with blackish colony edges. These characteristics are by the statement of Pitt & Hocking (2009) where *A. niger* colonies have white mycelium, dark brown-black conidia heads are round. The culture of *C. albicans* is bright white with the appearance of the shape according to the results of Mutiawati's research (2016).

Microscopic identification was carried out on the two test fungi to ensure that the characteristics of the fungi matched not only from their visible appearance but also from the microscopic appearance. The identification process was carried out at the Pharmacy Laboratory, Institut Teknologi Bandung. The results indicated that the two test fungi had similar morphological characteristics to the reference fungi. These results were obtained after staining using LPCB and viewed using a microscope with a magnification of 1000 times. The results identification of the fungi *A. niger* are

shown in Figure 4 and the results identification of *C. albicans* are shown in Figure 5.

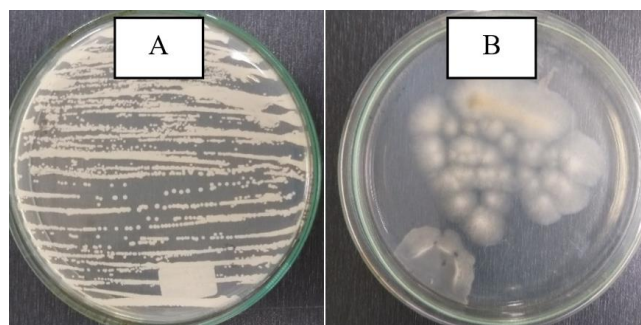


Figure 3. Macroscopic identification results (A) *C. albicans* (B) *A. niger*

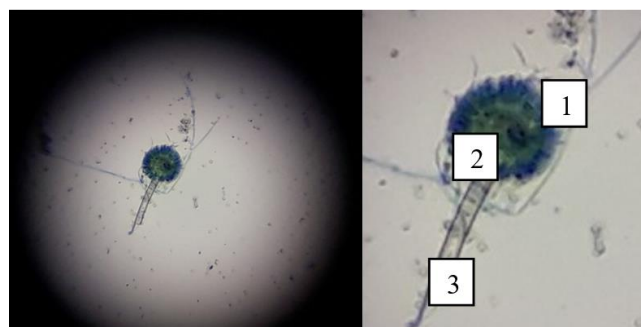


Figure 4. Microscopic identification of *A. niger*

The identified fungi *A. niger* showed that this fungi had an elongated shape with variations in the distinctive shape of each part of the fungi. The parts of the fungi that appear very clearly are marked with numbers 1, 2 and 3. Section number 1 shows conidia, number 2 shows vesicles and number 3 shows conidiophores. These parts of the fungi have characteristics that are very much in line with their theoretical characteristics. The staining process using LPCB makes the conidia color bluish so that the parts of the fungi can be distinguished more clearly.

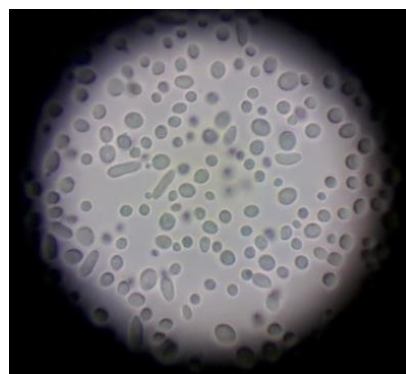


Figure 5. Microscopic identification of *C. albicans*

The microscopic identification of *C. albicans* showed that this microorganism had a different shape from *A. niger*. *C. albicans* has a perfectly round shape, oval round to elongated round with a clear bluish color. The size of each cell that appears on the magnification was not uniform, some cells are quite large while there are very small cells. One cell represents a whole microorganism because *C. albicans* is a single-celled microorganism.

Antifungal Activity Analysis

The antifungal activity in fraction 1 of patchouli oil fractionation with 38.8% α -guaiene content can be seen from the size of the inhibition zone formed. The results showed antifungal activity in fraction 1 patchouli oil which was indicated by the presence of an inhibitory zone in several of the concentrations tested. The inhibition zone formed was caused by the influence of the test substance on fungal growth activity. The inhibition mechanism occurred due to destruction of the cell membrane thus the activity in the cell is disrupted and has an impact on the inhibition of fungal growth or even killing it (Parwata *et al.*, 2008).

Fraction 1 of patchouli oil used in this study did not consist of 100% α -guaiene. There were other constituent components which may also exhibit antifungal activity. This is by Wulandari's (2016) statement that patchouli oil can inhibit fungal growth due to the synergistic effect of several of its constituent compounds. However, it can be seen that fraction 1 patchouli oil can be re-examined, especially in the development of antifungal products.

The diameter of the inhibition formed in the two test fungi had different values. The large difference in the inhibition zone formed indicated that each type of fungi had different resistance and the effectiveness of fraction 1 patchouli oil in inhibiting fungal growth. The comparison of DDH values in the two test fungi is presented in Figure 6.

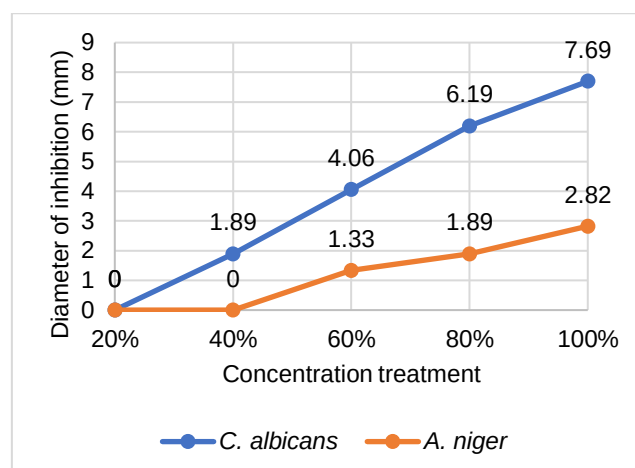


Figure 6. The comparison of DDH values in the two test fungi

The value of DDH formed in the fungi *C. albicans* was greater than that of the fungi *A. niger* at each concentration treatment tested. This can be caused by differences in the level of resistance of a fungi to antifungal substances. Each fungi has different characteristics, fungi can be single cells or consist of many cells (Entjang, 2001). *A. niger* is a multicellular microorganism (Putra *et al.*, 2020) while *C. albicans* is a unicellular microorganism (Mutiawati, 2016). The analysis of the measurement is presented in Table 1.

Table 1. Analysis of the variety of DDH test results

Source of Variation	SS	df	MS	F	F crit
Between Groups	191.85	9	21.31	326.61	3.46
Within Groups	1.3053	20	0.065		
Total	193.15	29			

Note: SS = Sum of squares
df = Degree of freedom
MS = Mean squares
F = F count
Fcrit = F table 1%

Table 2. Duncan test results

Concentration	Duncan Test Results of	
	<i>A. niger</i>	<i>C. albicans</i>
20%	0.593 ^a	0.636 ^{abc}
40%	0.619 ^a	1.89 ^{def}
60%	1.333 ^d	4.733 ^h
80%	1.89 ^{de}	6.874 ⁱ
100%	2.823 ^g	7.696 ^j

The analysis results showed that the F count was greater than F table 1%, and it was found a significant effect on the treatment carried out. This proves that fraction 1 patchouli oil with 38.8% α -guaiene content significantly inhibits the growth of the two test fungi. Duncan's test was carried out as a follow-up test. Duncan test results are presented in Table 2.

The results of Duncan's test showed that the all treatments of the concentration of the test substance resulted in significantly different amounts of DDH. Treatment concentrations of 20% and 40% on the fungi *A. niger* also the treatment concentration of 20% on the fungi *C. albicans* were not significantly different. Treatment of 60% and 80% of *A. niger* and 80% concentration of *C. albicans* were not significantly different. The other

concentration treatments had significantly different results on the two test fungi. These results are in accordance with the statement of Pelczar (1986) where the concentration of antimicrobial substances is one of the factors that determine whether a substance is good or bad in inhibiting microbial growth.

Inhibitory Diameter

The results of DDH observations on the fungi *A. niger* showed that the diameter of the inhibition began to form at a concentration of 60%. The concentration treatment of 20% and 40% did not produce the diameter of the inhibitory power. The positive control treatment with 2% ketoconazole produced the largest average diameter of inhibition with a

value of 20.78 mm while the negative control n-hexane did not form an inhibitory zone. DDH measurement results obtained from the test results are presented in Table 3.

Table 3. DDH measurement results in *A. niger*

Treatments	Average Inhibition zone (mm)	Standard deviation
20%	0	0
40%	0	0
60%	1.33	0.144
80%	1.89	0.128
100%	2.82	0.127
Ketoconazole 2%	20.78	0.348
n-heksana	0	0

The inhibition zone of each treatment given was not perfectly spherical so that the measurement was carried out 3 times to take the average. The 20% and 40% concentration treatments had an inhibition zone of 0 mm, this was due to the unaffected fungal culture of the test substance so that the fungi could still grow under the given conditions. The 60% concentration treatment resulted in an average diameter of inhibition of 1.33 mm. The 80% concentration treatment resulted in an average diameter of inhibition of 1.89 mm while the largest DDH value was obtained from the 100% concentration treatment with a value of 2.82 mm.

The largest DDH value obtained lies in the range of values 0-4 mm so that its antifungal activity was categorized in the weak group (Kandoli *et al.*, 2016). The striking difference in DDH values could also be seen from the comparison of the size of the inhibition zone with the positive control treatment which the difference in value reaches 17.86 mm, in the same categorization system 2% ketoconazole is included in the substance with very strong antifungal activity because the value exceeds 20 mm. The results of studies related to the antifungal activity of fraction 1 patchouli oil against the fungi *A. niger* are still higher than similar studies with other substances. Karlina *et al.* (2016) stated that the water extract of sappan wood with a pH of 3 and a concentration of 20% resulted in an inhibition zone of 9.69 mm.

The measurement results of DDH formed in the fungi *C. albicans* are presented in Table 4. Based on the value of DDH formed, fraction 1 patchouli oil with 38.8% content has antifungal activity.

Table 4. DDH measurement results in *C. albicans*

Treatments	Average Inhibition zone (mm)	Standard deviation
20%	0	0
40%	1.89	0.207
60%	4.06	0.236
80%	6.19	0.318
100%	7.69	0.632
Ketoconazole 2%	32.28	0.190
n-heksana	0	0

The concentration treatments of 40%, 60%, 80% and 100% each resulted in an average diameter of inhibition of 1.89 mm, 4.06 mm, 6.19 mm and 7.69 mm respectively.

The largest DDH value obtained was in the range of 5-10 mm so that the antifungal activity was categorized in the moderate group (Kandoli *et al.*, 2016). This value is still lower than the positive control treatment of 2% ketoconazole, under the same conditions this treatment produces an average DDH value of 32.28 mm which proves that ketoconazole which is used as a conventional antifungal currently has very strong antifungal activity against fungi *A. niger*.

CONCLUSION

Fraction 1 from patchouli oil fractionation with 38.8% α -guaiane content had antifungal activity against *A. niger* and *C. albicans*. The DDH value formed is directly proportional to the increase in the concentration of the test substance. The largest DDH value was obtained from treatment with 100% concentration on both fungi. The largest DDH value for *A. niger* was 2.82 mm while for *C. albicans* the value was 7.69 mm. Ketoconazole 2% formed an average diameter of 20.78 mm for *A. niger* and 32.28 mm for *C. albicans*, while n-hexane did not form an inhibition zone.

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