Antifungal Activity of Citronella Oil Against Clinical Isolate

*Candida albicans*

Potensi Antifungi Minyak Atsiri Serai Wangi terhadap *Candida albicans* Isolat Klinis

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Received 15-03-2022   Accepted 29-06-2022   Available online 27-09-2022

ABSTRACT

Citronella (*Cymbopogon nardus* (L.) Rendle) is commonly claimed as a multifunction plant. Citronella produces an essential oil whose quality standard was set in SNI 06-3953-1995. Citronella oil is used for traditional medication, such as an antimicrobial, antipyretic, analgesic, and many other functions. This study evaluated the *in-vitro* antifungal activity of citronella oil obtained from the farm in Menderek, Pintu Rime Gayo, Bener Meriah, Aceh, against clinical isolates of *Candida albicans*. The citronella oil analyzed using chromatography-mass spectrometry (GC-MS) showed chemical components of citronellal (26.06%), β-citronellol (26.314%), and geraniol (17.90%). Antifungal activity test of citronella oil against *Candida albicans* by well diffusion method showed the highest activity at 40% v/v concentration with the diameter of inhibition zone of 23.31±0.04 mm. The result of this study indicated that the citronella oil has a good potential to inhibit clinical isolate *Candida albicans* growth.

Keywords: Antifungal activity, citronella oil, *Cymbopogon nardus*, clinical isolates of *Candida albicans*.

ABSTRAK

*Serai wangi* (*Cymbopogon nardus* (L.) Rendle) telah diklaim sebagai tanaman multifungsi. *Serai wangi* memproduksi minyak atsiri serai wangi, dimana standar kualitas dari minyak atsiri serai wangi berdasarkan SNI 06-3953-1995. Minyak atsiri serai wangi digunakan sebagai pengobatan tradisional, contohnya sebagai
antimikroba, antipireutik, analgesic, dan banyak fungsi lainnya. Berdasarkan manfaat- 
manfaat tersebut, pada penelitian ini dilakukan pengujian secara in-vitro mengenai 
minyak atsiri serai wangi sebagai pengobatan. Minyak atsiri serai wangi yang diperoleh 
dari perkebunan di Kampung Menderek, Kecamatan Pintu Rime Gayo, Kabupaten Bener 
Meriah, Provinsi Aceh, telah dilakukan pengujian aktivitas antijamur terhadap Candida 
albicans isolat klinis. Analisis kandungan kimia dari minyak atsiri serai wangi 
menggunakan instrumentasi kromatografi gas-spektrofotometri massa, dengan hasil 
menunjukkan kandungan citronellal 26,06%, β-citronelol 26,314%, dan geraniol 17,90%. 
Uji aktivitas antijamur minyak atsiri serai wangi terhadap Candida albicans 
isolat klinis menggunakan metode sumuran menunjukkan hasil tertinggi pada konsentrasi 40% v/v 
dengan rata-rata diameter zona hambat sebesar 23,31±0,04 mm dengan kategori 
sangat kuat. Hasil tersebut mempresentasikan bahwa minyak atsiri serai wangi 
mempunyai potensi yang baik dalam menghambat pertumbuhan dari Candida albicans.

Kata kunci: Aktivitas antijamur, minyak atsiri serai wangi, Cymbopogon nardus, Candida 
albicans isolat klinis.

Introduction

Candidiasis is an infectious disease caused by fungi. It is the most common case in tropical countries and is 
caused by Candida albicans (Nugraha et al., 2020). Candida albicans is a normal 
microbiota in the mucous of the mouth, digestive tract, and female vital organs. 
However, it will be pathogenic if the amount is excessive and occurs when 
the human immune system is diminishing (Kurniawati & Nastiti, 2020).

Candidiasis generally occurs due to a lack of attention to the early symptoms that have been experienced, 
as well as poor hygiene. It can manifest worse if it invades a larger area of the 
body. Thus, the cases require antifungals which are indicated either to prevent or 
as a medication for candidiasis symptoms.

One of the herbal plants that are convenient to discover and have been utilized in traditional medicine since 
ancient times is citronella (Cymbopogon nardus). Citronella is a plant that is 
frequently used as a cooking spice, analgesic, antipyretic, antimicrobial, and several other benefits. 
Citronella is essential oils that contains geraniol, citronellal, and citronellol. Additionally, 
the quality of essential oils has been determined based on SNI 06-3953-1995 
(Badan Standarisasi Nasional (BSN), 1995).

The compound of Cymbopogon nardus oil has antifungal activity against Candida albicans. The inhibition 
diameter zone test for citronella oil against Candida albicans ATCC 10231 at 
50% concentration was 26.02 mm (Ahmad et al., 2020). Furthermore, the 
result of the inhibition zone test against Candida albicans MTCC 3958 at 100 
g/mL concentration was 62.0±2.2 mm (Kandimalla et al., 2016). In addition, 
according to Saputra et al. (2020), the result of the test against Candida albicans 
ATCC 90028 at 100 g/mL
concentration was obtained 65.0±2.2 mm.

In general, isolates utilized were non-clinical isolates, whereas the clinical isolates of *Candida albicans* in antifungal research were still limited. The clinical isolates and non-clinical isolates were assessed to have different physiological responses. According to Aguayo et al. (2017), clinical isolates of *Candida albicans* were more virulent than *Candida albicans* ATCC 10231, which had been cultured in the laboratory. The adhesion forces and the capability of *Candida albicans* clinical isolate showed various degrees of virulence compared to *Candida albicans* ATCC 10231. The adhesion of the clinical isolate strain was more than 10 times higher than the ATCC 10231. It indicated that clinical isolates have a varied capability to survive in blood and less sensitivity to many antifungal variants.

According to the descriptions above, it was necessary to conduct research on the antifungal activity of citronella oil against clinical isolates of *Candida albicans*. The antifungal activity test utilized the well method. The main compounds of citronella oil were determined using the Gas Chromatography-Mass Spectrometry (GC-MS) analysis method.

**Research Method**

**Instruments and Materials**

- Laminar air flow (LAF), autoclave, incubator, light microscope, GC-MS (Pyrolysis Shimadzu QP1020), the UV-Vis spectrophotometry, pycnometer, refractometer, and vortex.
- Citronella oil was obtained from Bener Meriah, Aceh, Indonesia. The clinical isolate of *Candida albicans* was obtained from the Microbiology Laboratory of Doctor Zainal Abidin General Regional Hospital, 0.5 Mc. *Farland* solution, potato dextrose agar (PDA), potato dextrose broth (PDB), dimethylsulfoxide (DMSO), and Micafungin 50 mg/vial.

**Experiments**

1. **Determination of citronella oil organoleptic**
   - The tests evaluated citronella oil’s color, odor, and taste (Lely et al., 2017).
2. **Determination of citronella oil specific gravity**
   - Specific gravity is carried out according to the ratio between the weight of the oil at a specified temperature and the weight of water in the same volume of water as the volume of the oil temperature (Badan Standarisasi Nasional (BSN), 1995).
3. **Determination of citronella oil solubility in ethanol**
   - Solubility in ethanol is carried out according to the solubility of oil in ethanol (Badan Standarisasi Nasional (BSN), 1995).
4. **Determination of citronella oil refractive index**
   - The Refractive index is carried out according to direct measurement of the refraction angle
of the oil maintained at a constant temperature (Badan Standarisasi Nasional (BSN), 1995).

5. Analysis of essential oil components with GC-MS

Identification of organic chemical components from *Cymbopogon nardus* essential oil conducted Gas Chromatography-Mass Spectrometry instrument. The condition of GC–MS was as follow: helium (carrier gas), the linear velocity of 30 cm/sec, column temperature of 50°C and injection temperature of 280°C, the pressure of 26.7 kPa, as well as 60 minutes contact time (Saputra et al., 2020). According to this test, the compounds of citronella oil are produced along with the percentage. The specified requirements for the quality of the essential oil content of citronella are 35% citronellal and 85% geraniol (Badan Standarisasi Nasional (BSN), 1995).

6. Gram staining of *Candida albicans*

Gram staining of the *Candida albicans* fungal was obtained by sterilizing the object glass utilizing 70% alcohol for the initial treatment. Furthermore, insert one drop of physiological NaCl into the object glass. One swipe of fungal was taken from pure culture and placed on fungal isolates. The fungal isolate was obtained, stained with purple dye (crystal violet), and waited for 2 minutes. Furthermore, the fungal isolate was flowed with sterile distilled water and dried. Added 1 drop of Lugol on the fungal isolate and occupied for 1 minute. It then flowed with sterile distilled water and desiccated. Alcohol of 96% concentration was added, and the fungal isolate was occupied for 30-40 seconds. Then flowed with sterile distilled water and desiccated again. Added 1 drop of Safranin, then flowed with sterile distilled water and desiccated. Added 1-2 drops of immersion oil. In addition, the object glass was observed on a light microscope with a magnification of 40x and 100x for observation of fungal morphology (Indrayati & Sari, 2018).

7. Preparation of test sample solution and suspension of the *Candida albicans*

Citronella oil test solution was prepared by diluting the oil in 10% DMSO at concentrations of 2.5, 5, 10, 20, and 40% (v/v). Positive control was Micafungin 50 mg/10 mL at 5% (w/v) and the negative control was 10% DMSO.

The suspension of the *Candida albicans* was obtained from one swipe fungal previously cultured. One swipe of fungal was transferred into a tube containing 10 mL of sterile distilled water and then homogenized. Standard McFarland 0.5 was prepared by mixing 0.1 mL of 1.175% BaCl₂ and 9 mL of 1% H₂SO₄.

8. Antifungal assay

Antifungal test with well diffusion method with 2.5, 5, 10, 20,
and 40% in triple replication (Kandimalla et al., 2016).

Data Analysis

The data obtained in this study were analyzed using the descriptive analysis method.

Results and Discussion

Organoleptic Characteristics of The Essential Oil

The organoleptic inspection consisted of citronella oil’s color, odor, and taste. According to Table 1, the color of *Cymbopogon nardus* essential oil was pale yellow or clear. Thus, that color test met the quality standard of SNI 06–3953–1995. The result of the *Cymbopogon nardus* essential oil odor test was representative of the citronella plant. Furthermore, the result of *Cymbopogon nardus* essential oil taste determination is spicy. The results in this study are also supported by Lely et al. (2017). The citronella oil tested organoleptically had a clear yellow oil color, spicy taste, and characteristic odor as citronella plant.

<table>
<thead>
<tr>
<th>No.</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Color</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>2.</td>
<td>Odor</td>
<td>Typical citronella</td>
</tr>
<tr>
<td>3.</td>
<td>Taste</td>
<td>Spicy</td>
</tr>
</tbody>
</table>

Specific Gravity, Refractive Index, and Ethanol Solubility of the Oil

According to Table 2, the results of determining the value of specific gravity, refractive index, and solubility in ethanol reached the standard of SNI 06-3953-1995. Specific gravity describes the function of its constituent components and each amount. Every component will have different specific gravity. The concentration and specific gravity values are directly proportional in that the greater concentration of certain oil components will also have a higher specific gravity of the oil (Khasanah, 2015). In accordance with the amount value, specific gravity is also influenced by the value of impurities in the oil.

The refractive index is influenced by the length of the carbon chain and the number of double bonds, which affects the value of the refractive index. The effect of carbon chain length and the number of double bonds is directly proportional to the refractive index enhancement. According to the research result conducted by Nuryoto et al. (2011), clove oil with a larger refractive index is considered better since eugenol content within the oil is greater. The refractive index value is affected.

This is presumably because terpene compounds dominate the components contained in the dry windmilled essential oil. If the essential oil contains many terpenoid group compounds, the refractive index value will increase directly to the number of increasing terpenoid group compounds (Khasanah, 2015). The value of the refractive index can also be affected because of the water content in the essential oil. The water component in the oil can be caused by the refining
process, especially in separating oil from water when conducted manually. Components of water in oil can reduce the value of the refractive index because of water characteristic, which quickly refracts light (Jayanudin & Hartono, 2011).

The refractive index is considered good in guaranteeing the quality of essential oils because it is estimated to contain more terpene group compounds. The greater the value of the refractive index, the greater the percentage of the analysis of the main content of the essential oil. It has greater quality and benefits, especially if used in medicine that utilizes secondary metabolites from the terpene group (Khasanah, 2014). In addition, the refractive index value reached the SNI range is considered to have guaranteed quality because it does not contain the water, which by the duration of storage of kaffir lime leaves, where the refractive index value produced from leaves treated with the dry milled wind has a higher refractive index value than the oil produced from kaffir lime leaves with intact and ripening treatment can reduce the refractive index value (Jayanudin and Hartono, 2011).

The solubility value of essential oils in alcohol can be caused by the presence of unoxygenated and oxygenated terpenes in essential oils. If the content of oxygenated terpene compounds is contained excessively in the essential oil, thus it influences the enhancement of the essential oil solubility within alcohol. The greater solubility of the essential oil in ethanol, the better quality of the essential oil will be (Wibowo et al., 2016).

**Analysis of Citronella Oil Composition**

The main components of *Cymbopogon nardus* essential oil have been claimed to have an antifungal ability obtained from the terpenoid and monoterpenes groups. According to SNI 06-3953-1995, the essential oil of *Cymbopogon nardus* must reach the quality for a minimum content of 35% citronellal and 85% geraniol. Reaching out to the quality requirements according to SNI is a fundamental assessment concerning the quality of citronella oils that suits to be distributed in export-import stages. The results of the GC–MS is convenient in Table 3 below. The *Cymbopogon nardus* essential oil in this study contains 26.06% citronellal, 26.314% -citroneol, and 17.90% geraniol. If the citronellal and geraniol within citronella oil do not reach the quality requirements of SNI, the essential oil cannot be traded through international trade. This makes producers only export raw materials of citronella (Laswatty et al., 2019). The chemical compounds of the citronella oil represented the quality of that oil.

There are several things that can affect the quality of citronella oil, one of which is the location of where the citronella grows.
Table 1. Citronella oil quality test results according to SNI

<table>
<thead>
<tr>
<th>No.</th>
<th>Test Parameters</th>
<th>Test Result</th>
<th>SNI 06 – 3953 – 1995 value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Specific gravity</td>
<td>0.884 g/mL</td>
<td>0.880 – 0.992 g/mL</td>
</tr>
<tr>
<td>2.</td>
<td>Refractive index</td>
<td>1.475</td>
<td>1.466 – 1.475</td>
</tr>
<tr>
<td>3.</td>
<td>Solubility in ethanol</td>
<td>1:1 clear</td>
<td>1:2 clear</td>
</tr>
</tbody>
</table>

The growth is influenced by soil fertility, climate, and the height of the location above sea level. Citronella can grow in various locations with a planting height of up to 1,200 meters above sea level and the optimal height at 250 meters above sea level (Djazuli et al., 2011). The temperature of the planting site will diminish while the altitude rises. Although citronella can grow in different environmental temperatures, the best for citronella growth is a lower temperature. Soil fertility is generally characterized by high nutrient availability. Nonetheless, the nutrients in the soil are dynamic and change according to the seasons, the method of the soil process, and the types of plants (Dacosta et al., 2017). The harvest time of the citronella plant will also affect the oil quality obtained. Late harvest time will cause flower growth, making the oil quality lower.

Antifungal Activity of Citronella Oil

Table 4 indicates that a higher concentration of citronella oil enhances the greater inhibition zone (mm) value. Terpenoids are monoterpenoids with a compound consisting of 2 isoprene units and 10 carbon atoms.

This is related to citronella capability, which is improved greater strength in inhibiting the fungal growth by higher oils concentrations. According to Figure 2, citronella oil with 40% concentration, moreover for three repetitions, obtained the largest inhibition zone measurement value with 23.31 mm on average.

The result was classified in a strong category for an antibiotic inhibition zone diameter against the test isolate. The potency of antifungal activity obtained from *Cymbopogon nardus* essential oil was caused by the presence of bioactive compounds terpenoid, namely monoterpenoids. The main ingredients are citronellal, geraniol and citronellol. According to Toledo et al. (2016), essential oils derived from plants can be an alternative as antifungals due to secondary metabolites such as terpenes, tannins, alkaloids, and flavonoids. They worked synergistically similarly in azole antifungal drugs (such as fluconazole, metronidazole, and itraconazole), which interacted with C-14α demethylase (cytochrome P450 enzyme) for inhibiting the lanosterol demethylase from being ergosterol, a crucial sterol for the fungal cell membranes formation. This inhibition process will disrupt the fungal function because the membrane’s protein structure becomes damaged and enhances the permeability of the fungal cell membrane, which will cause fungal death cell (Mustamim, 2013; Nurmansyah, 2010).
Tabel 3. The components of essential oil

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Retention Time (minutes)</th>
<th>Concentration (%)</th>
<th>Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.293</td>
<td>0.02</td>
<td>Cyclopentane, 1-methyl-3-(1-methyl ethyl)</td>
</tr>
<tr>
<td>2</td>
<td>11.103</td>
<td>0.10</td>
<td>6-Methyl-5-hepten-2-one</td>
</tr>
<tr>
<td>3</td>
<td>11.397</td>
<td>0.09</td>
<td>β-Myrcene</td>
</tr>
<tr>
<td>4</td>
<td>13.542</td>
<td>3.40</td>
<td>d-Limonene</td>
</tr>
<tr>
<td>5</td>
<td>13.777</td>
<td>0.02</td>
<td>p-Menthan-8-ol</td>
</tr>
<tr>
<td>6</td>
<td>13.944</td>
<td>0.16</td>
<td>cis-Ocimene</td>
</tr>
<tr>
<td>7</td>
<td>14.543</td>
<td>0.10</td>
<td>1,3,6-Octatriene, 3,7-dimethyl-, (E)- (CAS)</td>
</tr>
<tr>
<td>8</td>
<td>14.881</td>
<td>0.06</td>
<td>5-Heptenal, 2,6-dimethyl- (CAS) Melonal</td>
</tr>
<tr>
<td>9</td>
<td>15.943</td>
<td>0.03</td>
<td>Cyclohexene 4-methyl-1-(methyleneylethyl)</td>
</tr>
<tr>
<td>10</td>
<td>16.823</td>
<td>0.09</td>
<td>α-Terpinolene</td>
</tr>
<tr>
<td>11</td>
<td>17.781</td>
<td>1.84</td>
<td>Linalool L</td>
</tr>
<tr>
<td>12</td>
<td>19.654</td>
<td>0.03</td>
<td>β-Sitronelol</td>
</tr>
<tr>
<td>13</td>
<td>20.781</td>
<td>3.36</td>
<td>Isopulegol 2</td>
</tr>
<tr>
<td>14</td>
<td>21.408</td>
<td>26.06</td>
<td><strong>Sitronelal</strong></td>
</tr>
<tr>
<td>15</td>
<td>21.549</td>
<td>0.96</td>
<td>Isopulegol 1</td>
</tr>
<tr>
<td>16</td>
<td>21.875</td>
<td>0.07</td>
<td>Cyclohexanone, 5-methyl-2-(1-methylethyl)</td>
</tr>
<tr>
<td>17</td>
<td>22.145</td>
<td>0.24</td>
<td>(-)-Isopulegol</td>
</tr>
<tr>
<td>18</td>
<td>22.135</td>
<td>0.02</td>
<td>Cyclohexen, 1-(ethoxy methyl)-4-methylethyl</td>
</tr>
<tr>
<td>19</td>
<td>22.692</td>
<td>0.07</td>
<td>Isopulegol 1</td>
</tr>
<tr>
<td>20</td>
<td>22.855</td>
<td>0.07</td>
<td>3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)</td>
</tr>
<tr>
<td>21</td>
<td>23.786</td>
<td>0.15</td>
<td>3-Cyclohexen -1-methanol, alpha.alpha</td>
</tr>
<tr>
<td>22</td>
<td>24.632</td>
<td>0.13</td>
<td>Decanal (CAS) n-Decanal</td>
</tr>
<tr>
<td>23</td>
<td>25.315</td>
<td>0.03</td>
<td>γ-gamma.-Isogeraniol</td>
</tr>
<tr>
<td>24</td>
<td>25.841</td>
<td>0.21</td>
<td>Nerol</td>
</tr>
<tr>
<td>25</td>
<td>26.314</td>
<td>13.39</td>
<td><strong>β-Sitronelol</strong></td>
</tr>
<tr>
<td>26</td>
<td>26.717</td>
<td>0.32</td>
<td>Z-Sitral</td>
</tr>
<tr>
<td>27</td>
<td>28.008</td>
<td>17.90</td>
<td><strong>Geraniol</strong></td>
</tr>
<tr>
<td>28</td>
<td>28.708</td>
<td>0.37</td>
<td>Sitral</td>
</tr>
<tr>
<td>29</td>
<td>29.135</td>
<td>0.03</td>
<td>β-Sitronelol</td>
</tr>
<tr>
<td>30</td>
<td>31.474</td>
<td>0.02</td>
<td>Carvenolide</td>
</tr>
</tbody>
</table>

Table 4. Citronella oil antifungal test

<table>
<thead>
<tr>
<th>No</th>
<th>Conc. (% v/v)</th>
<th>Inhibition zone (mm)</th>
<th>Activity category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>18.38±0.01</td>
<td>Strong</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>20.54±0.04</td>
<td>Very Strong</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>21.37±0.03</td>
<td>Very Strong</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>22.04±0.05</td>
<td>Very Strong</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>23.31±0.04</td>
<td>Very Strong</td>
</tr>
<tr>
<td>6</td>
<td>K(+)</td>
<td>19.42±0.06</td>
<td>Strong</td>
</tr>
<tr>
<td>7</td>
<td>K (-)</td>
<td>0±0</td>
<td>Not Inhibiting</td>
</tr>
</tbody>
</table>

Conc.: Concentration
In accordance with this study, the results of the antifungal activity test utilizing citronella oil against clinical isolates of *Candida albicans* indicated the capability to inhibit fungal growth. Thus, it can be claimed that citronella oil from Menderek Village, Bener Meriah Regency, Aceh Province has the potential activity as an antifungal.

**Conclusion**

Citronella oil showed inhibition activity against clinical isolate *Candida albicans*, with the largest inhibition zone of 23.31 mm at a concentration of 40%. The main compounds of citronella oil were citronellal, geraniol, and citronellyl.

**References**


