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Antibacterial Activity of Citronella Essential Oil from *Cymbopogon nardus (L.) Rendle)* Against *Methicillin-Resistant Staphylococcus aureus*

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Abstract. Irrational antibiotic use in the treatment of infectious diseases can emerge antibiotic-resistant bacterial strains, such as *Methicillin Resistant Staphylococcus aureus* (MRSA). This study aimed to determine the physical properties of citronella essential oil (*Cymbopogon nardus* (L.) Rendle) and assess its antibacterial activity against MRSA. Citronella essential oil was extracted using a water-steam distillation method, yielding 0.5%. Citronella essential oil has a specific gravity of 0.904, a refractive index of 1.470, and is slightly soluble in 80% ethanol. All these parameters were in accordance with the Indonesian Standard Requirement for citronella essential oil. The disc diffusion assay was used to test the antibacterial activity of this citronella essential oil. This study indicated that citronella essential oil has an activity to inhibit MRSA in-vitro.

Keywords: Antibacterial activity, Citronella Essential Oil, *Cymbopogon nardus*, Methicillin Resistant Staphylococcus aureus

Abstrak. *Penggunaan antibiotik yang tidak rasional dalam pengobatan penyakit infeksi dapat menyebabkan munculnya strain bakteri yang resisten terhadap antibiotik seperti bakteri Methicillin Resistant Staphylococcus aureus (MRSA). Penelitian ini bertujuan untuk mengetahui sifat fisik dan mengevaluasi aktivitas antibakteri minyak atsiri serai wangi (Cymbopogon nardus (L.) Rendle) terhadap MRSA. Minyak atsiri serai wangi diperoleh dengan metode destilasi air-uap dan memberikan rendemen 0,5%. Sifat fisik serai wangi menunjukkan berat jenis 0,904, indeks bias 1,470 dan minyak sedikit larut dalam etanol 80%. Semua parameter ini memenuhi persyaratan Standar Nasional Indonesia (SNI) untuk minyak atsiri serai wangi. Aktivitas antibakteri dari minyak atsiri serai wangi ini dilakukan dengan uji difusi cakram. Penelitian ini menunjukkan bahwa minyak atsiri sereh wangi memiliki aktivitas menghambat MRSA secara in-vitro.*

Kata Kunci: *Aktivitas Antibakteri, Cymbopogon nardus, Minyak Atsiri Serai Wangi, Methicillin Resistant Staphylococcus aureus*

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1 Introduction

Infectious disease is a major problem that increases morbidity and mortality throughout the world. *Methicillin-Resistant Staphylococcus aureus* (MRSA), is one of the main nosocomial pathogenic germs in the world that is resistant to an entire class of β-lactam antibiotics which affect increasing patient morbidity, mortality, and hospital costs [1]. Today, it has limited antibiotics for treating the infection caused by MRSA. Finding a new drug to treat MRSA infection is challenging, especially from natural resources.

Essential oils derived from aromatic plants have great biological properties to prevent and treat human systemic diseases. They have been reported as good therapeutic agents for chemoprevention, cancer suppression, antidiabetic, and lowering serum cholesterol and triglycerides [2]. Many of them are highly active against Gram-positive and Gram-negative bacteria, as well as viruses and fungi. Essential oils, due to their anti-infective content, can be used to combat drug-resistant bacteria and to prevent the formation of resistance in pathogenic microbes.

Cymbopogon nardus (citronella grass), is a perennial aromatic plant from the family Poaceae, originating in tropical Asia, containing volatile oil that has antifungal and antibacterial properties. Citronellal, geraniol, and citronellol are the major constituents of citronella essential oil. The citronella essential oil compound can differ depending on where the sample grows. Geographical location can influence the production of secondary metabolite content due to seasonal differences, resulting in differences in the amount of secondary metabolite content that alters the pharmacological activity and oil quality [3].

Previous research reported antibacterial activity from citronella essential oil against *Micrococcus luteus, Bacillus cereus,* and *Staphylococcus aureus* at Minimum Inhibitory Concentration (MIC) 2.0 µL/mL [4], proving that the dominant citronellal, trans-geraniol, and citronellol compounds in citronella essential oils have antibacterial activity. Antibacterial activity against *S. aureus* produced a 1.23 mm inhibition zone at MIC 7.81 µL/mL [5]. Other research has shown citronella oil inhibited the growth of clinical isolates of *Staphylococcus aureus* at a concentration of 20; 30; 40 and 50% with inhibition zones 16.6, 20.2, 26.7, and 31 mm, respectively [6]. Citronella essential oil (*C. nardus* L) also has activity on MRSA with a diameter inhibition zone of 19 mm at a concentration of 30% [7].

2 Material and Methods

2.1 Plant Determination

The determination of the citronella plant was carried out at the Herbarium Bogoriense for Botanical Research Center for Biology-LIPI Bogor. The purpose of the determination was to determine the correct identity of the plants used in this study.

2.2 Essential Oil Isolation

Fresh leave of *Cymbopogon nardus* (6 kg) was isolated by water and steam distillation apparatus for three hours. The distillate was separated and then dried with magnesium sulfate to rest the remaining water. The pure essential oil was weighed to calculate the yield. The oil was stored in a refrigerator at a temperature of 4°C in a sealed vial for further analysis.

2.3 Physico-chemical analysis

Specific gravity was determined at 25 °C using a 25 ml capacity pycnometer. The refractive index was measured with an Abbe refractometer, at 20ºC. Solubility in ethanol was tested by complete solubilization of 1 mL of essential oil in 85% dilute ethanol, at 20ºC.

2.4 Microbial strains

The MRSA strains used in this study were clinical isolates from patients of dr. Zainoel Abidin Regional Public Hospital, Banda Aceh. The isolates were identified as MRSA according to gram staining, positive catalysis, and methicillin-resistant test.

2.5 Antibacterial Activity

The bacteria isolate was grown on Nutrient Agar (Hi-Media M001A) at 37°C. The antimicrobial activity of the Citronella essential oil was determined by the agar diffusion method *(Kirby-Bauer)* using disc paper. Each Petri dish was divided into 8 parts. As much as 0.5 ml bacteria suspension containing approximately 106 CFU/mL dispersed over agar on a petri dish. 30 mL of Mueller-Hinton Agar (Hi-Media M-173) was poured into a sterile Petri dish and allowed to set. 20 µL of citronellal essential oil was absorbed by a sterile paper disc (d=6 mm) were placed on agar. Ciprofloxacin (5 µg) was used for positive control and tween 80 (10%) as a negative control. Inhibition zones were determined after incubation for 24 h at 37°C. All tests were done for each citronella essential oil concentration at 2.5, 5, 10, 15, and 20% in triplicate.

3 Result and Discussion

3.1 Plant Determination

The results showed that the citronella plant used in this study was a species of *Cymbopogon nardus* (L.) Rendle from the family Poaceae (Figure 1). Determination aimed to ensure the correctness of the plant species used as samples in the study.

Figure 1. Fresh leaf of *Cymbopogon nardus* (L) Rendle

3.2 Essential Oil Yield

Citronellal essential oil obtained by water-steam distillation was 30.13 ml (0.5%) with a pale yellow color and pleasant odor. As previously reported, citronellal essential oil obtained by water-steam distillation method from Lembang, West Java, Indonesia, yielded the same yield (0.5 %), with the major constituents of this Citronella oil being citronella, δ-cadinene, methyl Isoeugenol, caryophyllene, geranyl butyrate, geranyl acetate, citronellyl propionate, germacrene, α-bergamoten, and eugenol [2].

Plant chemical constituents are influenced by the geographical conditions in which the plants grow. Citronella grass from this study grows in Takengon, middle Aceh, Indonesia, which shares geographic, climatic, and ecological parameters with Lembang, west Java, Indonesia. Furthermore, the chemical constituents of the plant's leaves can be affected by the maturity stage and harvesting time.

3.3 Physical Properties

Citronella essential oil is slightly soluble in ethanol, which indicates the presence of oxygenated terpenes with polar ends such as citronellal and geraniol in fragrant citronella essential oil. [8]. Citronella essential oil’s refractive index is 1.470. The refractive index is used mainly to measure how fast light travels through citronella oil to confirm the purity of this oil. It depends on their molecular weight, chain length, degree of unsaturation, and degree of conjugation. Citronella essential oil’s specific gravity value obtained 0.904 is less than 1 indicating that the oil is less dense than water.

Table 1. Physicochemical properties of Citronella essential oil

Physicochemical Properties	**Result**
Colour	Pale yellow
Specific Gravity	0.904
Refractive Index	1.470
Solubility in 80% ethanol v/v	Slightly soluble

Figure 2. Citronella Oil

3.4 Antibacterial Activity

Zone inhibition MRSA by citronella essential oil is listed in Table 2. Antibacterial activity of citronellal oil against MRSA showed that 10% of citronellal oil gave moderate antibacterial activity (diameter of inhibition = 13.20 mm) but antibacterial activity was decreasing at concentrations 15 and 20% (diameter of inhibition = 12.55 and 11.68 mm). However, ciprofloxacin 5 µg as positive control still gave strong activity against MRSA (diameter inhibition=28.54 mm) [9].

Table 2. Zone inhibition MRSA by citronella essential oil

Citronella Oil (%)	Inhibition Zone Diameter (mm)
2.5	0± 0
5	0± 0
10	13.20±1.13
15	12.55±0.76
20	11.68±1.56
25	10.03±1.75
Positive Control (Ciprofloxacin 5 µg)	28.54±0.27
Negative Control	0± 0

Several factors, including technical factors, biological factors, antibacterial compound content, extract diffusion power, and the type of bacteria used, can influence the results of antibacterial activity tests [10]. The amount of inoculum, incubation time, incubation temperature, and the composition as well as thickness of the agar medium are all technical factors. Biological factors, specifically the nature of bacterial resistance. Researchers can influence technical factors, but biological factors are uncontrollable.

The presence of antibacterial compounds is usually related to the environment in which the plant grows. Because differences in temperature, light, humidity, rainfall, soil type, and nutrients absorbed by plants affect secondary metabolite production, the location of growth determines the type and amount of secondary metabolite content in plants [11]. This is supported by a comparison of citronellal, geraniol, and citronellol levels from GC-MS results of citronella essential oil conducted by several researchers. Citronella, geraniol, and citronellol levels in the Gayo Lues area were 51.41, 20.06, and 11.01%, respectively. [12]. Meanwhile, the levels of citronellal, geraniol, and citronellol in citronella from Java were 32-45%, 12-18%, and 11-15%, respectively [13]. These secondary metabolites also play a role in providing antibacterial activity.

The antibacterial mechanism of essential oils work by interfering with the process of forming cell walls or bacterial cell membranes, causing the bacterial cell walls to be formed but imperfectly [14]. The mechanism of antibacterial activity of terpenoids such as citronellal compounds, citronellol, geraniol, geranyl acetate, and citronellyl acetate is still unknown, but it is thought to involve the disruption of bacterial cell membranes by lipophilic compounds [15]. The monoterpenes group includes citronellal, geraniol, and citronellol, which are dominant in citronella essential oil. The mechanism of monoterpene compounds as an antibacterial is by providing a toxic effect on the structure and function of bacterial cell membranes. The lipophilic nature of monoterpenes causes these compounds to move from the polar phase to the non-polar bacterial cell membrane structure [5].

As essential oils are hydrophobic, their chemical components may not diffuse optimally in the hydrophilic environment such as present in the disc diffusion assay. As a result, it should be re-evaluated using an assay that optimizes its potential antimicrobial activity.

Conclusion

Citronella essential oil has antibacterial activity against MRSA with the largest inhibition zone diameter at a concentration of 10%.

Conflict of interest

We declare that we have no conflicts of interest to disclose.

REFERENCES

[1] Aqil F, Zahin M, Ahmad I, Owais M, Khan MSA, Bansal S, Farooq S. Antifungal Activity of Medical Plant Extracts and Phytocompounds: A Review. Combating Fungal Infections. Springer, Heidelberg. 2010. 449-484.

[2] Wibowo DP, Febriani Y, Riasari H, Aulifa DL, Chemical composition, antioxidant and antibacterial activities of the essential oils of medicinal plant Cymbopogon nardus from Lembang West Java. Research Journal of Chemistry and Environment 2018. Vol.22

[3] Biavatti MW. Synergy: an Old Wisdom, a New Paradigm for Pharmacotherapy. Brazilian Journal of Pharmaceutical Sciences. 2009.45 (3): 371-378.

[4] Simic A, Rancic A, Sokovic MD, Ristic M, Grujic-Jovanovic S, Vukojevic J, Marin PD. Essential Oil Composition *of Cymbopogon winterianus* and *Carum carvi* and Their Antimicrobial Activities. Pharmaceutical Biology. 2008. 46(6): 437-441.

[5] Brugnera DF, de Oliveira MMM, dan Piccoli RH. Essential Oils of Cymbopogon sp. In the Control of Foodborne Pathogenic Bacteria. Alimentos e Nutricao (Brazilian Journal of Food and Nutrition). 2011. 22(3): 339-344.

[6] Riska, M. Uji Aktivitas Antibakteri Minyak Atsiri Sereh Wangi (*Cymbopogon nardus* L.) Terhadap Isolat Klinis *Staphylococcus aureus* Secara In Vitro. Skripsi. Fakultas Kedokteran Universitas Syiah Kuala, Banda Aceh. 2013.

[7] Chao, S., Young, G., Oberg, C., dan Nakaoka, K. Inhibition of *Methicillin- Resistant Staphylococcus aureus* (MRSA) by Essential Oils. Flavour and Fragrance Journal. 2008. 23(6): 444-449.

[8] Armando, R. Memproduksi 15 Minyak Atsiri Berkualitas. Penerbit Niaga Swadaya, Jakarta. 2009.

[9] Morales G, Sierra P, Mancilla, A., Paredes, A., Loyola, L. A., Gallardo, O., dan Borquez, J. Secondary Metabolites From Four Medicinal Plants From Northern Chile: Antimicrobial Activity and Biotoxicity Against Artemia salina. Journal of the Chilean Chemical Society. 2003.48(2): 13-18.

[10] Moja, F. K. Uji Aktivitas Antibakteri Ekstrak Metanol Daun Mangga Bacang (Mangifera Foetida L.) Terhadap *Salmonella typhi* Secara in Vitro. Jurnal Mahasiswa PSPD FK Universitas Tanjungpura. 2015. 3(1).

[11] Fatchurrozak., Suranto., dan Sugiyarto. Pengaruh Ketinggian Tempat terhadap Kandungan Vitamin C dan Zat Antioksidan pada Buah Carica pubescens di Dataran Tinggi Dieng. Kelola Mina Laut Unit Sumenep. EL-VIVO. 2013. 1(1): 15-22.

[12] Ikhwan. Aktivitas Antijamur Minyak Atsiri Sereh Wangi (*Cymbopogon nardus*) Terhadap Penicillium sp. Dan Trichoderma sp. Skripsi. Fakultas Pertanian Universitas Syiah Kuala. 2013. Banda Aceh.

[13] Sastrohamidjojo, H. Kimia Minyak Atsiri. Penerbit Universitas Gadjah Mada, Yogyakarta. 2004.

[14] Ali, S., Baharuddin, M., dan Sappewali., S. Pengujian Aktivitas Antibakteri Minyak Atsiri Jahe (Zingiber officinale roscoe) Terhadap Bakteri *Staphylococcus aureus dan Escherichia coli*. Al-Kimia. 2013. 1(2): 18-31.

[15] Cowan, M. M. Plant Products as Antimicrobial Agents. Clinical microbiology reviews. 1999. 12(4): 564-582.