

A Potent *Staphylococcus Aureus* Growth Inhibitor Of A Dried Flower Extract Of *Pinus Merkusii* Jungh & De Vriese And Copper Nanoparticle

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Abstract. The paper report antibacterial activity of flower extract from *Pinus merkusii* Jungh Et De Vriese and its mixture with copper nanoparticle on *Staphylococcus aureus*. This finding revealed the potency of pine forestry waste to overcome a bacterial-resistance problem on some commercially antibiotics. The extract was prepared by hot water extraction of a dried powder of pine flower. Copper nanoparticle was synthesized following “green synthesis technique” using phenolic-rich extract of pine’s flower as a reduction and capping agent. In short, a mixture of pine’s flower extract and copper nanoparticle importantly was able to inhibit the growth of *Staphylococcus aureus* four times higher than that using water extract.

Keywords: pinus merkusii, phenolic, antibacterial, nanoparticle, green synthesis, staphylococcus aureus

1. Introduction

Resistant of pathogenic bacterial such as *Staphylococcus aureus* in some commercially antibiotic have become a serious health problem in Indonesia [1–3], not only for human but also for animal [4,5]. This leads the way on finding new kind of antibacterial compounds and antibacterial herb from local natural sources. In the same time, the forests of *Pinus merkusii* Jungh et De Vriese was expanded recently. In total more than 800 kHa in East Java region, and this has paid development strategy to improve domestic income. Turpentine oil and oleoresin has become the main product, but the forests also provide a huge amount of flower cone-waste. Previous finding indicated that antibacterial activity of essential oil isolated from pine’s sap. Some terpenoid groups of alpha-pinene, beta-pinene, delta-carene, d/l-limonene consist of oil [6,7]. However, this mixtures still provide a low antibacterial activity. In addition, other compounds were also reported from the extract of spruce and knot pine tree. Some phenolic groups detected such as abietic acid, dehydroabietic acid, pimaric acid, isopimaric acid, notrachelogenin, liovil, pinosylvlin, pinocembrin, methoxypinosylvlin, dimethoxypinosylvlin[8], hydroxymatairesinol, allo-hydroxymatairesinol [9]. However, these had low fungal activity and also no antibacterial activity reported. Recently, we have found that incorporating of compound as well as



in the extract with metal and metal nanoparticle could improve antibacterial activity [7,10]. The paper reports a new finding incorporating of water extract of pine's flower with copper nanoparticle for inhibiting the growth of pathogenic *Staphylococcus aureus*.

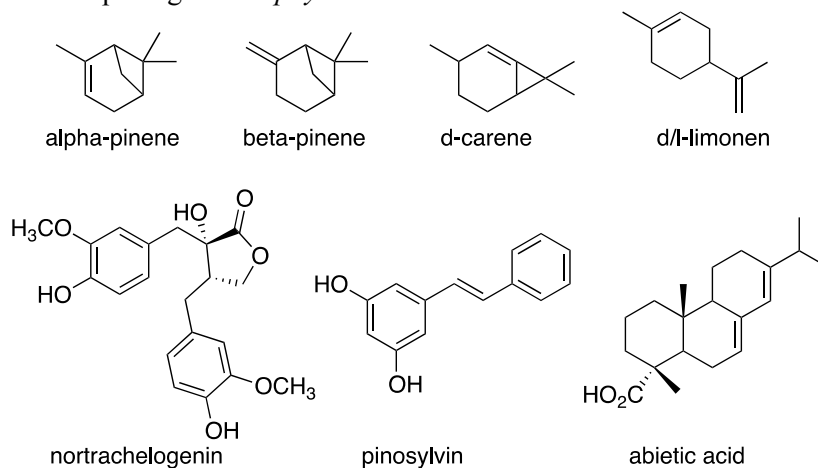


Figure 1. Some representative compounds isolated from pine tree

2. Experiment

2.1. Material and Chemicals

A dried-pine flower prepared from a pine's forestry waste of *Pinus merkusii* Jungh et De Vrise from local forestry in Malang, East Java. Plant taxonomy of the sample was determined by Dr. Djati Batoro (Laboratory of Plant Taxonomy, Brawijaya University). Chemicals used for research include copper sulfate hexahydrate (Merck), ethanol (Merck), acetone (Merck), and demineralized water (Bratachem)

2.2. Extraction Procedure

Water extract was prepared by extracting of a 50 g of dried powder of pine's flower with 250 mL water. This was boiled for 30 min and filtered of the aqueous extract of pine flower. Phenolic compounds in the extract was detected as precipitate or color changing of solution to violet or green-black [11], and its quantity was determined compared to gallic acid.

2.3. Nanoparticle Synthesis

Green synthetic of copper nanoparticle was undertaken by using phenolic-rich of pine's flower extract following reported procedure [12]. Nanoparticle analysis was performed using infrared spectrophotometry (Shimadzu FTIR 8400 series) and SEM-EDAX (FEI Inspect-S50).

2.4. Antibacterial Testing

Antibacterial activity was tested on *Staphylococcus aureus* local strain following Kirby-Bauer testing method [13]. Activity was determined by measuring (mm) clear zone surround the disc.

3. Result and Discussion

Extraction of a dried powder of pine flower cone produced a yellowish-brown of concentrated extract. Previous researchers reported some secondary metabolite composed the extracts from some part of tree, such as lignin [9], phenolic [8], terpenoids [6,14] groups, and oleoresin [15]. Moreover the result is found phenolic content 341.8 GAE/g which is able to be used for reduction and capping copper nanoparticle. Figure 2 (A) shows infrared spectra both of the extract and mixture of extract and copper

nanoparticle. Incorporating nanoparticle was detected in wavenumber 550 and 760 cm^{-1} for bond stretching of Cu--O. These both peaks are not observed in the extract sample. Interaction of copper nanoparticle with functional group of extract was detected in 3400 cm^{-1} (Cu---OH) and 1750 cm^{-1} (C=O---Cu). In addition, SEM image (Fig. 1B) display a clear nanoparticle size between 20-35 nm.

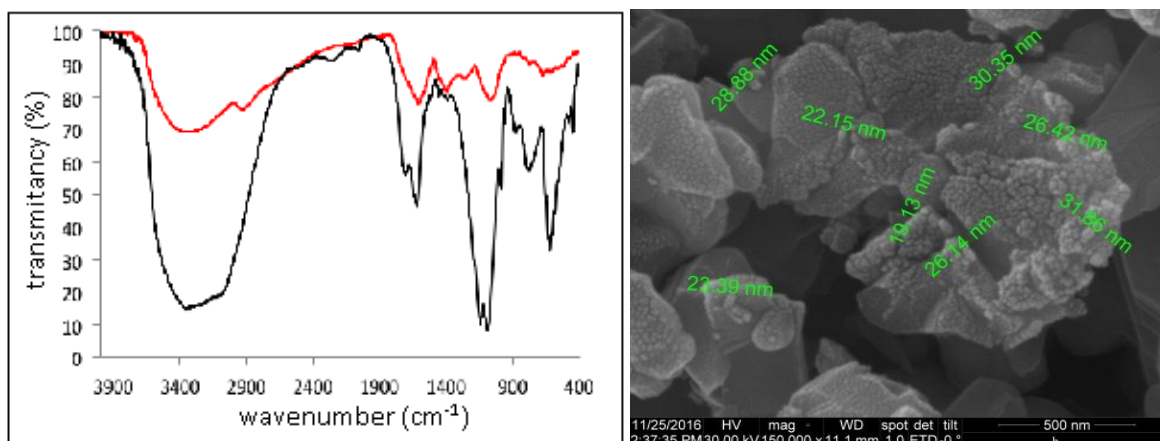


Figure 2. Infrared spectra of water extract pine's flower (red line) and its mixture with copper nanoparticle (black line) (A), and SEM image of copper nanoparticle (B)

The antibacterial activity was evaluated on *Staphylococcus aureus* and Kirby-Bauer Test [13] was applied to measure biological activity of the extract and its mixture with copper nanoparticle. The result is tabulated in Table 1 and compared to other finding previously [10,16]. The growth inhibition activities of the extract in various concentrations are recorded between 6.08 and 12.21 mm. The highest value using 100% of pine flower cone's extract, and the lowest using extract in 12.5%. This activity increases to 29.67 mm for 100% concentration of mixture the extract with copper nanoparticle. Similarly, results are provided for concentration 12.5% doubles to 12.21 mm. Visualization of the growth inhibition is displayed in Fig. 3. A clear zone surrounds the disc-loaded sample of extract contained-copper nanoparticle getting wider by increasing the concentration of sample from 12.5% to 100%. Conversely, a control-contained solvent did not have activity for *S. aureus*.

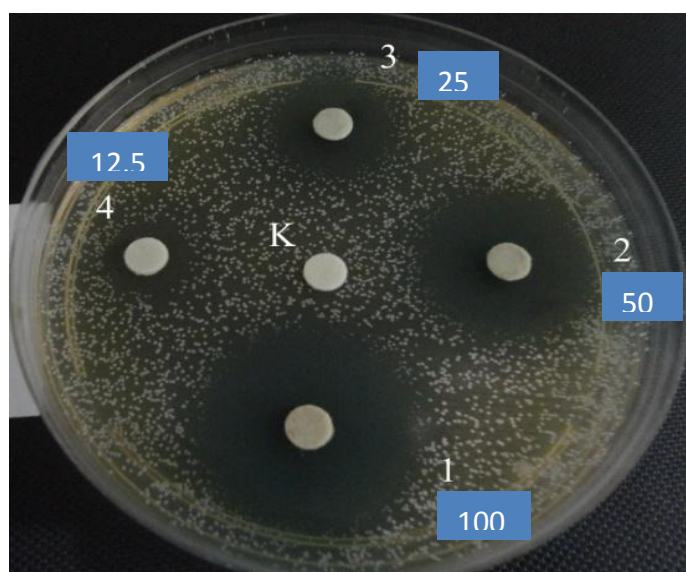


Figure 3. Antibacterial evaluation on *Staphylococcus aureus* of a mixture of pine flower cone's extract with copper nanoparticle in various concentrations, K is symbol for control.

Table 1. Growth inhibition activity of extract and its mixture with copper nanoparticle comparing to some references

| Sample | Growth inhibition diameter (mm) at concentration | | | |
|--|--|------------|------------|------------|
| | 100% | 50% | 25% | 12.5% |
| Extract | 12.21±0.02 | 9.48±0.01 | 7.12±0.01 | 6.08±0.01 |
| Mixture of extract-copper nanoparticle | 29.67±0.01 | 25.44±0.01 | 22.46±0.00 | 12.21±0.02 |
| Copper nanoparticle | 6.15 [16] | - | - | - |
| Amoxillin 10 mg | 7.50 [10] | - | - | - |
| Amoxillin-silver nanoparticle | 14.0 [10] | - | - | - |
| Erytromycin 5 mg | 10.0 [10] | - | - | - |

4. Conclusion

The water extract of pine flower cone contained phenolic compound can be used for reduction and capping copper nanoparticle. Its mixture with copper nanoparticle importantly improved antibacterial activity by inhibiting the growth of *Staphylococcus aureus*. This finding opens the potency of pine flower waste as antibacterial agent.

5. Acknowledgment

Author acknowledges to some institution for fund the research; PUPT Universitas Brawijaya and Ministry of Research, Technology and Higher Education from Republic of Indonesia. Mr. Widji Sulistjo and Hadi Kurniawan from Instrumentation Laboratory, Chemistry Department of Brawijaya University.

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