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# Inhibitory properties of panduratin A and 4-hydroxypanduratin A isolated from *Kaempferia pandurata* against some pathogenic bacteria

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## ABSTRACT

Two chalcone derivatives panduratin A (PA) (**1**) and 4-hydroxypanduratin A (4-HPA) (**2**) were isolated from the rhizome of *Kaempferia pandurata* Roxb, along with two flavanones pinostrobin (**3**) and pinocembrin (**4**). These compounds were screened for determining their growth inhibitory properties against nine bacterial pathogens: four Gram-(+) (*Bacillus cereus* ATCC 21772, *Bacillus subtilis* ATCC 663, *Listeria monocytogenes* ATCC 15313, and *Staphylococcus aureus* ATCC 29737) and five Gram-(-) (*Escherichia coli* 0157:H7, *Klebsiella pneumoniae* ATCC 13733, *Proteous mirabilis* ATCC 21100, *Salmonella typhi* ATCC 14028, and *Vibrio parahaemoliticus* ATCC 17802) bacteria. The results showed that only PA (**1**) and 4-HPA (**2**) exhibited significant inhibitory properties against these bacteria. Further antibacterial test indicated that these two compounds were active against all the tested bacteria, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values in the range of 1.2-75 µg/mL and 2.3-150 µg/mL, respectively. The Gram-(-) bacteria were found to be more affected by PA (**1**) and 4-HPA (**2**), and in general the antibacterial properties of PA (**1**) were two-fold higher than 4-HPA (**2**). Hence, PA (**1**) and 4-HPA (**2**) have the potential to be developed as new bioactive natural products that may serve as leads in new antimicrobial agents.

**KEYWORDS:** antibacterial activity, panduratin A, 4-hydroxypanduratin A, *Kaempferia pandurata*

## INTRODUCTION

*Kaempferia pandurata* Roxb. (syn. *Boesenbergia rotunda* Roxb.) (Zingiberaceae) is a plant of South East Asia, and in Indonesia is locally known as 'Temu Kunci'. The fresh rhizome has been used as a food seasoning and also as a folk medicine for the treatment of colic disorder, aphrodisiac, dry cough, rheumatism and muscular pains [1, 2]. Some studies have reported various biological activities of *K. pandurata*, including anti-inflammatory, antitumor, antidiarrhea, antidysentery, antiflatulence and antiepidermophytid effects [3, 4]. Previous phytochemical investigations of the rhizomes of this plant have succeeded in isolating an essential oil [5] and a number of flavonoid derivatives, including alpinetin, boesenbergins A and B, cardamonin, 2',6'-dihydroxy-4'-methoxychalcone, pinocembrin, pinostrobin, panduratin A (**1**) and 4-hydroxypanduratin A (**2**) [1, 6-9]. Panduratin A (**1**) and 4-hydroxypanduratin A (**2**) have shown antioxidative, antimutagenic, and antivirus activities [1, 10-12]. In addition, panduratin A (**1**) has also been reported to have anticancer [13-15], anti-inflammatory [16, 17],

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and antibacterial properties [2, 18-20]. Its antibacterial properties have been studied against *Entercoccus faecalis*, *E. faecium*, *Prevotella intermedia*, *Prevotella loescheii*, *Porphyromonas gingivalis*, *Propionibacterium acnes*, and *Streptococcus mutans.* This compound also has antibiofilm activities against multispecies oral biofilms, as well as antistaphylococcal activities [21, 22].

This study is aimed to find new bioactive natural products from traditional Indonesian medicinal plants. A systematic investigation of the constituents of *K. pandurata* was carried out. As a result, in addition to panduratin A (PA) (**1**), another promising antibacterial component, namely 4-hydroxypanduratin A (4-HPA) (**2**) was also isolated. We herein report the antibacterial activity of these two compounds.

## MATERIALS AND METHODS

### Plant materials

Rhizomes of *K. pandurata* were purchased from the herbal shop 'Babah Kuya', Pasar Baru, Bandung, Indonesia, in 2011, and were deposited at the Laboratory of Natural Product, Institut Teknologi Bandung.

### Isolation procedure

The dried and powdered rhizomes of *K. pandurata* (2 kg) were macerated with 10 L of acetone for 3 × 24 hours at room temperature to give a dark brown extract (256 g). A part of the extract (20.0 g) was fractionated by vacuum liquid chromatography (VLC, Si gel), and eluted using *n*-hexane-EtOAc of increasing polarity (10:0, 9:1, 8:2, 7:3, 1:1, 4:6, and 0:10) to give 16 fractions (F1-F16). Fractions F6 and F7 were combined and the combined fraction was recrystalized in methanol to give pinostrobin (PinS) (**3**) (1.8 g). Fraction F11 (400 mg) was purified by radial chromatography (Si gel, firstly eluted with an eluent of $CHCl_3$, and secondly with *n*-hexane-EtOAc at 17:3 and 8:2) yielding pinocembrin (PinC) (**4**) (120 mg). Purification of panduratin A (PA) (**1**) (80 mg) from fraction F9 was achieved using the same method (first run with *n*-hexane-EtOAc as eluent at 17:3; second run with *n*-hexane-$CHCl_3$ as eluent at 1:1). The purification of 4-hydroxypanduratin A (4-HPA) **(2)** (12 mg) from fraction F13 was also done using the same method (first run with *n*-hexane-$CHCl_3$ as eluent at 1:4; second run with *n*-hexane-EtOAc as eluent at 4:1). Compounds **1**-**4** were identified by NMR data.

### Bacterial strains

Nine bacterial species used in this study were obtained from the American Type Culture Collection (Rockville, MD, USA). They include *Bacillus cereus* ATCC 21772, *Bacillus subtilis* ATCC 6633, *Escherichia coli* 0157:H7, *Klebsiella pneumoniae* ATTC 13733, *Listeria monocytogenes* ATCC 15313, *Proteous mirabilis* ATCC 21100, *Salmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC 29737, and *Vibrio parahaemolyticus* ATCC 17802. All the bacteria were grown in Muller Hinton Agar (Difco Becton Dickincon, Sparks, MD, USA) and incubated at 37 °C for 24 h.

### Paper disc diffusion assay

Disc diffusion assay was done according to the method recommended by Clinical Laboratory Standards Institute [23]. In brief, a 100 μL of bacteria inoculum of approximately $10^6$ CFU (colony forming units)/mL was spread onto Mueller Hinton agar (MHA) plate using a sterile cotton swab. A sterile filter paper disc of 6 mm diameter was then placed on the top of MHA plate, and 10 μL of 0.1% solution (1000 μg/mL) of the tested compound containing 10% DMSO (dimethyl sulfoxide) in water was loaded onto the paper disc. The plate was incubated at 37 °C, and the clear zone was measured (in mm) after 24 h incubation. A solution containing 10% DMSO was used as a negative control, while a solution of chlorhexidine (500 μg/mL) was used as a positive control.

### Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC values were determined according to the previous standardization as recommended by the Clinical Laboratory Standards Institute [23]. Two compounds, PA (**1**) and 4-HPA (**2**), were tested for antibacterial activity against nine species of bacteria mentioned above in a 96-well microtiter plate using two-fold broth microdilution method, with an inoculum of approximately $10^6$ CFU/mL. In short, a 100 μL solution of each tested compounds (1200 μg/mL) was stirred and diluted two-folds with the test organism in 100 μL of Mueller Hinton

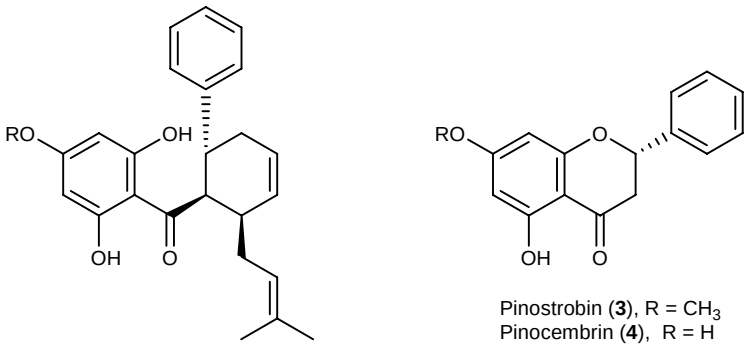
broth (MHB, Difco, Sparks, MD, USA). The highest concentration of the compound (600 µg/mL) was in column 12 of the microtiter plate, while column 3 contained the lowest concentration (1.2 µg/mL). The first column served as negative growth control (only MHB, no inoculum and tested compounds) while the second column was the positive growth control for all samples (only MHB and inoculum). The microtiter plate was then incubated aerobically at 37 °C for 24 h. The MIC was defined as the lowest concentration of tested compounds that resulted in no visible growth of the bacteria.

To determine MBC values, a 10 µL of aliquot was taken from each wells of MIC test system and was transferred onto MHA plates. The plates were incubated at 37 °C for 24 h until growth was seen in the growth control plates. MBC was defined as the minimum concentration required to completely kill the bacteria. In this assay, 500 µg/mL of chlorhexidine (CH) was used as a positive control.

## RESULTS AND DISCUSSION

Isolation work on the rhizomes extract of *K. pandurata* has led to the purification of four flavonoid derivatives. Based on their NMR spectral data these compounds were identified as panduratin A (PA) (**1**) [9], 4-hydroxypanduratin A (4-HPA) (**2**) [1], pinostrobin (PinS) (**3**) [6], and pinocembrin (PinC) (**4**) [4] (Figure 1). A preliminary test was carried out on these compounds for determining their growth inhibitory properties against nine pathogenic bacteria, including four Gram-(+) (*Bacillus cereus* ATCC 21772, *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes* ATCC 15313, and *Staphylococcus aureus* ATCC 29737) and five Gram-(-) (*Escherichia coli* 0157:H7, *Klebsiella pneumoniae* ATCC 13733, *Proteous mirabilis* ATCC 21100, *Salmonella thypi* ATCC 14028, and *Vibrio parahaemoliticus* ATCC 17802) bacteria. The test was done using paper disc diffusion method and the results are shown in table 1. Only PA (**1**) and 4-HPA (**2**) showed significant inhibitory properties against these tested bacteria (Table 1) and were comparable to the positive control, chlorhexidine (CH).

The two compounds PA (**1**) and 4-HPA (**2**) were subjected to antibacterial assay to determine their minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) values, which are shown in table 2. MIC values of these compounds against nine bacteria species ranged from 1.2 to 75 µg/mL. The strongest inhibitory effect was achieved by both compounds against the Gram-(+) *S. aureus* ATCC 29737, with an MIC value of 1.2 µg/mL. PA (**1**) also gave the same MIC value against the Gram-(-) *P. mirabilis* ATCC 21100. The data in table 2 also reveals that PA (**1**) and 4-HPA (**2**) showed significant activities against *E. coli* 0157:H7, *K. pneumonia* ATCC 13733, *B. subtilis* ATCC 6633, and *S. typhi* ATCC 14028, with MIC values of 2.3 and 4.7 µg/mL, respectively. A significant activity was also demonstrated by PA (**1**) against *V. parahaemoliticus* ATCC 17802 (MIC value of 9.4 µg/mL). Furthermore, moderate activities were shown by 4-HPA (**2**) against *V. parahaemoliticus* ATCC 17802, *B. cereus* ATCC 21772, and




Panduratin A (**1**), R = $CH_3$
4-Hydroxypanduratin A (**2**), R = H

Pinostrobin (**3**), R = $CH_3$
Pinocembrin (**4**), R = H

**Figure 1.** Structures of isolated compounds from *K. pandurata*.

**Table 1.** Growth inhibition of compounds isolated from *K. pandurata* against nine bacteria.

| Bacteria | CH[1)] | PA[2)] | 4-HPA | PinS | PinC |
|---|---|---|---|---|---|
| | | (**1**) | (**2**) | (**3**) | (**4**) |
| Gram-(+) | | | | | |
| *Bacillus cereus* ATCC 21772 | ++ | ++ | ++ | - | - |
| *Bacillus subtilis* ATCC 6633 | +++ | +++ | +++ | - | + |
| *Listeria monocytogenes* ATCC 15313 | ++ | ++ | ++ | - | - |
| *Staphylococcus aureus* ATCC 29737 | +++ | +++ | +++ | - | - |
| | | | | | |
| Gram-(-) | | | | | |
| *Escherichia coli* 0157:H7 | ++ | +++ | +++ | - | - |
| *Klebsiella pneumonia* ATCC 13733 | ++ | +++ | +++ | - | - |
| *Proteous mirabilis* ATCC 21100 | +++ | +++ | +++ | - | - |
| *Salmonella typhi* ATCC 14028 | ++ | +++ | +++ | - | - |
| *Vibrio parahaemoliticus* ATCC 17802 | ++ | ++ | ++ | - | - |

"-", < 9 mm; "+", 9-11 mm; "++", 12-13 mm; "+++", > 13 mm.
[1)]CH, chlorhexidine at 500 μg/mL.
[2)]PinS, Pinostrobin; PinC, pinocembrin; PA, panduratin A; 4-HPA, 4-hydroxypanduratin A at 1000 μg/mL.

**Table 2.** MIC and MBC values (in μg/mL) of panduratin A (PA) (**1**) and 4-hidroxypanduratin A (4-HPA) (**2**).

| Bacteria | PA (**1**) | | 4-HPA (**2**) | |
|---|---|---|---|---|
| | MIC | MBC | MIC | MBC |
| Gram-(+) | | | | |
| *Bacillus cereus* ATCC 21772 | 75 | 150 | 37.5 | 75 |
| *Bacillus subtilis* ATCC 6633 | 2.3 | 2.3 | 4.7 | 4.7 |
| *Listeria monocytogenes* ATCC 15313 | 37.5 | 37.5 | 37.5 | 37.5 |
| *Staphylococcus aureus* ATCC 29737 | 1.2 | 1.2 | 1.2 | 2.3 |
| | | | | |
| Gram-(-) | | | | |
| *Escherichia coli* 0157:H7 | 2.3 | 4.7 | 4.7 | 4.7 |
| *Klebsiella pneumonia* ATCC 13733 | 2.3 | 18.8 | 4.7 | 4.7 |
| *Proteous mirabilis* ATCC 21100 | 1.2 | 4.7 | 2.3 | 4.7 |
| *Salmonella typhi* ATCC 14028 | 2.3 | 18.8 | 4.7 | 4.7 |
| *Vibrio parahaemoliticus* ATCC 17802 | 9.4 | 18.8 | 18.8 | 18.8 |

*L. monocytogenes* ATCC 15313 (MIC values of 18.8-37.5 μg/mL) and a weak activity of PA (**1**) against *B. cereus* ATCC 21772 (MIC value of 75 μg/mL). In general, the Gram-(-) bacteria were more susceptible to both PA (**1**) and 4-HPA (**2**) than the Gram-(+) bacteria, and the inhibitory properties of PA (**1**) were higher than 4-HPA (**2**). Table 2 also presents MBC values of PA (**1**) and 4-HPA (**2**) against all tested bacteria, which reveals that they were the same or two-fold higher than MIC

values, indicating that both PA (**1**) and 4-HPA (**2**) can be considered as bactericidal agents [24].

Finding new antibacterial agents is a matter of urgency as antibiotic resistance in infectious diseases has become a global issue [25]. Although traditionally these agents have been obtained from microbes, in recent years much attention has been devoted to plants as an alternative source of the agents [26, 27]. Amongst plant-derived compounds, PA (**1**) isolated from the rhizome of *K. pandurata* has been proven as a promising new antibacterial agent. Antimicrobial studies of this compound have been reported by many researchers [2, 18-22, 28]. However, there are no antimicrobial studies on its de-*O*-methyl analogue, 4-HPA (**2**). Thus, this paper reports for the first time the antibacterial properties of 4-HPA (**2**). The results presented in table 2 indicates that both PA (**1**) and 4-HPA (**2**) are strong antibacterial and bactericidal agents against the Gram-(+) *B. subtilis* ATCC 6633 and all four tested Gram-(-) bacteria. Although less active by two-fold, a structural change from PA (**1**) to 4-HPA (**2**) by de-*O*-methylation still retains its antibacterial properties. Furthermore, the results of antibacterial evaluation of PA (**1**) in this study were comparable to those reported previously [20, 21].

## CONCLUSION

Phytochemical study combined with biological assay has led to the isolation and identification of two active components, namely panduratin A **(1)** and 4-hydroxypanduratin A **(2)** as bacterial growth inhibitors, from the rhizome *K. pandurata*. Further antibacterial test showed that these two compounds were strong antibacterial and bactericidal agents against five tested bacteria: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29737 [Gram-(+)], *Escherichia coli* 0157:H7, *Klebsiella pneumonia* ATCC 13733, *Proteous mirabilis* ATCC 21100, and *Salmonella typhi* ATCC 14028 [Gram-(-)]. The results suggested that both PA (**1**) and 4-HPA (**2**) are promising chemical constituents of the rhizome of *K. pandurata* that can be used as antibacterial agents and may serve as leads for developing new antimicrobial agents.

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## CONFLICT OF INTEREST STATEMENT

No conflicts of interest.

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