TWO FLAVANONES FROM FINGER-ROOT (*Curcuma rotunda*) AND ITS ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Finger-root (*Curcuma rotunda*) belongs to Zingiberaceae family, commonly used as a medicinal plant but has not been widely reported for its secondary metabolites and bioactivities. This study aims to isolate and characterize secondary metabolites contained in the semi-polar extract of *C. rotunda* rhizomes and evaluate its antibacterial activities. Simplicia of *C. rotunda* rhizomes was macerated using acetone, fractionated using vacuum liquid chromatography followed by radial chromatography, and purified using preparative thin layer chromatography to yield two isolated compounds. Based on FTIR and NMR spectroscopic data, two isolated compounds were elucidated as flavanone derivatives i.e. pinocembrin (1) and pinostrobin (2). Nevertheless, both of two isolated compounds did not show antibacterial activities toward *Staphylococcus aureus* and *Pseudomonas* sp

Keywords: Antibacterial, *Curcuma rotunda*, Finger-root, Flavanone, Pinocembrin, Pinostrobin

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INTRODUCTION

The resistance of antibiotics has become a serious and global concern in developed and developing countries since it is causing high mortality in the last years.¹ The emergence of bacterial strains that exhibit resistance to various antibiotics including multi-drug and inappropriate antibiotic usage is becoming the most significant factor of treatment failure.² Antibiotic resistance impacts the effective action of antibiotics resulting in prolonged illness and increased mortality.³ Therefore, alternative therapeutic agents from plants should be explored to manage these problems.

As part of our continuing efforts to discover responsible secondary metabolites from *Curcuma* genus plants in exhibiting some pharmacological activities^{4,9}, we found that extract from *Curcuma rotunda* rhizomes displayed antibacterial activities toward foodborne pathogenic bacteria, cariogenic bacteria, and acne-causing bacteria.¹⁰ *C. rotunda* (Zingiberaceae), also known as “temu kunci” in Indonesia or finger-root, is a famous traditional medicinal plant used in Indonesia to treat various ailments.¹¹ According to phytochemical studies, the rhizomes of *C. rotunda* are a rich natural source of flavonoids, which have demonstrated wide-range of biological activities such as antioxidant, anticancer antibacterial, antiviral, anti-inflammatory^{10,12}, anti-periodontal, anti-aging, antiulcer, anti-obesity, anti-hyperpigmentation¹⁰, antihypertensive¹³, anti-platelet aggregation¹⁴, anti-autoimmune¹⁵, antidepressant¹⁶, anti-Alzheimer’s¹⁷, anti-diabetic^{12, 18}, neuro- and cardioprotective properties^{12, 19}. However, the secondary metabolites having antibacterial activity in the rhizomes of *C. rotunda* have not yet been explored in detail. Therefore, the isolation of two flavanones (1-2) from *C. rotunda* rhizomes and their antibacterial activities toward *Staphylococcus aureus* and *Pseudomonas* sp are described in this paper.

EXPERIMENTAL

Material and Methods

The dried powdered of *C. rotunda* rhizomes were purchased and identified in January 2017 from Pusat Studi Biofarmaka Tropika (Trop BRC) LPPM-IPB, West Java, Indonesia. FTIR spectra were acquired

with Bruker Tensor 37 FTIR spectrometer. NMR spectra were performed on Agilent 500 instrument operating at 500 and 125 MHz for ^1H and ^{13}C , respectively. The melting point was measured using Mel Temp Model 1202D Barnstead® apparatus. Vacuum Liquid Chromatography (VLC) was carried out using Si 60 G and Si 60 0.2-0.5 mm (Merck) for column packed and sample adsorbed, respectively. Radial Chromatography (RC) and Preparative Thin Layer Chromatography (P-TLC) were accomplished with Si 60 PF₂₅₄ containing gypsum and Si 60 GF₂₅₄ (Merck), respectively. Pre-coated silica gel plates (Merck, Si 60 GF₂₅₄, 0.25 mm thickness) were used for TLC. TLC and P-TLC spots were identified under UV light (254 and 366 nm) or by heating after spraying with $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$ 1.5% in H_2SO_4 2N.

General Procedure

Sample (1.24 kg) were macerated three times in acetone and filtered. The filtrate then was concentrated using rotavapor to obtain crude extract (161 g). The crude extract (30 g) was fractionated via VLC using an *n*-hexane-EtOAc gradient (from 9:1 to 1:1, v/v) to yield eight major fractions (A-H). Fraction C (11.9 g) was solvent partitioned with acetone to give acetone-soluble fraction (1.75 g). The acetone-soluble fraction (1.22 g) was further isolated via RC using *n*-hexane-EtOAc (7:3, v/v) to afford eight fractions (C1-C8). Fraction C2 (240 mg) then was subjected to repeat radial chromatography using the same solvent to yield nine sub-fractions (C21-C29). Sub-fraction C23 (59.6 mg) was purified using preparative TLC (*n*-hexane-EtOAc = 8.5:1:5, v/v) to furnish pinocembrin (21.1 mg, 1.7% yield) as compound 1. Fraction C5 (243.6 mg) was re-separated by radial chromatography using an *n*-hexane-EtOAc (7:3, v/v) to yield eight sub-fractions (C51-C58). Fraction C4 (208.5 mg) and sub-fraction C54 (57.7 mg) then were consolidated and further purified via radial chromatography followed by preparative TLC using the same solvent to afford pinostrobin (2, 11.1 mg, 0.9% yield). Antibacterial activities of all isolated compounds were performed using disc-diffusion method by measuring the diameter of a clear zone formed in millimeters²⁰ with two bacteria, including *S. aureus* and *Pseudomonas sp.*, purchased from Department of Biochemistry IPB. Chloramphenicol and DMSO were selected as a positive and negative control, respectively. Duplicates were maintained and the experiment was repeated twice.

Pinocembrin (1)

$\text{C}_{15}\text{H}_{12}\text{O}_4$; yellow powder; mp. 189-190°C; Rf: 0.12 in *n*-hexane-EtOAc (9:1, v/v), 0.46 in *n*-hexane- CH_2Cl_2 (1:1, v/v), 0.81 in CH_2Cl_2 -EtOAc (9:1, v/v); FTIR (KBr) ν_{max} : 3434, 2962, 1631, 1604, 1488, 1302, 1261, 1168, 861, 825 cm^{-1} ; ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl_3 spectroscopic data, see Table 1.

Pinostrobin (2)

$\text{C}_{16}\text{H}_{14}\text{O}_4$; colorless-white powder; mp. 99-101°C; Rf: 0.28 in *n*-hexane-EtOAc (9:1, v/v), 0.83 in *n*-hexane- CH_2Cl_2 (1:1, v/v), 0.95 in CH_2Cl_2 -EtOAc (9:1, v/v); FTIR (KBr) ν_{max} : 3449, 3060, 2971-2910, 1647, 1621-1580, 1497-1444, 1383, 840 cm^{-1} ; ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CDCl_3 spectroscopic data, see Table-1.

RESULTS AND DISCUSSION

Phytochemical investigation of the acetone extract of *C. rotunda* rhizomes using successive various chromatography techniques resulted in the isolation and elucidation of two flavanones, i.e. pinocembrin (1) and pinostrobin (2) (Fig.-1). Pinocembrin (1) was obtained as a yellow powder with a melting point of 189-190°C while pinostrobin (2) was isolated as a colorless-white powder with a melting point of 99-101°C. FTIR spectra was used to identify the functional groups on both isolated compounds including O-H (3400 cm^{-1}), C-H sp^2 (3060 cm^{-1}), C-H sp^3 (2970, 2910 cm^{-1}), C=O (1630 cm^{-1}), C=C aromatic (1480-1620 cm^{-1}), and C-O-C (1150-1250 cm^{-1}).

Flavanone skeleton of two isolated compounds was identified by proton signal from ^1H NMR analysis at 5.42 (*dd*, $J = 3.1, 13.0$ Hz), 2.83 (*dd*, $J = 3.1, 17.1$ Hz), and 3.10 ppm (*dd*, $J = 13.0, 17.1$ Hz) representative for ABX system in flavanones²¹. The strong intramolecular hydrogen-bonded appeared in both isolated compounds at a proton signal of 12.0 ppm (*s*). A pair of doublets multiplicity ($J = 2.0$ Hz) in the aromatic region at a chemical shift of 6.0 ppm assigned for proton in ring A indicated tetrasubstituted benzene with three oxy-aryl carbon atoms from ^{13}C NMR data. Moreover, the presence of five protons

aromatic at chemical shifts of 7.29-7.45 ppm allocated for phenyl group in ring B which correspond to monosubstituted benzene. The selected HMBC correlation for both isolated compounds was shown in Fig.-2.

Fig.-1: Chemical Structure of Compounds 1-2

Fig.-2: Selected HMBC Correlation of Compounds (a) 1 and (b) 2

Table-1: ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) Data for Compounds 1-2 (CDCl₃)

C	1	δ_C	2	δ_C
	δ_H		δ_H	
2	5.42 (1H, <i>dd</i> , 3.1, 13.0 Hz)	79.4	5.42 (1H, <i>dd</i> , 3.1, 13.0 Hz)	79.4
3	2.83 (1H _{eq} , <i>dd</i> , 3.1, 17.1 Hz) 3.10 (1H _{ax} , <i>dd</i> , 13.0, 17.1 Hz)	43.5	2.83 (1H _{eq} , <i>dd</i> , 3.1, 17.1 Hz) 3.10 (1H _{ax} , <i>dd</i> , 13.0, 17.1 Hz)	43.5
4	-	195.9	-	195.9
5	-	164.5	-	164.3
6	6.0 (1H, <i>d</i> , 2.0 Hz)	96.9	6.07 (1H, <i>d</i> , 2.0 Hz)	95.3
7	-	163.3	-	162.9
8	6.0 (1H, <i>d</i> , 2.0 Hz)	95.7	6.09 (1H, <i>d</i> , 2.0 Hz)	94.4
9	-	103.3	-	103.3
10	-	165.0	-	165.0
1'	-	138.5	-	138.5
2' & 6'	7.29 (2H, <i>m</i>)	126.3	7.29 (2H, <i>m</i>)	126.3
3' & 5'	7.43 (2H, <i>m</i>)	129.0	7.43 (2H, <i>m</i>)	129.0
4'	7.45 (1H, <i>m</i>)	129.0	7.45 (1H, <i>m</i>)	129.0
5-OH	12.03 (1H, <i>s</i>)	-	12.02 (1H, <i>s</i>)	-
7-OCH ₃	-	-	3.81 (3H, <i>s</i>)	55.8

According to FTIR and NMR spectra data, there was no doubt that two isolated compounds were identified as pinocembrin (**1**) and pinostrobin (**2**) with the molecular formula of C₁₅H₁₂O₄ and C₁₆H₁₄O₄, respectively. Both of isolated compounds had been identified previously from the rhizomes of *C. rotunda* cultivated in Surabaya²¹ and Yogyakarta, Indonesia²². These findings and literature studies revealed the first study which reported that pinostrobin (**2**) is the major flavanone in *C. rotunda*²³.

Based on the antibacterial assay, all of the isolated compounds did not show antibacterial activities toward both of bacteria tests. It can be assumed because of little concentration of the isolated compounds during the antibacterial assay since both of isolated compounds are slightly nonpolar compounds having low solubility in water. There are four major factors affecting the antibacterial activity results including type, concentration, and diffusion property of the compounds, as well as the bacteria type²⁴. Recent

studies reported that pinocembrin (**1**) exhibited antibacterial activity toward *Escherichia coli* with an inhibition concentration of 250 µg/mL²². Moreover, pinostrobin (**2**) showed wide-range antibacterial activities towards *E. coli*, *Bacillus subtilis*, *Shigella dysenteriae*, and *Helicobacter pylori* with MIC values of 37.5, 37.5, 75, and 125 µg/mL, respectively²⁵. According to antibacterial activity studies, both isolated compounds displayed antibacterial activities in a limited number of bacteria which many scholars have not yet been explored in detail with other bacteria. Therefore, these findings demonstrated that two flavanones are inactive toward *S. aureus* and *Pseudomonas* sp.

CONCLUSION

In this study, we report further experimental scientific to isolate secondary metabolites from *C. rotunda* rhizomes and examine for antibacterial activities. Phytochemical analysis of the acetone extract of *C. rotunda* rhizomes led to the isolation of two flavanones, namely pinocembrin (**1**) and pinostrobin (**2**). However, both isolated flavanones did not show antibacterial activities toward *S. aureus* and *Pseudomonas* sp. Further studies will need to be conducted to assay in other bacteria.

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