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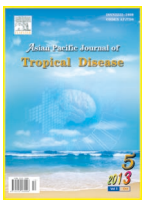

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Antioxidant and cytotoxic agent from the rhizomes of *Kaempferia pandurata*

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PEER REVIEW

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Comments

This is a good study in which the authors explained the isolation of flavonoid compounds from the rhizomes of *K. pandurata*. This paper discusses the structure elucidation of the two flavanones. Also, structure-activity relationship against DPPH radical and cytotoxic activity
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ABSTRACT

Objective: To determine antioxidant and cytotoxic activity of two flavanones, pinocembrin (1) and pinostrobin (2) from the rhizomes of *Kaempferia pandurata*. The chemical structures of both compounds were determined based on spectroscopic data, including UV, IR, MS and NMR spectra.

Methods: The antioxidant activities of pinocembrin (1) and pinostrobin (2) were assayed by using 2,2-diphenyl-1-picrylhydrazyl. Cytotoxic assay was done by using brine shrimp lethality test, and cytotoxic properties was tested against murine leukemia P-388 cells.

Results: Compounds 1-2 were evaluated for their antioxidant properties against DPPH, showing their IC_{50} were 5816 and 6268 μmol/L; brine shrimp lethality test: LC_{50} 23.3 and 60.5 μg/mL; murine leukemia P-388: IC_{50} 176.3 and 218.5 μmol/L.

Conclutions: The results indicated that pinocembrin (1) was slightly more active than pinostrobin (2).

KEYWORDS

Flavanone, Pinocembrin, Pinostrobin, *Kaempferia pandurata*, Antioxidant, Cytotoxic

1. Introduction

Kaempferia pandurata Robx. (*K. pandurata*) syn. *Boesenbergia pandurata* Robx. (local name: Temu Kunci) belongs to the family Zingiberaceae. In Indonesia, the rhizomes of this plant are extensively used as a flavouring in traditional food, and it is also used in traditional medicine as an aphrodisiac, and for the treatment of asthma, diarrhea, fever, and colic disorder. This plant has been shown to produce a number of flavonoid and essential oil compounds[1-3]. In continuation of these chemical investigations, we have examined *K. pandurata* Robx. and succeeded in isolating two flavanones, namely pinocembrin (1) and pinostrobin (2). This paper discussed the structure elucidation of the two flavanones. Also, free radical scavenging and cytotoxic properties of compounds 1-2 against DPPH radical, brine shrimp, and murine leukemia P-388 cells are briefly described.

2. Materials and methods

2.1. General experimental procedures

UV and IR spectra were measured with a Beckman DU 7500 and an FT-IR Spectrum One Perkin-Elmer instrument,

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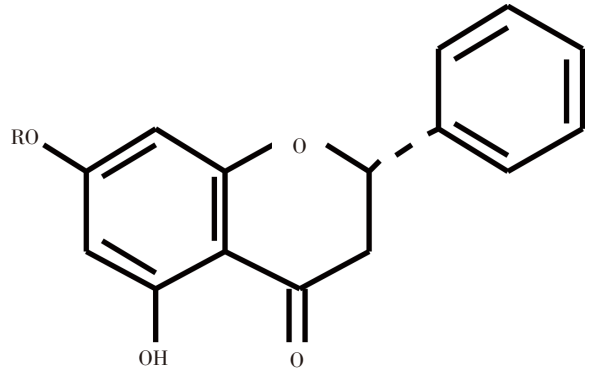
respectively. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL ECA400 spectrometer operating at 400 (^{1}H) and 100 (^{13}C) MHz, using residual and deuterated solvent peaks (δ_H 2.04 and δ_C 29.8, respectively) as reference standards. Mass spectra were obtained with a VG Autospec mass spectrometer (EI mode). Vacuum liquid chromatography and column chromatography were carried out using Si gel 60 G, and for TLC analysis, precoated Si gel 60 F254 plates were used. Solvents used for extraction and separation were of technical grades that were distilled before use.

2.2. Plants material

Samples of rhizomes of *K. pandurata* were collected from research garden, Faculty of Science and Technology District, Airlangga University, Surabaya, Indonesia. The plant was identified by the staff at the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia. and a voucher specimen had been deposited at the herbarium. The rhizomes were cleaned, air dried under the shade, cut into small pieces and milled.

2.3. Extraction and isolation of pinocembrin(1) and pinostrobin (2)

The dried and powder of rhizomes *K. pandurata* (1.0 kg) were macerated with *n*-hexane and then with methanol two times at room temperature, after *n*-hexane solvent evaporation gave a solid fraction, and recrystallization with methanol gave a needle like crystal of pinostrobin (2). Futhermore, methanol extract was redissolved in methanol-water (9:1) and partitioned into ethylacetate. The ethylacetate extract fraction was then fractionated using vacuum liquid chromatography eluting with mixtures of *n*-hexane-ethylacetate (9:1, 4:1 and 7:3) to give three major fractions A-C. Fraction B was separated with column chromatography and eluting with mixtures of *n*-hexane-ethylacetate (9:1, and 4:1) gave a yellow solid. Recrystallization with methanol to yield a yellow solid of pinocembrin (1).



(1) R=H
(2) R=CH_3

Pinocembrin (1), yellow solid, m.p. 202-204 °C, UV (MeOH) λ_{maks} nm (log ε) : 228 (3.91), 292 (4.06), and 323 sh (3.67) nm, (MeOH+$AlCl_3$) λ_{maks} nm (log ε) : 225 (3.99), 297 (4.11), and 382 sh (3.16) nm. IR (KBr) ν_{max}: 3435 (OH), 3000, 2910 (CH aromatic), 1641 (conj. C=O), and 1595, 1570 (C=C aromatic) cm^{-1}. EIMS: *m/z* (256, M^+, 100, base peak), 213 (8.9), 197 (4.5), 179 (76.2), 152 (80.1), 124 (38.7), 104 (18.8), and 77 (16.5). 1H NMR (400 MHz in acetone *d6*), δ_H ppm: 12.20 (1H, *br*, *s*, 5-OH), 9.75 (1H, *br*, *s*, 7-OH), 7.57 (3H, *m*, H-3′,4′,5′), 7.44 (2H, *m*, H-2′,6′), 5.92 (1H, *d*, *J*=2.0 Hz, H-8), 5.86 (1H, *d*, *J*=2.0 Hz, H-6), 5.49 (1H, *dd*, *J*=4.0; 12.0 Hz, H-2), 3.06 (1H, *dd*, *J*=12.0; 14.0 Hz, H-3_{ax}), and 2.78 (1H, *dd*, *J*=4.0; 14.0 Hz, H-3_{eq}). 13C NMR (100 MHz in acetone *d6*), δ_C ppm: 197.3 (C-4), 168.5 (C-7), 165.4 (C-5), 164.7 (C-8a), 140.4 (C-1′), 129.7 (C-3′,5′), 129.6 (C-4′), 127.3 (C-2′,6′),

Pinostrobin (2), white crystal, m.p. 96-98 °C, UV (MeOH) λ_{maks} nm (log ε) : 232 (3.93), 290 (4.09), and 325 sh (3.68) nm. EIMS: *m/z* (270, M^+, 100, base peak), 193 (76.2), 166 (80.4), 138 (40.2), 10^3 (21.1), and 77 (16.9). 1H NMR (400 MHz in acetone *d6*), δ_H ppm: 12.18 (1H, *br*, *s*, 5-OH), 7.57 (3H, *m*, H-3′,4′,5′), 7.45 (2H, *m*, H-2′,6′), 6.08 (1H, *d*, *J*=2.2 Hz, H-8), 6.04 (1H, *d*, *J*=2.2 Hz, H-6), 5.60 (1H, *dd*, *J*=3.8; 12.8 Hz, H-2), 3.76 (3H, *s*, 7-OCH_3), 3.12 (1H, *dd*, *J*=12.8; 16.4 Hz, H-3_{ax}), and 2.80 (1H, *dd*, *J*=3.8; 16.4 Hz, H-3_{eq}). ^{13}C NMR (100 MHz in acetone d6), δ_C ppm: 196.8 (C-4), 168.1 (C-7), 164.4 (C-5), 163.8 (C-8a), 140.0 (C-1′), 129.5 (C-3′,5′), 129.3 (C-4′), 127.2 (C-2′,6′), 103.0 (C-4a), 95.0 (C-8), 94.0 (C-6), 79.2 (C-2), 56.0 (7-OCH_3), and 43.6 (C-3).

2.4. DPPH scavenging activity test

The antioxidant activity of two flavanones and ascorbic acid (positive control) were measured in triplicate, based on the method used by Muller. The pinocembrin, pinostrobin, and ascorbic acid were diluted with methanol to prepare sample solution equivalent to 10 000, 5 000, 2 500, 1 000, and 500 μmol/L. A methanolic solution (100 μL) was placed in a cuvette, and 100 μL acetate buffer (100 mmol/L, pH 5.5) then 50 μL 5.10^{-4}mol/L in methanol was added. The mixture was incubated at 20 °C for 30 min[4]. Absorbance of the pinocembrin, pinostrobin, and ascorbic acid were measured at 517 nm. The inhibition percentage (%) of radical scavenging activity was calculated using the following equation: Inhibition (%)=$(A_o-A_s/A_o)\times100$

Where A_o is the absorbance of the control reaction (containing all reagents except the test compound), and A_s is the absorbance of the test compound.

2.5. BSLT bioassay

The cytotoxic effect of pinocembrin, and pinostrobin were evaluated by LC_{50} of brine shrimpt lethality test. *Artemia salina* Leach (brine shrimpt eggs) were placed in 1 L of sea water, aerated for 2 d at 37 °C for the shrimpt to hatch become nauplii. After 48 h, ten brine shrimp nauplii were placed in a small container filled with sea water. The compound (1), and (2) were dissolved in dimethylsulphoxide (DMSO) separately and 3 graded doses 1, 5, 10, 25, 50, and 100 μg/mL respectively were used for 5 mL sea water containing

10 brine shrimpt nauplii in each group. The lethality of brine shrimp was observed after 24 h of treatment was given[5]. Probity analysis was used to determine lethal concentration (LC_{50}) of pinocembrin, and pinostrobin on nauplii.

2.6. MTT assay

Living cells 3×10^3/mL were plated in 96-well culture dishes. Plates was incubated at 37 °C in humidified CO_2 incubator for 24 h. After the cells adhered to the plates, 10 μL medium containing one of five different concentrations of compound 1-2 were added. Plates was incubated incubated at 37 °C in humidified CO_2 incubator for 48 h. After incubation, medium was removed from the wells and 150 μL of fresh medium+50 μL MTT was added. Plates was incubated at 37 °C in humidified CO_2 incubator for 4 h. Four hours later, MTT was removed and insoluble formazan was dissolved in 50 μL DMSO. Optical density was measured on micro plate reader at 550 nm[6]. IC_{50} was calculated according to One-way analysis of variance (ANOVA).

2.7. Statistical analysis

Statistical analysis was performed using One-way analysis of variance (ANOVA) and followed by least square difference. Results were expressed as mean±SD from three replications. $P<0.01$ was considered significant.

3. Results

3.1. Phytochemical

Extraction of the dried milled rhizomes of *K. pandurata* with *n*-hexane and methanol gave a fraction which was separated by column chromatography to give pinocembrin (1), and pinostrobin (2). The molecular ion at *m/z* 256 one of flavanone had a formula $C_{15}H_{12}O_4$ and was identified as pinocembrin (5,7-dihydroxy flavanone) by comparing data with reported values[7]. The UV spectrum of 1 showed absorption maxima at 228, 292, and 323 sh nm, and the 1H NMR spectrum the proton signal at 5.49 (*dd*, *J*=4.0; 12.0 Hz), 3.06 (*dd*, *J*=12.0; 14.0 Hz), and 2.78 (*dd*, *J*=4.0; 14.0 Hz). characteristic for H-2, H-3_{ax}, and H-3_{eq} a typical ABX system for a flavanone structure. The ^{13}C NMR of 1 suggested the presence of three oxyaril carbon atoms, and therefore 1 is a dihydroxy derivative of a flavanone. The presence of one downfield signals at δ_H 12.20 ppm was assignable to 5-OH strongly hydrogen-bonded intramolecularly to the 4-carbonyl group. The presence of the proton signals of a pair of doublets (*J*=2.0 Hz) in the aromatic region at δ_H 5.92 and 5.86 ppm, assignable to the H-6 and H-8 proton signals of the ring A. Furthermore, in the 1H NMR spectrum, the appearance of five proton aromatic (δ_H 7.57 and 7.44) assignable to the signals of a phenyl group of the ring B.

Pinostrobin (2) was isolated as a white crystal. The molecular formula ($C_{16}H_{14}O_4$) of compound 2, showing one more oxygen atom than 1 were obtained from its EIMS, ^{1}H and ^{13}C NMR data. Its UV, IR, EIMS, 1H and 13C NMR spectrum were very similar to those of compound 1. The presence of one methoxyl at δ_H 3.76 and δ_C 56.0 ppm in the 1H and 13C NMR spectrum of 2 was identified methoxy group at C-7. The compound 2 was suggested as pinostrobin (7-methoxy-5-hydroxy flavanone)[8].

The radical scavenging against DPPH, brine shrimpt lethality test toward *Artemia salina* Leach and cytotoxic properties against murine leukemia P-388 cells were evaluated according to the method of MTT assay of pinocembrin (1), and pinostrobin (2) are presented in Table 1.

Table 1
Antioxidant and cytotoxic activities of pinocembrin (1), and pinostrobin (2)

Compound	DPPH(μmol/L)	BSLT (μg/ mL)	Cytotoxic (μmol/L)
Pinocembrin	5816±20.563	23.3	176.3±5.6
Pinostrobin	6268±28.132	60.5	218.5±9.8
Ascorbic acid	0.329±0.001	–	–

4. Discussion

The ginger family contains about 50 genus and 1300 species, which are distributed in tropical regions. *K. pandurata* Robx. used as flavouring agents, spices and herbal medicine.

Two flavonoid compounds of flavanone type have been isolated from the rhizomes of *K. pandurata* and were identified pinocembrin (1), and pinostrobin (2). The structure of both compounds has been elucidated based on spectroscopic methods and comparison of their physical data. The results indicate that compounds 1-2 to give very weak activities as radical scavenging than positive control (ascorbic acid). Preliminary cytotoxic evaluation of compounds 1-2 was carried out against brine shrimp lethality test showed potent activities[7]. However, on cytotoxic evaluation against murine leukemia P-388 cells using MTT assay of compounds 1-2 was inactive[9]. The structure-activity relationship of compounds 1-2 against radical scavenging, brine shrimp, and cytotoxic data against murine leukemia P-388 cells suggested that the presence of hydroxyl group at C-7 on pinocembrin structure tend to be more active than the methoxyl group at C-7 on pinostrobin structure.

Two flavanones, pinocembrin (1), and pinostrobin (2) have been isolated from the rhizomes of *K. pandurata* Robx., a species belongs to the family Zingiberaceae. The radical

scavenging and cytotoxic activities of compounds 1–2 were evaluated against DPPH, brine shrimp, murine leukemia P–388 cells which showed that compound 1 is slightly more active than compound 2.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

The research is an investigation of phytochemical work of Indonesian medicinal plants aiming to find flavonoid compounds from the rhizomes of *K. pandurata* Robx. with antioxidant and cytotoxic activities.

Research frontiers

This research include phytochemical, elucidation structure of both flavonoids, antioxidant and cytotoxic activities, and structure–activity relationship of flavonoid from rhizomes of *K. pandurata* Robx. The cytotoxic effect of the isolated compounds was evaluated against P–388 and by using brine shrimp lethality test while the antioxidant activities was carried out using 2,2–diphenyl–1–picrylhydrazyl.

Related reports

Flavonoid compunds from of *K. pandurata* Robx. and their biological activity have been reported. However, the reported about antioxidant and cytotoxic activities from pinocembrin and pinostrobin which has not been investigated by other workers.

Innovations & breakthroughs

K. pandurata Robx belongs to medicinal plants used in traditional medicine as an aphrodisiac, asthma, diarrhea, and fever. In the present study, authors have explained the phytochemical, elucidation structure, antioxidant and cytotoxic activities of *K. pandurata* Robx.

Applications

K. pandurata Robx belongs to medicinal plants in Indonesia. This herb contains a lot of active compound that have activity as antioxidant, anticancer, and inflammatory. The isolation of two flavonoids is interesting to study of structure–activity relationship.

Peer review

This is a good study in which the authors explained the isolation of flavonoid compounds from the rhizomes of *K. pandurata*. This paper discusses the structure elucidation of the two flavanones. Also, structure–activity relationship against DPPH radical and cytotoxic activity.

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