Proceedings of the 4th ITB Graduate School Conference
Innovation and Discovery for Sustainability
July 6, 2023

Phenolic derivatives from heartwood of *Cryptocarya pulchrinervia* (Lauraceae)

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Abstract. *Cryptocarya* is a genus of plants in the Lauraceae family with 350 species. This genus has many economic values, such as building materials, perfume, and traditional medicines. *Cryptocarya* were reported to contain flavonoids, pyrones, and alkaloids as main secondary metabolites, as well as phenyl propanoids, lignans, stilbenoids, terpenoids, and steroids. Isolation and bioactivity of secondary metabolites of *C. pulchrinervia* leaves have been recently reported. Meanwhile, the isolation of other plant parts, such as the heartwood, has never been conducted. Therefore, the objective of this research was to isolate secondary metabolites from the heartwood of *C. pulchrinervia* and determine their bioactivity against murine leukemia P-388 cells. The structures of the isolated compounds were determined based on 1D-NMR spectroscopy data (^{1}H-NMR and ^{13}C-NMR). In this research, three secondary metabolites were isolated, consisting of three phenolic compounds, i.e., syringaldehyde **(1)**, coniferaldehyde **(2)**, and sinapaldehyde **(3)**. The IC_{50} values of the extract and the compound **1**–**3** was 57.91 µg/mL, 28.0 µg/mL, 26.47 µg/mL, and 24.00 µg/mL, respectively. The extract and all compounds were inactive against P-388 cells.

Keywords: *cryptocarya pulchrinervia; isolation; heartwood.*

1 Introduction

Cryptocarya is a plant genus consisting of 350 species from the family Lauraceae.[1] Several species from this genus are used as raw materials for pulp in the paper industry (*C. ferrea*), so they have economic values. In other uses, they have been used as traditional medicines to cure various diseases, such as muscle pain, joint pain, and fever (*C. massoy*), infections due to fungi and bacteria (*C. alba*), headaches, and nausea (*C. latifolia*).[2] Phytochemical studies on the genus

[1] Kostermans in [1]
[2] Juliawaty, *et.al* in [2]

Cryptocarya have revealed that this genus produces various secondary metabolites with a unique framework. Flavonoids, pyrones, and alkaloids are the main secondary metabolites of the genus *Cryptocarya*.[3] The other secondary metabolites are phenyl propanoids, stilbenoids, lignans, terpenoids, and steroids. The isolated compounds were obtained from various tissues, such as stem bark, leaves, roots, and branches. Several compounds have been obtained from stem bark, such as 7-hydroxy-5,6-dimethoxyflavanone (*C. costata*),[4] 3-hydroxy-5-methoxy stilbene (*C. idenburgensis)*,[5] linderan (*C. densiflora*),[6] and taraxerol (*C. cassinervia*).[7] Meanwhile, goniothalamin and *β*-sitosterol[8] were isolated from the leaves of *C. aschersoniana* and *C. latifolia*, respectively. Goniothalamin was also isolated from the roots of *C. caloneura*[9] and *C. mochata*,[10] whereas compounds yielded from branches are 9,9'-O-di-feruloyl-(-)-secoisolariciresinol and β-sitosterol (*C. impressinervia*).[11]

One of the species that grows in Indonesia is *C. pulchrinervia*. The phytochemicals in the leaves of *C. pulchrinervia* have been studied and reported. Several pyrone and amide derivatives have been obtained. The pyrones are *(S)*-rugulactone, pulchrinervialactone A, pulchrinervialactone B, and cryptobrachitone C.[2] While, the amide derivatives are *N-tran*s feruloyltryramine, *N-trans* feruloyl-3-methoxytyramine, and *N-trans* feruloyltyramine.[2] Three pyrone derivatives showed significant activity against murine leukemia P-388 cells. However, there is no phytochemical work on other tisues of this species. In this paper, as a continuation of the research on Indonesian *Cryptocarya*, we report the isolation, structural elucidation, and its bioactivity of isolated compounds obtained from the heartwood of *C. pulchrinervia*

2 Experimental

2.1 General

Vacuum liquid chromatography (VLC) used Si gel (Merck 60 GF_{254} art 7731). Gravity column chromatography (GCC) used Si gel (Merck 60 GF_{254} art 7734). Thin-layer chromatography (TLC) analysis used precoated Si gel plates (Merck

[3] Kurniadewi, *et.al.* in [3]
[4] Usman, *et.al.* in [4]
[5] Juliawaty *et.al.* in [5]
[6] Achmad in [6]
[7] Achmad *et al.* in [7]
[8] Hamza *et.a.l* in [8]
[9] Hlubucek, *et.al.* in [9]
[10] Cavalheiro, *et.al.* in [10]
[11]Xiong *et.al.* in [11]

Kieselgel 60 $GF_{254,}$ 0.25 mm thickness). Solvents (MeOH, acetone, EtOAc, CH_2Cl_2, and *n*-hexane) for extraction, fractionation, and purification were of technical grades, which were distilled before used, while $CHCl_3$ used was a pro-analysis grade. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with spectrometer of Agilent DD2 system operating at 500 (^{1}H) and 125 (^{13}C) MHz, using residual and deuterated solvent peaks as reference standards.

2.2 Plant materials

The heartwood of *Cryptocarya pulchrinervia* were collected from Bogor Botanical Garden, Bogor, West Jawa Province, Indonesia.

2.3 Extraction and isolation

The dried and powdered heartwood of *C. pulchrinervia* (2 kg) was extracted with acetone three times (each for 24 h) at room temperature. The extract was then evaporated under vacuum to give a dark brown acetone extract (13.2 g). The acetone extract was applied to a silica gel vacuum liquid chromatography (VLC) and eluted with solvent (*n*-hexane−EtOAc 10:0− 0:10, MeOH) to give sixteen major fractions (Frs. A−P). Fractions H and I were combined (136.6 mg) and they were further purified on gravity column chromatography (GCC) eluted with *n*-hexane: EtOAc (7:3 v/v) to give 36 fractions (HI.1−36). Fraction HI.16−24 (59.7 mg) was purified using GCC eluted with CH_2Cl_2: EtOAc (19:1 v/v) to give six fractions. One fraction was obtained as a pure fraction identified as syringaldehyde **(1)**. Besides that, other HI fractions, i.e., fr. HI (1−15), HI (25−36), HI (16−24) was recombined to give the HI' fraction (132.4 mg). It was then purified on GCC (*n*-hexane: CH_2Cl_2 (1: 9 v/v)) to give 16 fractions HI'. Fraction HI'-10 was obtained as a pure fraction determined as coniferaldehyde **(2)** (6.5 mg). Meanwhile, fraction HI'-13 (11.6 mg) was obtained as a pure fraction to yield sinapaldehyde **(3)**. The isolated compounds were identified structurally based on their spectral analysis, including ^{1}H-NMR and ^{13}C-NMR and compared to literature data.[12]

Compound 1 (7.0 mg): yellow solid; $C_9H_{10}O_4$; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm) : 7.15 (2H, *s*, H-2, H-6), 3.97 (6H, *s*, CH_3O-3, CH_3O-5), 9.82 (1H, *s*, CHO); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C (ppm): 128.3 (C-1), 106.6 (C-2, C-6), 147.2 (C3, C5), 140.7 (C-4), 190.6 (-CHO), 56.3 (CH_3O-3, CH_3O-5).

Compound 2. (6.5 mg): yellow oil; $C_{10}H_{10}O_3$; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm) : 7.07 (1H, *d, J* = 1.9 Hz, H-2), 6.96 (1H, *d, J* = 8.2 Hz, H-5), 7.12 (1H,

[12] Panyo, *et.al.* in [12]

dd, *J* = 8.2, 1.9 Hz, H-6), 6.59 (1H, *dd*, *J* = 15.8, 7.7 Hz, H-2'), 9.65 (1H, *d*, *J* = 7.7 Hz, CHO), 3.94 (3H, *s*, -OCH_3).

Compound 3. (11.6 mg): yellow solid; $C_{11}H_{12}O_4$; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm): 6.81 (2H, *s*, H-2, H-6), 7.38 (1H, *d*, J = 15.7 Hz, H-1'), 6.60 (1H, *dd*, *J* = 15.7, 7.7 Hz, H-2'), 3.93 (6H,*s*, CH_3O-3, CH_3O-5), 9.65 (1H, *d*, *J* = 7.7 Hz, CHO). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C (ppm): 153.1 (C-1), 105.5 (C-2, C-6), 147.3 (C-3, C-5), 138.1 (C-4), 153. 1 (C-1'), 126.7 (C-2'), 193.4 (CHO), 56.4 (CH_3O-3, CH_3O-5).

2.4 Murine leukemia P-388 assay

The cytotoxic activity test carried out was on murine leukemia P-388 cells. Determination of the cytotoxic properties of the isolated compounds followed the MTT (3-(4,5-dimethylthiazole-1-yl)-2,5-diphenyltetrazolium bromide) assay method.[13] Based on this method, the IC_{50} value of P-388 murine leukemia cancer cells can be determined. An extract is determined active if it has an IC_{50} value < 20.0 μg/mL. A pure compound is determined to be very active if it has an IC_{50} value < 2.0 μg/mL, active if an IC_{50} value is 2.0–4.0 μg /mL and inactive if an IC_{50} value is > 4.0 μg /mL. 5-Fluorouracil was used as a positive control in this assay.

3 Results and discussion

The structures of three phenolic derivatives (**1–3**), namely syringaldehyde **(1)**, coniferaldehyde **(2)**, and sinapaldehyde **(3),** isolated from heartwood of *C. pulchrinervia*, were described at **Figure 1.**

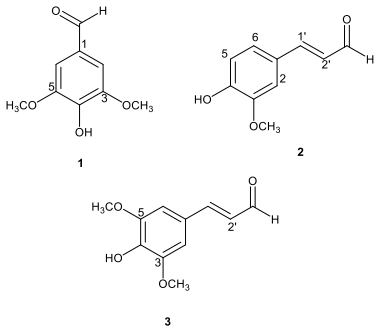

Figure 1 Three compounds isolated from heartwood of *C. pulchrinervia*.

[13] Alley, *et.al.* in [13]

Compound **1** was isolated as a yellow solid with the molecular formula $C_9H_{10}O_4$. This compound is an aldehyde phenolic compound that has a framework C_6–C_1, i.e., a tetrasubstituted benzene (C_6) having an aldehyde unit as a side chain. It has two mutually symmetrical protons with two symmetrical methoxy substituent groups flanking one hydroxy group and one aldehyde substituent. Based on the ^{1}H-NMR spectrum, a singlet signal appeared at δ_H 9.82 ppm, showing a characteristic signal for the aldehyde proton. In the aromatic region (δ_H 6–8 ppm), a singlet signal at δ_H 7.15 ppm appeared with 2H (H-2 and H-6), showing a symmetrical signal of methine protons. Then, a singlet signal appeared at a chemical shift of 3.97 ppm integrated 6H, indicating the presence of two methoxy groups substituted at C-3 and C-5. Meanwhile, a singlet signal appears at δ_H 6.07 ppm integrated 1.0, indicating the existence of a hydroxy group at C-4. The ^{13}C-NMR spectrum showed the presence of six signals. The signal at δ_C 190.6 ppm showed a typical carbon of an aldehyde carbon. Meanwhile, the signal appearing at δ_C 56.3 ppm indicated a typical carbon shift for two carbons of methoxy groups. In addition, the signal at δ_C 147.2 ppm showed the oxyaryl carbons substituted with the methoxy groups (C-3 and C-5). Besides that, the appearance of a signal at δ_C 140.7 ppm identified an oxyaryl carbon (C-4) substituted with a hydroxy group. In addition, a quarterner carbon substituted with C=O of an aldehyde group appeared at δ_C 128.3 ppm. While the presence of symmetrical methine carbons of aromatics (C-2 and C-6). Based on these NMR analysis, the structure of **1** was confirmed as syringaldehyde.

Compound **2** was isolated as a yellow oil and has molecular formula $C_{10}H_{10}O_3$. The ^{1}H-NMR spectrum showed a doublet signal appearing at δ_H 9.82 ppm with a value of J = 7.7 Hz, indicating a typical signal proton of a coupled aldehyde. The signal at δ_H 6.59 ppm as a double doublet (*dd*) with values J = 15.8 Hz and 7.7 Hz (C-2') showed the presence of a *trans* vinylic unit next to an aldehyde group. Next, a signal at δ_H 7.40 ppm appeared as a doublet (*d*) with a value of J = 15.8 Hz (C-1'), indicating the presence of a proton of a *trans* vinylic group. In the aromatic region (δ_H 6–8 ppm), the presence of three proton signals demonstrated the ABX system. The three signals consisted of one *meta* doublet (*dm*) signal at 7.07 (1H, H-2) with J = 1.9 Hz and the *ortho* doublet (*do*) signal at δ_H 6.96 ppm (1H, H-5) with J = 8.2 Hz. Meanwhile, the signal appeared at 7.12 ppm (1H, H-6) and showed *ortho* and *meta* doublet signals with J = 8.2 Hz and 1.9 Hz, respectively. In addition, a singlet signal at δ_H 3.94 ppm (1H), which confirmed the presence of the methoxy group. Then, the signal at δ_H 5.85 ppm (1H) indicated the presence of a hydroxy group. This compound was suggested to be a phenolic phenylpropanoid compound having a framework (C_6-C_3). It also had a trisubstituted pattern in the aromatic framework (C_6), named coniferaldehyde **(2).**

Compound **3** was obtained as a yellow solid with the molecular formula $C_{11}H_{12}O_4$.The ^{1}H NMR data of compound **3** was very similar to compound **2**, except for the disappearance of protons of the ABX system in the aromatic unit, which were replaced by only one singlet signal at δ_H 6.81 (2H, H-2, and H-6), which showed the presence of two symmetrical protons at the tetrasubstituted benzene ring. The ^{13}C-NMR spectrum of compound **3** also confirmed the presence of a tetrasubstituted benzene ring having a *trans* α,β-unsaturated aldehyde as a side chain, just like as compound **2**. However, the differences were one signal carbon at δ_c 147.3 ppm (C-3 and C-5) for two symmetrical oxyaryl carbons attached to methoxy groups, δ_c 138.1 for one oxyaryl carbon substituted with a hydroxy group (C-4), and δ_c 105.5 ppm indicating two symmetrical methine aromatic carbons (C-2 and C-6). Besides that, there was also one signal at δ_c 125.5 ppm of a quarternary carbon (C-1). According to these data, this compound was determined to be sinapaldehyde (**3**).

Biosynthetically, the compounds **1**–**3** followed the shikimic pathway. Moreover, syringaldehyde **(1)** was first isolated from *Cryptocarya* genus, but it had been reported from other plants. Besides that, coniferaldehyde **(2)** and sinapaldehyde **(3)** compounds have been reported from the heartwood of *C. massoy*.[14]

Furthermore, the results of cytotoxic tests of the acetone extract and isolated compounds (**1**–**3)** against P-388 cells can be seen in **Table 1**. It showed that the extract and all the compounds were inactive. The extract had IC_{50} value greater than 20.0 µg/mL, while the compounds **1**–**3** had IC_{50} values greater than 4.0 µg/mL. However, according to Xiong *et.al*. (2021), compounds **1–3** were also reported to have antibacterial activity against *C. albicans*, with MIC values (µg/L) of compound **1** of 62.5 µg/L, and compound **2**–**3** of were 125 µg/L.[12]

Table 1 The cytotoxicity of compounds **1**–**3** and positive control*

Compounds	IC_{50} (µg /mL)	Inhibition (P-388 cells)
Acetone extract	57.91	Inactive
1	28.08	Inactive
2	26.47	Inactive
3	24.00	Inactive
5-Fluorouracil*	0.22	Very active

[14] Siallagan in [14]

4 Conclusion

As a conclusion, three secondary metabolites have been isolated for the first time from the heartwood of *Cryptocarya pulchrinervia*. These compounds are known phenolic derivatives, characterized as syringaldehyde **(1)**, coniferaldehyde **(2)**, and sinapaldehyde **(3)**. The finding of compound **1** is for the first time from *Cryptocarya*. However, compounds **2** and **3** have been isolated previously from *C. massoy*. Then, the results of the murine P-388 cells assay showed that the acetone extract and compounds **1**–**3** were inactive, with IC_{50} values of 57.91 μg/mL, 28.08 μg/mL, 26.47 μg/mL, and 24.00 μg /mL, respectively. The phytochemisty and bioactivity studies of the compounds from *C. pulchrinervia* provided significant data on the study of the Indonesian *Crpyptocarya* plants.

Acknowledgments

The authors would like to thank the Ministry of Education, Culture, Research and Technology (puslapdik) and LPDP for providing scholarship and research fund. We are also grateful to Dr. Elvira Hermawati, Integrated Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung for NMR measurements.

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