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Chemical Constituent of *Acacia auriculiformis* Wood Extractives and Their Antioxidant Activity

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ABSTRACT

Acacia auriculiformis showed good potential to be developed as a raw material for bioactive compounds. The heartwood of *A. auriculiformis* is still rarely explored. The purpose of this study was to identify the bioactive components of the heartwood. Six compounds were identified from the heartwood through nuclear magnetic resonance and mass spectroscopy, namely the C-3,4',7,8 hydroxyl substituted flavonoids and chalcone. Two methylated teracacidins and chalcone-like teracacidin were identified for the first time in the *A. auriculiformis* heartwood, along with three known compounds. These compounds showed good radical scavenging and reduction activity, compared to crude extract and ascorbic acid, using three different antioxidant assays. The molecular structure-dependent activity was observed to affect the trend of the different antioxidant activities. This finding shows good potential for further development of plant parts of *A. auriculiformis* from Indonesia as new raw materials for medicines.

1. Introduction

A wood extractive is a wood component which potent to be developed. Several studies have shown that wood extracts serve as a source of biopharmaceuticals, being responsible for the natural resistance of wood and wood bonding in interactions with adhesives (Aulia et al. 2022; Kirker et al. 2013; Prayogo et al. 2022; Roffael 2016). *Acacia auriculiformis* is a fast-growing, resilient tree. *A. auriculiformis* trees can also be used as a source of biomass energy due to their rapid growth, ability to thrive in a variety of marginal environments, and ease of cultivation (Hendrarti et al. 2014). This tree has been harvested as an industrial raw material for sawn timber until now (Nirsatmanto and Sunarti 2019). Empirically, *A. auriculiformis* was used for treating rheumatic (root part), skin (seed part), malarial (seed part), and depressant (not specified part) (Rangra et al. 2019a; Subhan et al. 2018). Utilization of the *A. auriculiformis* tree has been the subject of extensive research regarding its pharmacological activity and potential as a source of medicinal raw materials in *in vitro* (Sathya and Siddhuraju 2012) and *in vivo* (Sathya and Siddhuraju 2013), bark and seed coat extracts demonstrated strong biological activity as an antioxidant and an anti-

diabetic. Bark extracted with different solvents, including ethyl acetate (Singh et al. 2007a), acetone (Singh et al. 2007b), and methanol (Singh et al. 2007c), has also been evaluated for its antioxidant activity in the past. Bark extract has also been evaluated for its antimutagenic and anticarcinogenic properties, but its activity is lower than that of *A. nilotica* (Kaur et al. 2002). The *A. auriculiformis* seed has also been evaluated for its antimutagenic properties, and the *A. auriculiformis* seed extract has the potential to be developed into a spermicide with no mutagenic effect (Pal et al. 2009). The auriculiformis tree has promising anticancer properties. Compounds isolated from the bark exhibit promising anticancer-related protein kinase inhibitor potential, particularly for leukemia treatment (Ahmadu et al. 2021).

The biological activity of each portion of the *A. auriculiformis* tree is dependent on the extractive components it contains. Diverse types of extractives have been discovered in various *A. auriculiformis* tree parts. Several chemical classes have been identified as extractives of the *A. auriculiformis* tree, including flavonoids, simple phenolics, steroids, terpenoids, and saponins. Examples of flavonoid compounds from the *A. auriculiformis* tree include flavonoids with hydroxyl substitution at positions C-4', 7, and 8 (flavanones, flavonols, flavanols, and flavones), kaempferol glycosides, myrisetin glycosides, quercetin glycosides, and flavan glycosides (Barry et al. 2005; Drewes and Roux 1966; Kalsom et al. 2001; Mihara et al. 2005; Prayogo et al. 2021; Sahai et al. 1980). Terpenoid, steroid, and saponin were identified from various parts of *A. auriculiformis*, such as betulin (bark), stigmasterol (leaf), α-sitosterol (leaf), β-sitosterol (leaf), γ-sitosterol (leaf), campesterol (leaf), proacaciaside (fruit) and acaciaside (fruit) (Ahmadu et al. 2021; Garai and Mahato 1997; Mahato et al. 1992; Pal et al. 2009; Rangra et al. 2019b; Sahai et al. 1980).

The investigation of the pharmacological activity of *A. auriculiformis* wood is still in its infancy. Previous research demonstrated that *A. auriculiformis* heartwood methanol extract possessed the second-highest antioxidant activity when compared to four other species, including *A. mangium*, *A. decurrens*, *A. crassicarpa*, and *A. leucophloea* (Prayogo et al. 2021). Antioxidant activity has been studied as a key treatment for degenerative diseases. Therefore, this study aimed to investigate the new potent raw material for medicine, *A. auriculiformis* heartwood from Indonesia, in order to isolate and identify the chemical constituent with good antioxidant activity.

2. Materials and Methods

2.1. Plant Materials

A. auriculiformis tree (diameter of 27 cm with the 66.4% of heartwood part and age of 10 years) was obtained from Parung Panjang District Forest, Bogor, Indonesia (6° 22' 30"–6° 23' 30" S and 106° 30' 30"–106° 31' 30" E). The species has been confirmed by the Center for Biological Research, the Indonesian National Research and Innovation Agency. The voucher specimen was deposited at the Tropical Biopharmaca Research Center IPB University (BMK0485072021).

2.2. Extraction and Isolation

Five hundred grams of *A. auriculiformis* air-dried heartwood sawdust was extracted in methanol for 24 h (repeated four times). The filtrate was concentrated using an evaporator. The liquid-liquid fractionation was then performed with three different polarity solvents to create four fractions: n-hexane-soluble fraction, ethyl acetate-soluble fraction, butanol-soluble fraction, and

water-soluble fraction (Snyder 1974). The ethyl acetate-soluble fraction (12 g) was subjected to silica gel column chromatography (CC) using $CHCl_3$/MeOH (0:100, 1:100, 3:100, 1:20, 1:10, 1:5, 1:1, 100:0) to give 16 fractions. Fraction 9 was subjected to sephadex LH-20 CC using MeOH/$CHCl_3$ to give 15 fractions. Fraction 9–10 (176.5 mg) was purified using preparative high-performance liquid chromatography (pHPLC) with 20% MeOH to yield compound 1 (11 mg). Fraction 9–15 (25.3 mg) was purified further using pHPLC with 50% MeOH to yield compound 2 (18.7 mg). Fraction 10 was subjected to sephadex LH-20 CC using MeOH/$CHCl_3$ to yield compounds 3 and 4 (30.7 and 147 mg, respectively). Fraction 11 was subjected to sephadex LH-20 CC using MeOH/$CHCl_3$ to yield compound 4 (96 mg). Fraction 12 was purified using pHPLC with 20% MeOH to yield compound 5 (22.8 mg). Fraction 16 was purified using pHPLC with 30% MeOH to yield compound 6 (22 mg). The butanol-soluble fraction was subjected to medium-pressure liquid chromatography with a YMC-ODS column using MeOH/Water (1:3, 1:1, 3:1) to give 5 fractions. Fraction 3 (46 mg) was then purified using pHPLC with 45% MeOH to yield compound 4 (10 mg).

2.3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The antioxidant activity of extracts, fractions, and isolated compounds was determined using a microplate reader (Epoch Biotek, USA). In a 96-well plate, 100 µL of DPPH radical solution in methanol with a concentration of 125 µM was mixed with 100 µL of sample solution. The mixtures were then incubated at room temperature for 30 min. Furthermore, the absorbance of each mixture was measured at a wavelength of 515 nm. The radical scavenging or antioxidant activities were reported as IC_{50} value. Ascorbic acid was used as the positive control or reference to compare the antioxidant activity.

2.4. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Assay

A 5 mL of 7 mM ABTS reagent (in water) was combined with 88 L of 140 mM $K_2S_2O_8$ (in water). The mixture was then stored in the dark for 16 h before being diluted with water at a 1:44 (v/v) ratio. In a 96-well microplate, 180 µL of ABTS reagent was mixed with 20 L of sample solution and incubated at room temperature for 15 min. After incubation, absorbance was measured using a microplate reader (Epoch Biotek, USA) at a wavelength of 734 nm. Then, the sample absorbance was equivalent to the standard trolox curve. The capacity obtained will be expressed in mmol trolox equivalent per gram extract or compound (mmol TE/g).

2.5. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP reagent was prepared by mixing 300 mM acetate buffer pH 3.6, 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (in 40 mM HCl), and 20 mM $FeCl_3$ (in deionized water) in a ratio of 10:1:1, respectively. Reducing power analysis was carried out by mixing 10 µL of an extract with 270 µL of FRAP reagent in a 96-well plate. The mixture was incubated for 30 min at 37°C in an incubator. The absorbance of the solution was observed at 593 nm using a microplate reader (Epoch Biotek, USA). Trolox was used as the positive control to make the calibration curve. The reduction power was reported as mmol trolox equivalent per gram extract or compound (mmol TE/g).

2.6. Compound Structure Elucidation

Extracts, fractions, and isolated compounds were analyzed using the Waters ACQUITY UPLC system coupled with a Waters Xevo QToF mass spectrometer. The C-18 column was employed as the stationary phase column. The stepwise elution was used with the 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) and an elution system of 0–25 min 5–100% B solvent, 25–29 min 100% B solvent, and 100–5% B solvent 29–29.10 min. The flow rate, detection temperature, detector wavelength, and sample injection amount were all set to 1 mL/min, 210–400 nm, and 5 µL, respectively. Detection by mass spectrometry was carried out in the range of 100 – 1500 Da in negative ionization mode. An ionizing electrospray and an ion trap analyzer were employed in the mass spectrometer. Annotation of the analyzed compounds was carried out using MSDial ver. 4.9.221218.

A nuclear magnetic resonance (NMR) spectrometer with a frequency of 600 MHz was employed. The ^{1}H and ^{13}C frequencies were 600 MHz and 150 MHz, respectively. The spectra were produced by dissolving the sample in the hydrogen-free solvent. The peak signals were corrected using a solvent signal of acetone-d_6 (δ_H 2.05 ppm and δ_C 29.84 ppm) or methanol-d_4 (δ_H 3.31 ppm and δ_C 49.00 ppm) as the reference. The 1D NMR spectra of ^{1}H and ^{13}C and 2D NMR spectra of COSY (correlated spectroscopy), HMQC (heteronuclear multiple quantum coherence), and HMBC (heteronuclear multiple bond correlation) were examined using NMR data processing tools (JEOL Delta version 6.0, JEOL, USA). Direct correlations between ^{1}H and ^{13}C were determined by HMQC spectra. Indirect correlations between ^{1}H and ^{13}C were determined by HMBC. The ^{1}H and ^{1}H correlations were determined by COSY. These 2D NMR spectra were used to identify the exact position of the hydrogen and carbon atom in isolated compounds. The chemical shift (δ, in ppm) of ^{1}H and ^{13}C, coupling constant (J), hydrogen amount, and position of carbon and hydrogen from the NMR spectra were summarized below.

Compound 1: (2S,3R,4R)-2-(3-hydroxyphenyl)-4-methoxychromane-4,7,8-triol
ESI-MS: m/z 271.0567 [M-CH_3OH-H]-
^{1}H NMR (600 MHz, methanol-d_4) δ 7.31 (d, J = 9 Hz, 2H, H-2',6'), 6.81 (d, J = 9 Hz, 2H, H-3',5'), 6.65 (d, J = 8.3 Hz, 1H, H-5), 6.43 (d, J = 8.2 Hz, 1H, H-6), 5.08 (d, J = 9.8 Hz, 1H, H-2), 4.20 (d, J = 3.2 Hz, 1H, H-4), 4.02 (dd, J = 9.8, 3.2 Hz, 1H, H-3), 3.45 (s, 3H, H-4-O ether).
^{13}C NMR (150 MHz, methanol-d_4) δ 158.67 (C-4'), 147.66 (C-7), 144.33 (C-9), 133.90 (C-8), 131.34 (C-1'), 130.37 (C-2',6'), 121.74 (C-5), 116.04 (C-3',5'), 114.02 (C-10), 108.75 (C-6), 78.44 (C-2), 77.93 (C-4), 71.61 (C-3), 57.00 (C-4-O methyl).

Compound 2: (E)-3-(4-hydroxyphenyl)-1-(2,3,4-trihydroxyphenyl)prop-2-en-1-one
ESI-MS: m/z 271.0568 [M-H]-
^{1}H NMR (600 MHz, methanol-d_4) δ 7.79 (d, J = 15.4 Hz, 1H, H-α), 7.61 (d, J = 9 Hz, 1H, H-2,6), 7.608 (d, J = 14.4 Hz, 1H, H-β), 7.56 (d, J = 9 Hz, 1H, H-6'), 6.85 (d, J = 8.4 Hz, 2H, H-3,5), 6.47 (d, J = 8.9 Hz, 1H, H-5').
^{13}C NMR (150 MHz, methanol-d_4) δ 194.13 (C-γ), 161.57 (C-4), 154.43 (C-2'), 153.30 (C-4'), 145.57 (C-α), 133.74 (C-3), 131.84 (C-2), 127.84 (C-1), 123.28 (C6'), 118.41 (C-β), 116.92 (C-3), 115.14 (C-1'), 108.53 (C-5')

Compound 3: trans-2,3-3,4',7,8-tetrahydroxyflavanone
ESI-MS: m/z 287.0489 [M-H]-
^{1}H NMR (600 MHz, methanol-d_4) δ 7.43 (dd, *J* = 8.9, 0.6 Hz, 2H, H-2'6'), 7.31 (d, *J* = 8.7 Hz, 1H, H-5), 6.85 (d, *J* = 8.8 Hz, 2H, H-3',5'), 6.57 (d, *J* = 8.7 Hz, 1H, H-6), 5.05 (d, *J* = 11.9 Hz, 1H, H-2), 4.58 (d, *J* = 11.8 Hz, 1H, H-3).
^{13}C NMR (150 MHz, methanol-d_4) δ 195.05 (C-4), 159.28 (C-4'), 154.25 (C-7), 152.34 (C-9), 133.87 (C-8), 130.72 (C-2',6'), 129.44 (C-1'), 119.57 (C-5), 116.12 (C-3',5'), 114.24 (C-10), 111.46 (C-6), 85.94 (C-2), 74.66 (C-3).

Compound 4: 3,4',7,8-tetrahydroxyflavone
ESI-MS: m/z 285.0199 [M-H]-
^{1}H NMR (600 MHz, acetone-d_6) δ 8.24 (d, *J* = 8.8 Hz, 2H, H-2',6'), 7.57 (d, *J* = 8.7 Hz, 1H, H-5), 7.02 (d, *J* = 8.6 Hz, 1H, H-6), 7.02 (d, *J* = 8.8 Hz, 2H, H-3',5').
^{13}C NMR (150 MHz, acetone-d_6) δ 174.15 (C-4), 160.59 (C-4'), 151.30 (C-7), 147.79 (C-9), 146.21 (C-2), 138.54 (C-3), 134.35 (C-8), 131.30 (C-2',6'), 124.76 (C-1'), 116.98 (C-5), 116.17 (C-3',5'), 115.98 (C-10), 115.47 (C-6).

Compound 5: teracacidin
ESI-MS: m/z 271.0610 [M-H_2O-H]-
^{1}H NMR (600 MHz, acetone-d_6) δ 7.44 (d, *J*=9 Hz, 2H, H-2',6'), 6.85 (d, *J* = 9 Hz, 1H, H-5) 6.82 (d, *J* = 9 Hz, 2H, H-3',5'), 6.48 (d, *J* = 8.4 Hz, 1H, H-6), 5.12 (s, 1H, H-2), 4.94 (d, *J* = 4.9 Hz, 1H, H-3), 3.99 (dd, *J* = 4.2 Hz, H-4)
^{13}C NMR (150 MHz, acetone-d_6) δ 157.88 (C-4'), 145.72 (C-9), 143.70 (C-7), 132.75 (C-8), 130.85 (C-1'), 129.57 (C-2',6'), 118.70 (C-5), 117.19 (C-10), 115.47 (C-3',5'), 108.99 (C-6), 79.81 (C-2), 70.33 (C-4), 68.15 (C-3).

Compound 6: (2R,3R,4R)-2-(3-hydroxyphenyl)-4-methoxychromane-4,7,8-triol
ESI-MS: m/z 271.0546 [M-CH_3OH-H]-
^{1}H NMR (600 MHz, acetone-d_6) δ 7.40 (d, *J* = 8.2, 2H, H-2',6'), 6.84 (d, *J* = 8.6 Hz, 2H, H-3',5'), 6.71 (d, *J* = 8.3 Hz, 1H, H-5), 6.48 (d, *J* = 8.2 Hz, 1H, H-6), 5.05 (s broading, 1H, H-2), 4.11 (d, *J* = 3.0, 1H, H-4), 3.97 (dd, *J* = 3.0, 1.3 Hz, 1H, H-3), 3.44 (s, 3H, H-4-O ether).
^{13}C NMR (150 MHz, acetone-d_6) δ 157.87 (C-4'), 146.64 (C-9), 144.55 (C-7), 133.34 (C-8), 130.52 (C-1'), 129.67 (C-2',6'), 122.98 (C-5), 115.53 (C-3',5'), 112.51 (C-10), 108.63 (C-6), 78.07 (C-4), 76.46 (C-2), 69.29 (C-3), 56.25 (C-4-O methyl).

3. Results and Discussion

3.1. Isolation and Identification of Compounds

The phytochemical profiles of the extracts and fractions show the presence of different phytochemical components. The chromatogram peaks of the chemical components of the extract and its fractions of the *A. auriculiformis* were observed at retention times below 10 min, except for the n-hexane fraction (**Fig. 1**). The peaks with low retention times indicated compounds with higher polarity. This showed that the crude extract, along with ethyl acetate, buthanol, and water-

soluble fractions, contained compounds with higher polarity and vice versa for the n-hexane soluble fraction.

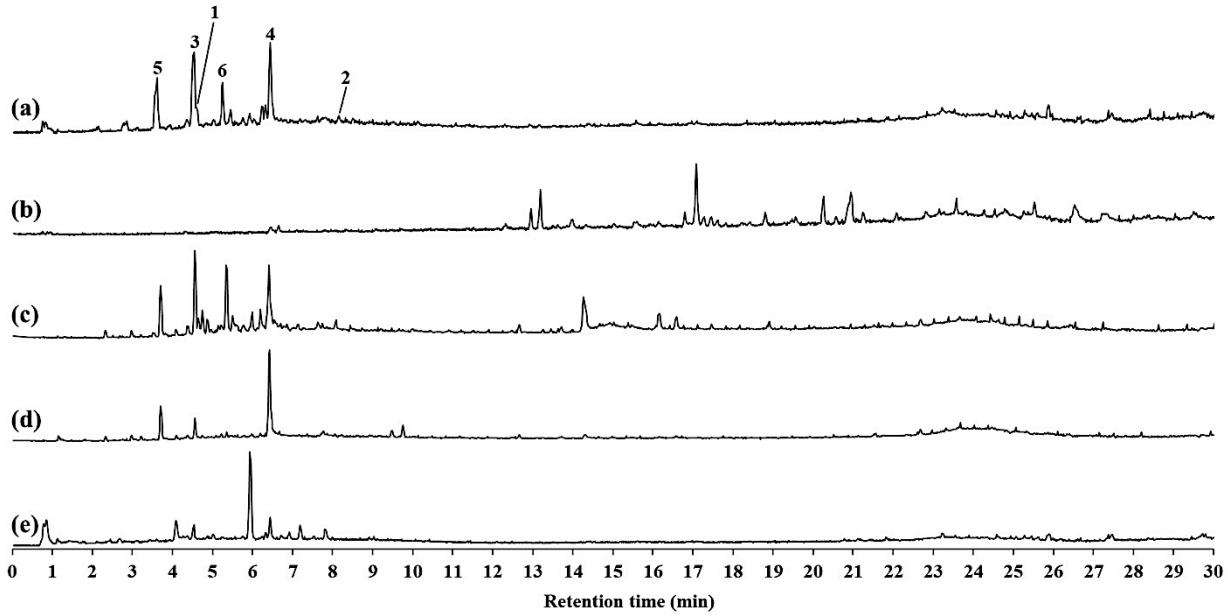

Fig. 1. Chromatogram profile of crude extract (a), n-hexane (b), ethyl acetate (c), butanol (d), and water (e) soluble fractions. Peak with number showed the peak position of the isolated compounds 1–6 in the chromatogram.

The identification of the phytochemical components of the extract and the fractions indicates the content of flavonoid compounds (**Table 1**). Some of these flavonoid components have been isolated and identified by looking at the mass fragmentation and NMR spectra. These compounds were (2S,3R,4R)-2-(4-hydroxyphenyl)-3-methoxychromane-4,7,8-triol (1), (E)-3-(4-hydroxyphenyl)-1-(2,3,4-trihydroxyphenyl)prop-2-en-1-one (2), trans-2,3-3,4',7,8-tetrahydroxyflavanone (3), 3,4',7, 8-tetrahydroxyflavone (4), teracacidin (5), and (2R,3R,4R)-2-(4-hydroxyphenyl)-3-methoxychromane-4,7,8-triol (6). All of these compounds were obtained from the ethyl acetate-soluble fraction, while compound 4 was also present in the butanol-soluble fraction.

In this study, all isolated compounds are flavonoids. The molecular structure of the identified flavonoids had a special characteristic, namely the presence of hydroxyl substitutions at C-4', C-7, and C-8 (**Fig. 2**). Teracacidin (5), 3,4',7,8-tetrahydroxyflavone (4), and trans-2,3-3,4',7,8-tetrahydroxyflavanone (3) had similar hydroxyl substitutions in rings A and B, but differ in ring C. In addition, these three isolated compounds had a relatively high relative abundance in the crude extract. Several previous studies have also succeeded in isolating and identifying these compounds, namely teracacidin and trans-2,3-3,4',7,8-tetrahydroxyflavanone isolated from heartwood of *A. auriculiformis* (Mihara et al. 2005), while 3,4',7,8-tetrahydroxyflavone is isolated from the bark of *A. auriculiformis* (Ahmadu et al. 2019). Analysis of the mass fragmentation of teracacidin showed a unique phenomenon. Teracacidin had an m/z of 271 Da with the adduct type [M-H_2O-H]. This is related to the instability of hydroxyl substitutions at C3 and C4, which form 3,4-diol, so teracacidin is identified as a dehydrated ion, and this phenomenon has been confirmed before (Barry et al. 2005). A similar phenomenon is observed in the mass fragmentation of other flavan-3,4-diols (melacacidin and isomelacacidin) from the heartwood of *A. burkitii* (Grace et al. 2009).

Table 1. The relative abundance of isolated compounds in crude extract and its fractions based on liquid chromatography mass spectrometry

Retention time (min)	m/z (Da)	Compound names	Molecular formula	Relative abundance (%)				
				Crude	n-Hexane	EtOAc	BuOH	Water
6.447	285.0371	3,4',7,8-tetrahydroxyflavone (4)	$C_{15}H_{10}O_6$	27.36	0.04	20.91	60.67	10.13
4.539	287.0537	trans-2,3-3,4',7,8-tetrahydroxyflavanone (3)	$C_{15}H_{12}O_6$	13.60	0.00	10.40	3.17	3.08
5.257	271.0608	(2R,3R,4R)-2-(4-hydroxyphenyl)-3-methoxychromane-4,7,8-triol (6)	$C_{16}H_{16}O_6$	10.00	0.00	1.94	0.34	0.41
3.605	271.0638	teracacidin (5)	$C_{15}H_{14}O_6$	6.72	0.00	4.97	5.92	0.32
4.599	271.0610	(2S,3R,4R)-2-(4-hydroxyphenyl)-3-methoxychromane-4,7,8-triol (1)	$C_{16}H_{16}O_6$	2.45	0.00	0.04	0.01	0.18
8.151	271.0558	(E)-3-(4-hydroxyphenyl)-1-(2,3,4-trihydroxyphenyl)prop-2-en-1-one (2)	$C_{15}H_{12}O_5$	1.19	0.00	0.25	0.05	0.01

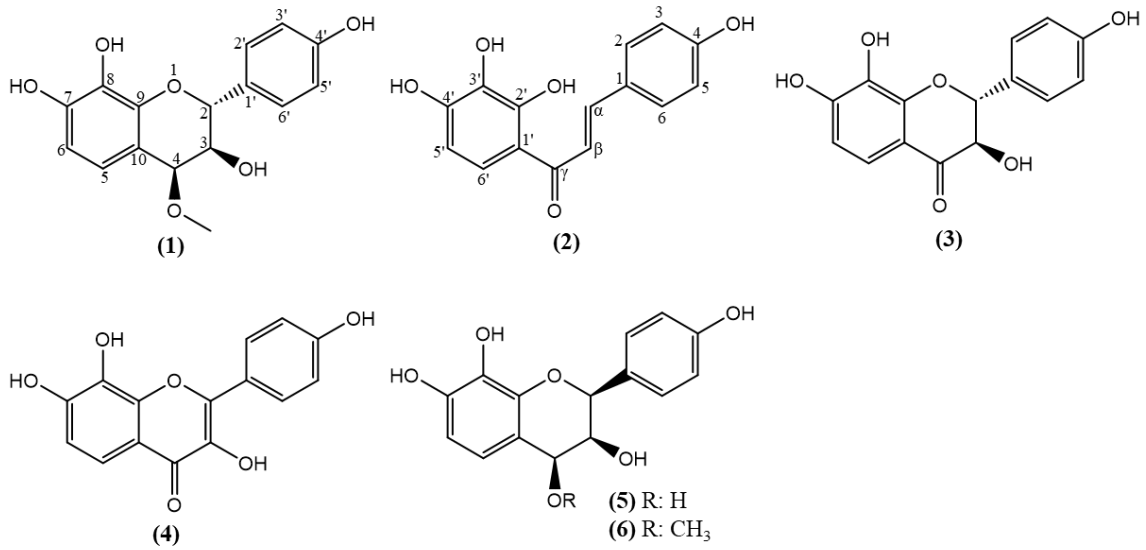

Fig. 2. Molecular structure of six identified compounds.

Teracacidin derivatives have been isolated and identified from the heartwood extract of *A. auriculiformis*. The compound was (2R,3R,4R)-2-(4-hydroxyphenyl)-3-methoxychromane-4,7,8-triol (6). This compound was a methylated form of teracacidin on the C-4 carbon via an ether bond. In addition, the stereoisomer was isolated and identified, namely (2S,3R,4R)-2-(4-hydroxyphenyl)-3-methoxychromane-4,7,8-triol (1). The determination of this methylation position was identified through the HMBC spectra, where the methyl hydrogen (δ_H 3.45 ppm) had a correlation with the C-4. Compound 1 had a different stereoisomer on C-2 than compound 6. The coupling constant value of the C-2 and C-3 in compound 1 was quite large (J = 9 Hz), which indicated a different stereochemistry between the two carbons compared to the low J value of

compound 6 (J = 3 Hz). Both compounds were identified with m/z 271 (M-CH_3OH-H)-, not 303 (M-H)-. This is similar to the phenomenon of teracacidin (3) fragmentation, which is associated with the presence of 3,4-diol (Barry et al. 2005; Grace et al. 2009). Compound 6 also had a greater relative abundance than compound 1. This teracacidin-derived compound was identified for the first time in the heartwood of *A. auriculiformis*.

The (E)-3-(4-hydroxyphenyl)-1-(2,3,4-trihydroxyphenyl)prop-2-en-1-one) (2) has quite a different structure compared to the other five compounds. Compared to the other 5 compounds, compound 2 had a very small relative abundance. Compound 2 was identified as a chalcone characterized by the absence of a C ring. Structurally, this compound had a substitutional position of the hydroxyl group similar to teracacidin, namely on carbons C-2', 3', 4', and 4. Stereochemistry on C-2 and C-3 showed a *trans* configuration, marked by a large coupling constant value (J = 15 Hz). Another chalcone with a different hydroxyl substitution position in the form of chalcone glycoside has been identified from the flower part of *A. dealbata* (Imperato 1982), so this compound was identified for the first time from the heartwood of *A. auriculiformis*.

3.2. Antioxidant Activity of the Extract, Fractions, and Isolated Compounds

A. auriculiformis heartwood extract has good antioxidant activity (**Table 2**). In comparison to the positive control, the crude extract exhibited lower antioxidant activity than ascorbic acid, but its IC_{50} value was satisfactory (**Table 2**). Additionally, the antioxidant activity of *A. auriculiformis* heartwood crude extract was superior to that of other species and *A. auriculiformis* parts. Based on the DPPH assay, *A. auriculiformis* heartwood crude extract exhibited greater antioxidant activity than *A. cyanophylla* flower extract and the leaf, flowers, and pods of *A. nilotica*, *A. seyal*, and *A. laeta* (Abdel-Farid et al. 2014; Ghribia et al. 2014). Based on the ABTS assay, the crude extract, ethyl acetate-soluble fraction, and butanol-soluble fraction exhibited superior antioxidant activity compared to the seed extracts of *A. farnesia*, *A. tortilis*, *A. mollissima*, *A. cyanophylla*, *A. horrida*, *A. salicina*, and *A. cyclops* (Hannachi et al. 2011). In addition, the reducing potency (based on the FRAP assay) of the heartwood crude extract, ethyl acetate-soluble fraction, and butanol-soluble fraction from *A. auriculiformis* was superior to that of the bark extracts from *A. mangium*, *A. auriculiformis*, and *A. crassicarpa* (Aulia et al. 2022).

Table 2. Antioxidant activity of the crude extract, fractions, and isolated compounds

Sample	DPPH (IC_{50}, µg/mL)	ABTS (mmol TE/g)	FRAP (mmol TE/g)
Crude extract	14.44 ± 0.22	2.76 ± 0.13	5.84 ± 0.14
n-Hexane soluble fraction	557.95 ± 44.44	0.48 ± 0.02	0.93 ± 0.03
Ethylacetate soluble fraction	9.50 ± 0.34	4.08 ± 0.06	6.67 ± 0.20
Buthanol soluble fraction	9.55 ± 0.31	2.56 ± 0.03	4.32 ± 0.20
Water soluble fraction	23.14 ± 1.22	1.20 ± 0.02	2.60 ± 0.13
Compound 1	4.57 ± 0.05	0.74 ± 0.06	4.00 ± 0.07
Compound 2	8.47 ± 0.11	4.07 ± 0.19	6.18 ± 0.24
Compound 3	6.82 ± 0.03	2.35 ± 0.10	5.90 ± 0.32
Compound 4	4.49 ± 0.04	2.03 ± 0.01	5.64 ± 0.44
Compound 5	8.28 ± 0.19	1.05 ± 0.01	4.62 ± 0.12
Compound 6	12.30 ± 0.05	0.63 ± 0.02	2.46 ± 0.03
Ascorbic acid	5.13 ± 0.06	-	-

The fractions of *A. auriculiformis* extract show good antioxidant activity. The ethyl acetate-soluble and butanol-soluble fractions had better antioxidant activity compared to the crude extracts and water-soluble fraction. However, these two fractions had lower antioxidant activity than the ascorbic acid. A different phenomenon was observed in the n-hexane fraction, which had poor antioxidant activity. This was related to the differences in chemical components contained in the n-hexane fraction compared to other fractions. This fraction contained many non-polar components dissolved in n-hexane. This was also confirmed in the chromatogram profile of the n-hexane fraction, which showed peaks at higher retention times (**Fig. 1**).

The antioxidant activity of the isolated compounds from *A. auriculiformis* shows good radical scavenging activity for ABTS and DPPH. When compared with the positive control, the six compounds had close values **(Table 2)**. In fact, compounds 1 and 4 had better IC_{50} values than ascorbic acid. The trend of antioxidant activity from high to low based on DPPH was compound 4 > 1 > 3 > 5 > 2 > 6. This trend was quite different compared to antioxidant activity based on ABTS but similar to FRAP. The trend from high to low antioxidant activity of ABTS and FRAP were compound 2 > 3 > 4 > 5 > 1 > 6 and 2 > 3 > 4 > 5 > 1 > 6, respectively.

The different effects of molecular structure on the radical scavenging activity of DPPH, ABTS, and the reducing activity of FRAP are observed by the different data trends. The hydroxyl group on C-3 and the stereochemistry on C-2 were thought to influence DPPH radical cleavage. The influence of the C-3 hydroxyl group was shown by the difference between the carbonyl functional groups at C4 or the double at C-2 and C-3, by maintaining the hydroxyl group at C-3, resulting in good antioxidant activity in compounds 1, 3, and 4. The effect of stereochemistry on C-2 on the cleavage of the DPPH radical was observed by comparing the compound 1 to compounds 5 and 6. Compound 1 with C2-C3 (R,S) stereochemistry gave better antioxidant activity than C-2 and C-3 (S,S) stereochemistry. Meanwhile, in the scavenging of ABTS radicals, the carbonyl functional groups at C-4 and the hydroxyl at C-3 (found in compounds 3 and 4) were thought to play a role in good antioxidant activity based on the trend of their antioxidant capacity values. Compound 2 showed the best activity on ABTS but not on DPPH. This similar phenomenon has been reported and is thought to be influenced by the absence of a C ring in the molecular structure of chalcone, as in flavonoids (Platzer et al. 2021). The influence of the molecular structure in FRAP assay showed a similar phenomenon that occurred in ABTS. The carbonyl functional groups at C-4 and the hydroxyl at C-3 (found in compounds 3 and 4) were thought to have the main effect on the reduction power in FRAP assay.

In general, these findings show remarkable potential for *A. auriculiformis* heartwood to become new medicine's raw material as a source of bioactive flavonoids. It was shown by both extract, fractions, and isolated compounds of *A. auriculiformis* heartwood, which had promising antioxidant activity. The isolated compound that showed good antioxidant activity could be used further as the marker compound in developing the *A. auriculiformis* standardized extract. Moreover, a deep study of the bioactivity of extract, fractions, and isolated compounds is needed by using cell (molecular level), preclinical, and/or clinical tests.

4. Conclusions

Six compounds were isolated from the heartwood of *A. auriculiformis*. Three compounds were isolated for the first time from the heartwood of *A. auriculiformis*, namely (2R,3R,4R)-2-(4-hydroxyphenyl)-3-methoxychromane-4,7,8-triol, (2S,3R,4R)-2-(4-hydroxyphenyl)-3-methoxy

chromane-4,7,8-triol, and (E)-3-(4-hydroxyphenyl)-1-(2,3,4-trihydroxyphenyl)prop-2-en-1-one. The isolated compounds had good antioxidant activity compared to ascorbic acid and crude extract. The molecular structure-dependent antioxidant activities were observed. These compounds had different antioxidant activities against three different antioxidant assays, which showed the influence of molecular structure on their activity.

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