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Antibacterial activity of five Indonesian medicinal plants and the isolation of compounds from *Plectranthus scutellarioides*

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ABSTRACT

The purpose of this study is to investigate the antibacterial properties of five Indonesian medicinal plant extracts, namely *Physalis angulata*, *Loranthus parasiticus*, *Plectranthus scutellarioides*, *Cyperus rotundus*, and *Terminalia catappa.* The isolation of compounds from *P. scutellarioides* against pathogenic bacteria was also investigated. The assessment of the antibacterial activity of the extracts was conducted using the microdilution-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method against Gram-positive bacteria (*Bacillus subtilis and Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*). The plant extract with the highest antibacterial activity was further purified using various chromatographic assays to isolate bioactive compounds. High Resolution-Electron Ionization Mass Spectroscopy/Electrospray Ionization Mass Spectroscopy (HR-ESIMS/EIMS) and Nuclear Magnetic Resonance (NMR) spectrometers were used to elucidate the structure of isolated compounds. Among all the plants tested, *P. scutellarioides* showed the strongest antibacterial activity against *S. aureus* [minimum inhibitory concentration (MIC 100 µg/ml)] and *B. subtilis* (MIC 200 µg/ml). Three compounds were isolated from *P. scutellarioides* including an abietane diterpene, 2,16-diacetoxy-6,11,12,14,17-pentahydroxy-abieta-5,8,11,13-tetraene-7-one (**1**), 5,6,7,3′,4′,5′-hexamethoxy flavone (**2**), and 5,6,7,8,3′,4′,5′-heptamethoxy flavone (**3**). Compounds **2** and **3** were recognized to be first isolated from this plant. Compound **1** exerted antibacterial activity against *S. aureus* with a MIC value of 60 µM, but was inactive against *B. subtilis* and *E. coli* (MIC > 200 µM). Compounds **2** and **3** were inactive against *S. aureus*, *B. subtilis*, and *E. coli* (MIC > 200 µM).

INTRODUCTION

Infectious diseases remain one of the top public health threats worldwide. In 2019, infectious diseases caused 13.7 million deaths in the world [1]. The leading causes of death in countries with lower and middle incomes, according to the WHO, include respiratory injuries, tuberculosis, and diarrhea [2]. There are many kinds of infectious diseases and some of them are caused by bacteria, such as tuberculosis, typhoid, pneumonia, cholera, and gonorrhea. With an increase in microbial drug resistance and a lack of novel antibacterial drugs being developed, bacterial infection is on the rise [3].

The search for new antibacterial to overcome infections and resistance problems has been a top priority for the pharmaceutical industry and academia. Plant secondary metabolites are considered as a potential source of new antibacterial agents [4]. It is estimated that 500–800 different secondary metabolites are contained in each plant species. Plant accumulates secondary metabolite with high antibacterial activities, such as alkaloids, coumarins, isoflavonoids, quinones, tannins, and terpenes. It is known that plant secondary metabolites can affect microbial cells through several mechanisms, such as disrupting cell membranes, interrupting bacterial transcription and replication, and inhibiting cell division [5,6]. Based on this knowledge, the search for antibacterial agents from plant sources is worth conducting.

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Medicinal plants such as *Physalis angulata*, *Loranthus parasiticus*, *Plectranthus scutellarioides*, *Cyperus rotundus*, and *Terminalia catappa* have been used as traditional medicine in Indonesia [7,8]. They possess very diverse ethnopharmacological uses. *Physalis angulata* is used as a traditional folk remedy and has been empirically used to treat rheumatic diseases, hepatitis, cervical cancer, and mouth and throat inflammatory conditions. Alkaloids, physalins, angulatins, withangulatin A, and withaferin A were known present in *P. angulata* [9,10]. While *L. parasiticus* has been used for centuries as a traditional remedy. Bioactive components of this plant mainly belong to triterpenes, biscotoxins, lectins, sesquiterpenes lactones, and flavonoid, which is the most important phenolic compounds [11,12]. *Plectranthus scutellariosides* are also widely used as a traditional medicine in Indonesia. It is used to treat stomach pain, diarrhea, hemorrhoids, and skin conditions [13,14]. Research on *P. scutellarioides* has resulted in the separation of flavonoid glycosides, caffeic acid, and abietane or labdane-type diterpenoids. β-sitosterol and stigmasterol, which belong to steroid compounds are also present in this plant [15–19]. *Cyperus rotundus*, a nut grass that belongs to the family Cyperaceae, is used to treat convulsions, amenorrhea, bronchitis, dysentery, leprosy, diarrhea, and gastric disorders in tropical and sub-tropical countries. This plant contains many secondary metabolites such as terpenoids including sesquiterpenes, alkaloids, fatty acids, steroids, saponins, and phenolic compounds including flavonoids [20]. *Terminalia catappa* (Combretaceae) is often found and planted in Asia, especially in India. This plant has multipharmacological purposes such as dressing for rheumatic joints and treatment of dermatitis, scabies, and leprosy. Previous reports stated that *T. catappa* contains flavonoid glycosides, tannin including punicalagin and punicalin, as well as terpenoids and coumarin [21,22].

As part of our ongoing effort to identify natural antimicrobials, we conducted research on the antibacterial properties of these five plants, as well as the isolation of bioactive constituents from a selected plant. The secondary metabolites present in these plants such as alkaloids, terpenes, tannins, saponins, flavonoids, and phenolics have been shown to have antimicrobial activity [23]. The antibacterial activity of the plants, as well as the isolated compounds, was assessed against three pathogenic bacteria, namely *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*.

MATERIALS AND METHODS

Materials

Five species of medicinal plants, namely *P. angulata* fruits, the aerial parts of *L. parasiticus*, *P. scutellarioides* leaves, *C. rotundus* rhizome, and *T. catappa* leaves were obtained from Yogyakarta, Indonesia. The authentication of all plants was determined by D. Santosa (Faculty of Pharmacy, Gadjah Mada University, Indonesia).

Antibacterial evaluation of plant extracts

Five species of Indonesian medicinal plants were extracted with methanol and chloroform (1:5) using the ultrasonication technique. These extracts were then tested to determine the antibacterial activities using standard microdilution-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test against *B. subtilis* NBRC 13719 and *S. aureus* NBRC 100910, which are Gram-positive bacteria, as well as *E. coli* NBRC 102203, a Gram-negative bacteria [24]. Yeast Polypeptone (YP) was used as a medium for bacterial culture which is comprised of 0.2% yeast extract (BD Difco™, USA), 1% polypeptone (Nihon Pharmaceutical Co., Ltd., Japan), agar (2%), and 0.1% hydrous magnesium sulfate (Nacalai Tesque Inc., Japan). Initially, bacteria were inoculated on YP agar plates under 37°C overnight incubation. Bacterial strains were then transferred to grow in liquid YP medium (without agar) and incubated at 37°C under shaking conditions for 12 hours. A stock sample solution (medicinal plant extracts) was prepared in dimethyl sulfoxide (DMSO) with a concentration of 10 mg/ml. Liquid YP medium and bacteria were put in 96-well plates and a sample solution was applied. Medium-contained microbial strains and samples were then further diluted into varying concentrations (200 and 100 µg/ml). The positive controls, ampicillin, and kanamycin (Nacalai Tesque) were also treated as the extract and finally, the plate was incubated at a temperature of 37°C overnight. 50 µl of MTT (Sigma-Aldrich, USA) solution (5 mg/ml in isopropanol-Cl) were added into each well and then incubated for 1 hour. The experiment was conducted in triplicates. The extract that showed inhibition of bacterial colony growth which could be observed visually (clear yellow color) both at concentrations of 200 and 100 mg/ml was selected for further isolation.

Antibacterial evaluation of isolated compounds

The antibacterial activity of compounds isolated from a selected plant, *P. scutellarioides*, was performed using the same method as the plant extracts. The sample stock solution in DMSO (5 mM) was first prepared and then diluted to various concentrations (200, 100, 80, 60, 50, 25, and 12.5 µM) with YP medium in 96-well plates containing bacterial culture. After incubation at 37°C overnight, the minimum inhibitory concentration (MIC) values were visually observed by the addition of 50 µl of MTT solution into each well, followed by 1 hour incubation at 37°C. The experiment was performed in triplicates. After the incubation, the result was observed by the unaided eye. The MIC of isolated compounds is defined as the lowest concentration of the compounds that completely inhibits the growth of the bacteria (clear yellow color).

Extraction and isolation of compounds from *P. scutellarioides*

Extraction of *P. scutellarioides* was performed using powdered leaves (300 g) with $CHCl_3$ as a solvent. The sample was macerated under sonication for 1.5 hours at room temperature using 2 l of solvents and then remacerated two times. The filtrate was collected and evaporated by a vacuum rotary evaporator to yield $CHCl_3$ extract (25.2 g). The extract was fractionated using normal phase medium pressure liquid chromatography (Büchi Labortechnik, Switzerland) with silica gel as stationary phase (100 × 460 mm sample column; 40–50 µm and 1.85 kg silica; flow rate = 30 ml/minute), and mixtures of n-hexane–ethyl acetate (EtOAc) from 1:0 to 0:1 as eluents. A total of 18 fractions were collected from the process. Fraction 11 (2,470 mg) was separated using reversed-phase column chromatography (Cosmosil 75C18-OPN, Nacalai Tesque Inc., Japan) and H_2O–methanol (MeOH)

(1:1) as eluent resulting in four subfractions (F11-1: 180 mg; F11-2: 83 mg; F11-3: 450 mg; F11-4: 812 mg). Purification of sub-fraction F11-1 by column chromatography with n-hexane:EtOAc (2:1) as solvent system followed by reversed-phase preparative Thin Layer Chromatography (TLC) (RP-18F254 plates, Merck, Germany, eluents $CH_3CN:H_2O$ 7:3) afforded known compound **1** (4.8 mg). Further purification of sub-fraction F11-2 using normal phase open column chromatography and solvent system of n-hexane–EtOAc (2:1) yielded a known compound **2** (2.1 mg). Subsequently, Fr. 13 was separated by reversed-phase column chromatography to afford several sub-fractions. Purification of sub-fraction F13-3 by semipreparative High Performance Liquid Chromatography (HPLC) (Agilent 1260 Infinity series G1311B) with $CH_3CN–H_2O$ (45:55) furnished known compound **3** (2.2 mg; flow rate = 2 ml/minute, Rt 35 minutes). The structure of all isolated compounds was analyzed and determined by 1D/2D NMR (Varian UNITY 600 spectrometer; ^1H NMR for 600 MHz, ^{13}C NMR for 150 MHz, and JEOL JNM-ECA500II; ^1H NMR for 500 MHz, ^{13}C NMR for 125 MHz) and MS spectra (JEOL MStation JMS-700 High-Resolution Electron Impact Mass Spectrometer and Waters SYNAPT G2-Si HDMS High-Resolution Electron Spray Ionization Mass Spectrometer), then cross-referenced with previously published data.

RESULTS AND DISCUSSION

Antibacterial activity of five Indonesian medicinal plants

Antibacterial activity screening of five species of Indonesian medicinal plants was performed by using microdilution-MTT assay against *B. subtilis*, *S. aureus*, and *E. coli* (Table 1). According to the screening result, the chloroform extract of *P. angulata*'s fruit exerted antibacterial activity against *B. subtilis* (MIC 200 µg/ml). The methanol extract of *T. catappa*'s leaves showed antibacterial activity against *S. aureus* (MIC 200 µg/ml). The chloroform extract of *P. scutellarioides'* leaves showed antibacterial activities against *B. subtilis* (MIC 200 µg/ml) and *S. aureus* (MIC 100 µg/ml). Hence, *P. scutellarioides* leaf extract was chosen to be further investigated.

Plectranthus scutellarioides (synonym: *Coleus scutellarioides*, *Coleus blumei*, *Coleus atropurpureus*) is an ornamental plant belonging to the Lamiaceae family. This plant is widely distributed in Indonesia, the Philippines, India, China, and Australia [25]. Previous research on the biological properties of the isolated compounds from *P. scutellarioides* showed the potential for antibacterial, anti-inflammatory, and antiproliferative properties as well as antioxidant properties [15,26–28]. The result of this study corresponds to the study by Bismelah *et al.* [29] which reported that *P. scutellarioides* extract showed antibacterial activity against some bacteria strains, including *S. aureus* and *B. subtilis* [29]. The extract of *P. scutellarioides* can disrupt the bacteria's cell wall, leading to cell death.

Isolation of compounds from *P. scutellarioides*

The $CHCl_3$ extract of *P. scutellarioides* was subjected to fractionation and purification to obtain known compounds **1**, **2**, and **3** (Fig. 1).

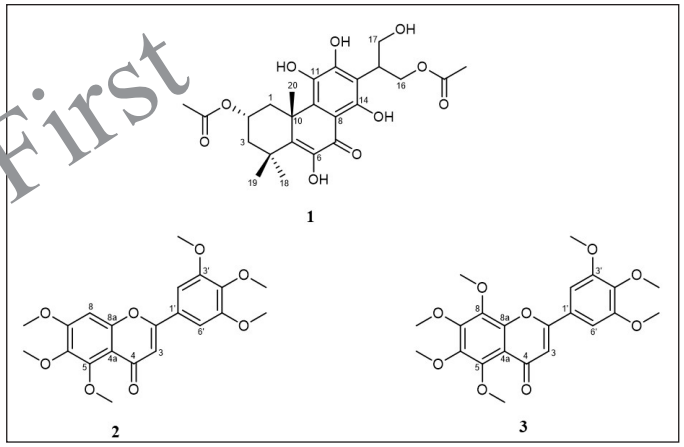

Figure 1. Structure of compounds isolated from *P. scutellarioides.*

Table 1. Result of antibacterial activity screening of Indonesian medicinal plants.

Plant species	Plant part	Extracts	*B. subtilis*	*S. aureus*	*E. coli*
P. angulata	Fruit	MeOH	–[a]	–	–
		$CHCl_3$	+ (200 µg/ml)	–	–
L. parasiticus	Aerial part	MeOH	–	–	–
		$CHCl_3$	–	–	–
P. scutellarioides	Leaves	MeOH	–	–	–
		$CHCl_3$	+ (200 µg/ml)	+ (100 µg/ml)	–
C. rotundus	Rhizome	MeOH	–	–	–
		$CHCl_3$	–	–	–
T. catappa	Leaves	MeOH	–	+ (200 µg/ml)	–
		$CHCl_3$	–	–	–

[a]No inhibition observed at the tested concentration (200 and 100 µg/ml).

Table 2. Comparison of NMR spectra of compound **1** and reference.

Position	δH ($CDCl_3$)	δH ref. ($CDCl_3$) [30]	δC ($CDCl_3$)	δC ref. ($CDCl_3$) [30]
1	1.51 (1H, m) 3.84 (1H, m)	1.45 (1H, m) 3.86 (1H, m)	34.1	34.07[a] 34.02[a]
2	5.41 (1H, dq, *J* = 6.3, 2.2)	5.41 (1H, dq, *J* = 6.5, 2.3)	68.2	68.2
3	1.68 (1H, dd, *J* = 14.9, 2.3) 2.3 (1H, dd, *J* = 15.5, 6.3)	1.70 (1H, dd, *J* = 15.9, 2.7) 2.35 (1H, dd, *J* = 15.9, 6.6)	42.38[a] 42.37[a]	42.4
4	-	-	35.9	35.9
5	-	-	142.21[a]	142.1
			142.16[a]	
6	-	-	141.09[a]	141.0
			141.07[a]	
7	-	-	182.8	182.7
8	-	-	105.3	105.4
9	-	-	133.5	133.5
10	-	-	40.5	40.4
11	-	-	135.8	135.8
12	-	-	150.6	150.5
13	-	-	109.8	109.8
14	-	-	152.3	152.3
15	3.89 (1H, m)	3.90 (1H, m)	36.6	36.57[a]
				36.62[a]
16	3.95 (1H, m) 4.79 (1H, q, *J* = 10.8)	3.95 (1H, m) 4.79 (1H, m)	61.25[a] 61.23[a]	61.5
17	3.91 (1H, m) 3.97 (1H, m)	3.92 (1H, m) 3.99 (1H, m)	61.7	61.62[a] 61.69[a]
18	1.44 (3H, s)	1.47 (3H, s)	28.6	28.5
19	1.49 (3H, s)	1.53 (3H, s)	29.5	29.5
20	1.607[b] (3H, s) 1.617[b] (3H, s)	1.632[b] (3H, s) 1.622[b] (3H, s)	24.8	24.69[a] 24.75[a]
2-OAc	-	-	170.8	170.7
	2.00 (3H, s)	2.01 (3H, s)	21.5	21.0
16-OAc	-	-	173.9	173.8
	2.179[b] (3H, s) 2.171[b] (3H, s)	2.184[b] (3H, s) 2.179[b] (3H, s)	21.1	21.4
6-OH	6.93 (1H, s)	6.94 (1H, s)	-	-
11-OH	6.07 (1H, br s)	6.081[b] (1H, s) 6.061[b] (1H, s)	-	-
12-OH	11.67 (1H, br s)	11.667[b] (1H, br s) 11.646[b] (1H, br s)	-	-
14-OH	13.078[b] (1H, s) 13.075[b] (1H, s)	13.081[b] (1H, br s) 13.077[b] (1H, br s)	-	-
17-OH	5.05 (1H, br s)	5.05 (1H, br s)	-	-

δC and δH in ppm, *J* (coupling constant) in Hz; Reference spectra: ^{1}H NMR 300 MHz, ^{13}C NMR 75 MHz; Compound 1 spectra: ^{1}H NMR 500 MHz, ^{13}C NMR 125 MHz.
[a]Carbon doublets due to diastereomerism.
[b]Proton doublets due to diastereomerism.

Compound **1** was isolated as yellow amorphous, and from the ESIMS analysis (*m/z* 478 $[M]^+$), the molecular formula of compound **1** was determined to be $C_{24}H_{30}O_{10}$ in accordance with NMR data. The ^{1}H NMR analysis (500 MHz, $CDCl_3$) revealed the signals for two aliphatic methylene groups [δH 3.84 (1H, m, H-1a), 1.51 (1H, m, H-1b), 2.31 (1H, dd, *J* = 15.5,

Table 3. Comparison of NMR spectra of compound **2** and reference.

Position	δH ($CDCl_3$)	δH ref. ($CDCl_3$) [31]	δC (2)	δC ref. ($CDCl_3$) [31]
2	-	-	160.9	161.0
3	6.62 (1H, s)	6.62 (1H, s)	108.4	108.3
4	-	-	177.3	177.2
4a	-	-	113.1	112.9
5	-	-	152.7	154.5
6	-	-	140.4	140.4
7	-	-	157.9	157.8
8	6.80 (1H, s)	6.81 (1H, s)	96.4	96.3
8a	-	-	154.6	152.6
1’	-	-	127.0	126.9
2’	7.08[a] (2H, s)	7.08[a] (2H, s)	103.7	103.4
3’	-	-	153.7	153.6
4’	-	-	141.4	140.9
5’	-	-	153.7	153.6
6’	7.08[a] (2H, s)	7.08[a] (2H, s)	103.7	103.4
5-OMe	3.99 (3H, s)	4.00 (3H, s)	56.5	56.4
7-OMe	4.00 (3H, s)	4.04 (3H, s)	62.3	62.2
3’-OMe	3.96[b] (6H, s)	3.96[b] (6H, s)	56.6	56.4
5’-OMe	3.96[b] (6H, s)	3.96[b] (6H, s)	61.7	61.6
4’-OMe	3.93[c] (6H, s)	3.93[c] (6H, s)	61.7	61.6
6-OMe	3.93[c] (6H, s)	3.93[c] (6H, s)	61.7	61.6

δC and δH in ppm; Reference spectra: ^{1}H NMR 400 MHz, ^{13}C NMR 125 MHz; Compound 2 spectra: ^{1}H NMR 600 MHz, ^{13}C NMR 150 MHz.
[a,b,c]Overlapping resonance within the same column.

6.3 Hz, H-3a), and 1.68 (1H, dd, J = 14.9, 2.3 Hz, H-3b)]; one oxygenated methine proton [δH 5.41 (1H, dd, J = 6.3, 2.2 Hz, H-2)], two oxygenated methylene groups [δH 4.79 (1H, q, J = 10.8 Hz, H-16a), 3.95 (1H, m, H-16b), 3.97 (1H, m, H-17a), and 3.91 (1H, m, H-17b)]; five hydroxyl groups [δH 6.93 (1H, s, 6-OH), 6.07 (1H, br s, 11-OH), 11.67 (1H, br s, 12-OH), δH 13.07 (1H, s, 14-OH), and 5.05 (1H, br s, 17-OH)]; methine proton [δH 3.89 (1H, m, H-15)], and five singlet methyl protons [δH 2.00 (3H, s, 2-OCOCH_3), 2.17 (3H, s, 16-OCOCH_3), 1.44 (3H, s, H-18), 1.49 (3H, s, H-19), and 1.61 (3H, s, H-20)]. Several proton signals at H-20, 14-OH, and 16-OCOCH_3 appeared as “doublets” with the difference of chemical shift not more than 0.01 ppm, but in fact, they were overlapping singlets. This data suggested the possibility that the compounds were a mixture of diastereomers.

The ^{13}C NMR (125 MHz, $CDCl_3$) indicated 24 signals including 2 methylenes [δC 34.1 (C-1), 42.3 (C-3)], 2 oxygenated methylenes [δC 61.2 (C-16), 61.7 (C-17)], methine carbon [δC 36.6 (C-15)], oxygenated methine carbon [δC 68.2 (C-2)], 2 quaternary carbons [δC 35.9 (C-4), 40.5 (C-10)], 5 methyl carbons [δC 29.5 (C-19), 28.6 (C-18), 24.8 (C-20), 21.5 (2-OCOCH$_3$), 21.1 (16-OCOCH$_3$)], 8 olefinic carbons [δC 142.2 (C-5), 141.1 (C-6), 105.3 (C-8), 133.5 (C-9), 135.8 (C-11), 150.6 (C-12), 109.8 (C-13), 152.3 (C-14)], ketone carbonyl [δC 182.8 (C-7)], and 2 ester carbonyls [δC 173.9 (16-OCOCH$_3$), 170.8 (2-OCOCH$_3$)]. Similar to the proton signals, the carbon signals of those at C-2, C-5, C-6, and C-16 appeared as “doublets” with the difference of chemical shift not more than 0.02 ppm, rather than singlets. The ^{1}H and ^{13}C NMR confirmed the possibility of the diastereomeric mixture presence of compound **1**. From NMR and ESIMS analysis, it is concluded that compound **1** is an abietane-type diterpene, identical to those of 2,16-diacetoxy-6,11,12,14,17-pentahydroxy-abieta-5,8,11,13-tetraene-7-one, published in previous literature (Table 2) [30]. The previous finding by Ragasa *et al.* [30] led to the conclusion that this compound was a 1:1 mixture of diastereomers and it was proposed to be diastereomeric at C-15.

Compound **2** was obtained as a pale-yellow amorphous solid. Compound **2** was analyzed with ESIMS and showed *m/z* 403 $[M+H]^+$, consistent with the molecular formula $C_{21}H_{22}O_8$. The ^{1}H and ^{13}C NMR data of **2** were nearly similar to those of reference (Table 3) [31]. Thus, compound **2** was determined to be 5,6,7,3′,4′,5′-hexamethoxy flavone.

Compound **3** was isolated as a yellow amorphous solid. The molecular formula was established as $C_{22}H_{24}O_9$ based on its EIMS peak at *m/z* 432 $[M]^+$. The ^{1}H NMR and ^{13}C NMR data of **3** showed similarity to compound **2** with the addition of one methoxy group. Moreover, the ^{1}H NMR data of **3** are similar to those of Rumbero *et al.* [32] (Table 4). Thus, compound **3** was determined to be 5,6,7,8,3′,4′,5′-heptamethoxy flavone (5′ -methoxynobiletin). Compounds **2** and **3** are

Table 4. Comparison of NMR spectra of compound **3** and reference.

Position	δH ($CDCl_3$)	δH ref. ($CDCl_3$) [32]	δC ($CDCl_3$)	δC ref. ($CDCl_3$) [32]
2	-	-	160.9	160.55
3	6.63 (1H, s)	6.62 (1H, s)	107.8	107.41
4	-	-	177.5	177.07
4a	-	-	115.1	114.63
5	-	-	151.7	151.35
6	-	-	138.1	137.80
7	-	-	147.9	147.52
8	-	-	144.3	143.96
8a	-	-	148.6	148.21
1’	-	-	126.9	126.48
2’	7.16[a] (2H, s)	7.16[a] (2H, s)	103.5	103.04
3’	-	-	153.8	153.38
4’	-	-	141.2	140.85
5’	-	-	153.8	153.38
6’	7.16[a] (2H, s)	7.16[a] (2H, s)	103.5	103.04
5-OMe	4.10 (3H, s)	4.09 (3H, s)	62.0	61.60
6-OMe	4.02 (3H, s)	4.02 (3H, s)	62.1	61.71
8-OMe	3.95 (3H, s)	3.95[b] (12H, s)	62.4	62.05
3’-OMe	3.94[b] (9H, s)	3.95[b] (12H, s)	56.4	56.02
5’-OMe	3.94[b] (9H, s)	3.95[b] (12H, s)	56.4	56.02
7-OMe	3.94[b] (9H, s)	3.95[b] (12H, s)	56.4	56.02
4’-OMe	3.92 (3H, s)	3.92 (3H, s)	61.2	61.31

δC and δH in ppm; Reference spectra: ^{1}H NMR 300 MHz, ^{13}C NMR 75.5 MHz; Compound 3 spectra: ^{1}H NMR 600 MHz, ^{13}C NMR 150 MHz.

[a,b]Overlapping resonance within the same column.

polymethoxyflavones, and as far as our knowledge they were first isolated from *P. scutellarioides.*

Antibacterial activity of extracts and isolated compounds

The isolated compounds from *P. scutellarioides* were tested for their antibacterial activities against Gram-positive bacteria *S. aureus* and *B. subtilis*, as well as Gram-negative bacteria, *E. coli* (Table 5).

The results of the antibacterial investigation suggested that compound **1**, which belongs to acylhydroquinone abietanoids, was found to be selectively active against *S. aureus* with a MIC value of 60 µM. This result corresponds to the previous studies on Coleon U, an acylhydroquinone isolated from *P. grandidentatus* and *P. forsteri* that displays potent antibacterial activities against *S. aureus* and other bacteria strains [33,34]. It has been proposed that the potent activities of the acylhydroquinone abietanoids are due to the presence of oxygenated functions in the chromophoric system on the B and C rings. A number of studies suggested that these specific characteristics were linked to the compound’s capacity to cross or degrade bacterial cell membranes [35,36]. In contrast, compound **1** was inactive against *B. subtilis* and *E. coli*.

Compounds **2** and **3** were inactive against all tested bacteria, *S. aureus*, *B. subtilis*, and *E. coli* (MIC > 200 µM). Previous research on the antibacterial activity of

Table 5. Antibacterial activities of isolated compounds from *P. scutellarioides.*

Sample	Minimum inhibitory concentration (MIC)		
	S. aureus	***B. subtilis***	***E. coli***
1	60 µM	>200 µM	>200 µM
2	>200 µM	>200 µM	>200 µM
3	>200 µM	>200 µM	>200 µM
Ampicillin[a]	<0.08 µg/ml	<0.08 µg/ml	-
Kanamycin[a]	-	-	5 µg/ml

[a] Positive control.

polymethoxy flavones mainly discussed the activity of those compounds on Gram-negative bacteria. Yao *et al.* [37] stated that polymethoxy flavones such as nobiletin and tangeretin were weak/inactive against *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. Other research showed that polymethoxylated flavones such as tangeritin and nobiletin are less active than flavanones in inhibiting the growth of *Helicobactor pylori* [38]. According to Shamsudin *et al.* [39], methoxylation at C3’ and C5 on flavonoid structure could decrease its antibacterial action [39]. This is due to the lipophilicity of polymethoxylated flavones that cause minimal or no antibacterial activities [40].

CONCLUSION

This research unfolds the antibacterial properties of *P. scutellarioides* extract and the isolated compounds. Among five Indonesian medicinal plants that are tested for antibacterial activity, *P. scutellarioides* extract exerted the most effective antibacterial activity against Gram-positive bacteria, *B. subtilis* (MIC 200 µg/ml), and *S. aureus* (MIC 100 µg/ml). An abietane diterpene was isolated, as well as two polymethoxy flavones which were recognized to be first isolated from this plant. The abietane diterpene compound possessed potential antibacterial activity against *S. aureus* (MIC 60 µM) but was inactive against *B. subtilis* and *E. coli*. Whereas two polymethoxy flavones were inactive against tested bacteria (*S. aureus*, *B. subtilis*, and *E. coli*) due to their lipophilicity. Further research regarding the antibacterial activity mechanisms of the abietane diterpene compound was needed to provide an alternative therapy for antibacterial infection.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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