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DRUG-DRUG INTERACTIONS BETWEEN GRISEOFULVIN AND A NEW PRENYLATED CHALCONE FROM ELATOSTEMA PARASITICUM AND ITS ANTIBACTERIAL ACTIVITY NORTRIPTYLINE AT BINDING SITES OF BOVINE SERUM ALBUMIN

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Abstract

A new prenylated chalcone (4,4',6' trihydroxy 3 methoxy 3' pentene chalcone) has been isolated from *Elatostema parasiticum* (Blume) Blume ex. H. Schroet. Its structure was elucidated based on UV, IR, mass and NMR spectra data. The isolated compound was evaluated against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, Candida albicans, *Aspergillus niger* and *Microsporum gypseum* using broth micro dilution method. This compound only inhibitted the growth of *Staphylococcus aureus* and *Bacillus subtilis* with the MIC values were 7.8 µg/ml and 1.95 µg/ml, respectively.

Key words: chalcone, antimicrobial, *Elatostema parasiticum*

Introduction

Chalcone is 1, 3-diphenyl-2-propene-1-one, consist of two aromatic rings linked by a three carbon α, β-unsaturated carbonyl system. Chalcone acts as precursors for a vast range of flavonoid derivatives found throughout the plant kingdom. The compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial, anti-inflammatory, analgesic, anticancer, antiviral and antioxidant activities. The presence of a reactive & unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity [1,2].

More than 300 new chalcones has been reported with various biological activities. Those compounds included chalcones and dihydrochalcones [3]. Licochalcone A, licochalcone C, 2,4,2-trihydroxychalcone and 2,4,2-trihydroxy-5-methylchalcone have been reported to posses antimicrobial activity [4]. From urticaceae plants, the new chalcon has been isolated from *Boehmeria rugulosa* Wedd. leaves, chalcone-6'-hydroxy-2',3,4-trimethoxy-4'-O-β-D-glucopyranoside which has an antibacterial activity against *Staphylococcus aureus*, *Streptococcus mutans* (bacteria) and *Microsporum gypseum, Microsporum canis, Trichophyton rubrum* (pathogenic fungi) [5].

Elatostema parasiticum (Blume) Blume ex. H. Schroet (urticaceae) is one of Indonesian urticaceae plants [6] It is used by Siwai and Buin communities, Bougainville islands, Papua New Guinea for fever and fobia disorder [7]. The antimicrobial activities from Indonesian urticaceae extracts (*Cypholophus lutescens* (Blume) Wedd., *Dendrocnide stimulants* (L. f) Chew., *Dendrocnide microstigma* (Gaud. ex Wedd.) Kuntze., *Debregeasia longifolia* (Burm. F.) Wedd., *Elatostema repens* (Lour.) Hallier f., *Elatostema sinuatum* (Blume) Hassk., *Elatostema parasiticum* (Blume) Blume ex. H. Schroet., *Elatostema integrifolium* (D. Don.) Wedd., *Myriocarpa longipes* Liebm., *Pilea repens* (Sw.) Liebm., *Pilea melastomoides* (Poir.) Wedd., *Villebrunea scabra* (Blume) Wedd. and *Villebrunea rubescens* (Blume) Blume) has been carried out. The extract of aerial parts of *Elatostema parasiticum* showed the best antimicrobial activity among all extracts [8].

Behalf of that, in this research was to isolate, characterize and elucidated the antimicrobial compounds from ethanolic extract of *Elatostema parasiticum* and to identify the antimicrobial activity of the isolated compounds.

Materials and Methods

Materials

The aerial parts of *Elatostema parasiticum* were collected and determined from Bogor botanic garden, Desember 2011. The plant materials were washed, dried and grounded to small pieces. Test microbes were *Staphylococcus aureus* (American Type Culture Collection ATCC (6538)), *Bacillus subtilis* (ATCC 6636), Escherichia coli (ATCC 8939), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404), and *Microsporum gypseum*. Those were obtained from Microbiology laboratory collection of Bandung Institute of Technology in February 2012.

Instrumentation

Melting poin was determined by an electrothermal apparatus. UV spectra were measured by spectrophotometer UV-VIS Beckman DU6501, IR spectra were determined by spectrometer JASCO FT/IR using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded by Agilent operating at 500 MHz (1D-NMR: ^{1}H, ^{13}C and TOCSY NMR; 2D NMR: COSY, HMQC, and HMBC) in CDacetone solution with TMS as an integral reference ^{1}H. Mass spectra were measured by ES1-MS-TOF water LCT Primier XE mode negative.

Procedure

Preparation of Extract and Fractions

About 500 g of powder of the dried plant material was macerated with ethanol 35 l for 24 hours. Maceration process were repeated for 7 times. The ethanol extract was dried using rotary evaporator, it was obtained 75.49 g of extract. That extract was dissolved in hot water, then filtered through filter paper. The filtrate was fractionated by liquid-liquid extracted with n-hexane and ethyl acetate solvents. All fractions were dried using rotary evaporator.

Isolation

The n–hexane fraction was partioned by Gravity Column Chromatography (GCC) using n-hexane-etilasetat-methanol (gradient eluent). By this process, it has resulted 10 subfractions. Sub-fraction 6 was portioned by Radial Chromatography (RC) using n-hexane-chloroform-methanol (n-hexane : chloroform 3:7, 4:6 then chloroform : methanol 7:3; 5:5 until 0:10). By this process, it has resulted 7 sub-subfractions (NS1, NS2, NS3, NS4, NS5, NS6, NS7) and recrystalisation from sub-subfraction 2 (NS2) yielded compound (1). Gravity Column Chromatography was carried out using Merck silica gel 60, radial chromatography was used Merck silica gel 60 GF_{254}, and for Thin Layer

Chromatography analysis were used precoated silica gel plate (Merck Kiesel-gel 60GF 254 0.25 mm).

Antimicrobial Activity Test

Determination of the Minimal Inhibitory Concentration (MIC)

Antimicrobial activities test of isolat were using by micro dilution broth method based on National Committee for Clinical Laboratory Standard (2012). A series of dilutions of extract were prepared in Mueller Hinton broth (MHB) or Sabouraud dextrose broth (SDB) at final concentrations ranging from 1.95 to 1000 µg/mL. The inocula of microorganisms were prepared from 24 hours cultures and suspensions were adjusted to 0.05 x 0.5 McFarland standard suspensions. The tubes were dispensed into 100 µL with different concentrations of extract and 10 µL inoculum. The control tubes contained only MHB or SDB and inoculums suspension. The positive or reference controls were prepared using tetracycline HCl, and ketoconazole. The inoculated tubes of bacteria were incubated at 37^0C for 24 hours, yeasts at 28^0C for 48 hours and fungi at 28^0C for 120 hours. The MIC was calculated as no visible growth of tested microorganism appeared, which were expressed in µg/ml. The tests were conducted in triplicate. The least concentration of each extract showing a clear of inhibition was taken as the minimal inhibitory concentration (MIC).

Determination of the Minimal Bactericidal/Fungicidal Concentration (MBC/MFC)

The minimal bactericidal/fungicidal concentration of the isolate was done according to the method highlighted in National Committee for Clinical Laboratory Standard (2000). Briefly 5µL that was pipetted from the microbe mixture obtained in the determination of MIC stage was streaked out on the nutrient agar/ Sabouraud dextrose agar at 37^0C for 24 hours, yeasts at 28^0C for 48 hours and fungi at 28^0C for 120 hours. The least concentration of the extract with no visible growth was taken as the minimal bactericidal/fungicidal concentration.

Results and Discussion

Compound (1): an orange neddle crystall (10.4 mg), m.p. 154.6-155.5^{0}C, Rf 0.5 ($CHCl_3$-MeOH, 10:0,1). The UV spectra showed absorptions at 235, 266 and 379 nm, indicating the likely presence of substituted aromatic rings and an α-β unsaturated ketone in the molecule [9,10]. The UV absorption showed a batochromic shift (73 nm, 379 to 456 nm) on addition NaOH solution, indicating that the compound is chalcone with 4-OH. Accurate mass analysis showed the $[M^+H]^+$ ion, $C_{21}H_{22}O_5$ plus H, at m/z 353.1378 (expected/theoretical 353.1344). The ^{1}H NMR spectrum showed signals for an isoprene (3-methyl-1-butenyl) fragment at δ 1.08; 1.08 for two simetry methyl groups as singlet corresponding to six protons, δ 6.76 (1H, dd, J= 7 and 6 Hz) and δ 6.65 (1H, d, J=16.5 Hz) were assigned to the olefinic protons. The singlet at δ 3.39 corresponds to a methoxyl group. A pair of doublets at δ 7.7 8 and 7.84 (1H, d, J=15.5 Hz) for the chalcone double bond, and a singlet for chelated hydroxyl at δ 14.5 for 2'OH chalcone system. The phenyl ring attached C-β with para-hydroxyl group A methoxyl group at C-3, as shown by the correlation between the methoxy protons and C-3. The substituent at the carbonyl group is a tetra-substituted benzene ring with two ortho-positioned hydrogen coupling with each other, ortho-coupled doublets are shown at δ 6.9 (1H, d, J=8.5 Hz) and δ 7.32 (1H, d, J=8 Hz) due to the 5' and 6' protons in B-ring. TOCSY-NMR spectrum showed signals for chalcone. 1H-NMR signals were splitting into three parts because of 2 benzene in chalcone. Literature survey indicates that compound (1) is a new compound and separated from *Elatostema parasiticum* for the first time.

Antimicrobial activity test

The results of the antimicrobial test of compound (1) are shown in table 2 and 3. Table 2 shows the MIC values of compound (1) of all tested microbes, table 2 shows the MBC/MFC values of them. Compound (1) only inhibited the growth of *S. aureus* and *B. subtilis* with the MIC values of 7.8 µg/ml and 1.95 µg/ml, respectively.

Conclusion

A new prenylated chalcone isolated from the ethanolic extract of *Elatostema parasiticum* and defined compound (1) was 4,4',6' trihydroxy 3 methoxy 3' pentene chalcone. This compound showed an antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* with the MIC values of 7.8 µg/ml and 1.95 µg/ml, respectively.

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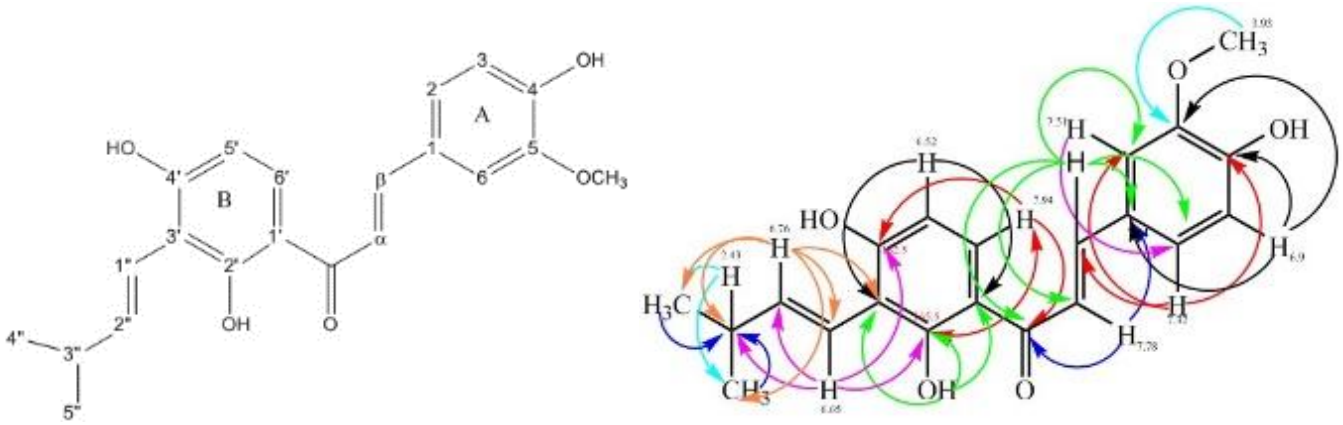

Figure 1. Structure of 4,4',6' trihydroxy 3 methoxy 3' pentene chalcone (compund 1)

Table 1. 1H and 13C chemical shifts of compound (1)

Position	δ ^{1}H (ppm)	δ ^{13}C	HMBC ^{13}C
1	-	128	-
2	7.51 (1H, d, J=2 Hz)	112.1	6
3	-	148.8	-
4	-	150.6	-
5	6.9 (1H, d, J=8.5 Hz)	116.2	1,3,4
6	7.32 (1H, k, J=8 Hz)	124.9	2,4, β
A	7.78 (1H, d, J=15.5 Hz)	118.5	1,C=O
B	7.84 (1H, d, J= 15.5 Hz)	145.6	1,2,6, α, C=O
C=O	-	193.2	-
1^{1}	-	114.2	-
2^{1}-OH	14.5	165.5	-
3^{1}	-	113.2	-
4^{1}-OH	-	162.5	-
5^{1}	6.52 (1H, d, J = 9 Hz)	108.2	1^{1},3^{1}
6^{1}	7.94 (1H, d, J = 9 Hz)	130.5	2^{1},4^{1},C=O
1^{11}	6.65 (1H, d, J=16.5 Hz)	117.6	2^{11},3^{11},4^{1},2^{1}
2^{11}	6.76	142.6	1^{11},3^{11},4^{11},5^{11}
3^{11}	2.43	33.9	4^{11},5^{11}
4^{11}	1.08	23.1	3^{11}
5^{11}	1.08	23.1	3^{11}
OCH_3	3.93	56.4	3

Table 2. Minimal inhibitory concentration of compound 1

No	Sample	Minimal inhibitory concentration (µg/mL) against						
		Sa	Bs	Ec	Pa	Ca	An	Mg
1	Compound 1	7.81	1.95	-	-	-	-	-
2	Tetracycline HCl	0.39	0.09	1.56	12.5	12.50	0.78	1.56
3	Ketokonazole							

Sa: *Staphylococcus aureus*; Bs: *Bacillus subtilis*; Ec: *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Ca: *Candida albicans*; An: *Aspergillus niger*; Mg: *Microsporum gypseum*

Table 3. Minimal bactericidal/fungicidal concentration of compound 1

No	Sample	Minimal bactericidal/fungicidal concentration (ppm) against						
		Sa	Bs	Ec	Pa	Ca	An	Mg
1	Compound 1	31.25	3.91	-	-	-	-	-
2	Tetracycline HCl	0.39	0.78	1.56	12.50	12.50	0.78	1.56
3	Ketoconazole							