NOTE:

Isolation and Evaluation of Antioxidant and Antibacterial Activity of Flavonoid from *Ficus variegata* Blume

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Abstract: Ficus variegata *Blume is a specific plant of East Kalimantan. Flavonoid compound of* F. variegata *Blume was isolated by vacuum liquid and column chromatography, with previously extracted by maceration method using n-hexane and methanol, and fractionation using ethyl acetate solvent. The eluent used in isolation were n-hexane:ethyl acetate (8:2). Based on the results of elucidation structure using spectroscopy methods (GC-MS, NMR, and FTIR), 5-hydroxy-2-(4-methoxy-phenyl)-8,8-dimethyl-8H-pyrano[2,3-f] chromen-4-one was obtained. This compound has antibacterial and antioxidant activity.*

Keywords: Ficus variegata *Blume; flavonoid; antibacterial activities; antioxidant activity*

■ INTRODUCTION

Ficus is a genus that has unique characteristics, and it grows mainly in tropical rainforest [1-3]. Secondary metabolite of the genus Ficus is rich in polyphenolic compounds, and flavonoids, therefore, the genus Ficus is usually used as traditional medicine for treatment of various illnesses [4]. One species in the genus Ficus is *Ficus variegate* Blume. The *F. variegata* Blume plant has the origin name in Indonesia area i.e. Kundang, Godang (Java, Bali); Kondang (Sunda); Ara, Arah, Aro, Barai Silai Unding, Haru Kucing (Sumatera); Akau, Adei Yeva, Gondal, Sesem, Kabato (Maluku); Ganang, Kanjilu (Sumba), Nyawai, Kara, Tentabau, Ayak, Tandilan, Kendang, (Kalimantan).

F. variegata Blume plant is a fast growing important species and belongs to the pioneer group. This wild plant is easily found naturally in natural forests of former fires in East and South Kalimantan [5]. The high of *F. variegata* Blume can reach up to 25 m and begin to bear fruit after the age of 3 years. The fruit of this plant is clustered in on stem bark and branches of tree. This plant does not know the season for fruiting and the abundance of fruit is very much every time it produces fruit. Due that, this plant is a potential plant to be used as medicinal plant.

F. variegata Blume is usually used as traditional medicine for treatment of various illnesses, i.e., dysentery and ulceration. Our research group has been reported the bioactivity of a secondary metabolite from leaves, stem bark, and fruit of *F. variegata* Blume, i.e. antibacterial and antioxidant activity [6-8]. However, until now is not yet reported the isolation of flavonoids compound from fruit of *F. variegata* Blume.

■ EXPERIMENTAL SECTION

Materials

Fruits of *F. variegata* Blume on this research were supplied from Gunung Pinang and Gunung Kelua, Samarinda subdistrict, East Borneo, Indonesia. The plant was identified by a botanist in Department of Foresty, Mulawarman University.

The chemicals used were n-hexane p.a. (Merck), methanol p.a. (Merck), ethyl acetate p.a. (Merck), TLC Silica gel 60 (Merck), nutrient agar (Merck), 1,1-

diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich), and distilled water.

The bacteria used were *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923.

Instrumentation

The instrumentation used were NMR JEOL JNMECA 500 MHz, GC-MS QP2010S Shimadzu, and FTIR Spectrophotometer 8201PC Shimadzu.

Procedure

Fruit of *F. variegata* Blume washed to remove impurities with flow of water. Fruit was dried at 80 °C for 48 h. The dried samples were grounded using an electric blender, and it was stored in airtight containers.

Two hundred grams of powder *F. variegata* Blume was extracted using maceration method in 1 L *n*-hexane to separate the latex. Then was re-extracted with methanol (1:5) using maceration method [6]. Methanol extract was then evaporated to separated methanol solvent using a rotary evaporator and water bath to obtain a crude extract.

The crude extract was dissolved in chloroform: methanol (1:1) and added to the silica gel then stirred to form a slurry. A slurry was then put in a chromatography column. Then eluted with *n*-hexane and followed by ethyl acetate solvent until the ethyl acetate fraction becomes clear to obtain a fraction of ethyl acetate dried extract.

The ethyl acetate dried extract was eluted by gravity column chromatography using *n*-hexane:ethyl acetate eluent 8:2. Silica gel 60 as a stationary phase is suspended with *n*-hexane. The stationary phase was inserted gradually. The stationary phase with eluent was left to soak in the column for overnight. Elution rate was set using the faucet until the eluate that came out not too fast and not too slow. The eluate was accommodated in a 5 mL vial bottle. The entire eluate was then dried with aerated.

The chemical structure of isolate was determined using NMR, GC-MS, and FTIR.

Screening antibacterial activity

Antibacterial activity test was used by TLC bioautographic. The method is a method based on the contact bioautographic diffusion process of the compound after being separated by TLC method in medium inoculated test bacteria. The eluent used was *n*-hexane:ethyl acetate 9:1. The test was done by using Nutrient agar (NA) against negative Gram bacteria (*E. coli*) and positive Gram bacteria (*S. aureus*). The bacteria used was in the form of bacterial suspension ratio (1:40) with the solvent NaCl. The fraction was dissolved in chloroform: methanol (1:1) to tested and spotted on a TLC plate and eluted using the eluent best, the plates were then dried. The bacterial suspension was inoculated into a petri dish and added to the culture medium and then homogenized. TLC plate was then placed on the surface of the medium for 30 min as a process of diffusion of compounds on TLC plates to the surface of the NA medium and then removed. Medium subsequently incubated in an incubator for bacteria suitable incubation period is 1×24 h at 37 °C later observation. Positive results were characterized by the formation of a clear zone at the former TLC plate attachment.

Screening antioxidant activity

The antioxidant activity was tested using DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The method used is a qualitative method that simply by spraying DPPH 80 ppm on a TLC plate which was eluted fractions isolates [6]. Positive results were characterized by their bright color changes to yellow after the spraying process DPPH on stains found on the TLC plate.

The concentration of DPPH 80 ppm was prepared by dissolving 0.02 g of DPPH in 25 mL of methanol. The eluent used to elute the TLC plate is *n*-hexane:ethyl acetate. Selection of comparison eluent used is the same as the eluent used when testing antibacterial eluent. The eluent is able to raise the stain so as to facilitate the observation and testing. The comparison used is *n*-hexane:ethyl acetate (8:2).

■ RESULTS AND DISCUSSION

Structural Elucidation

GC analysis is done to ensure that isolated is only one isolate compounds with one peak retention time of 31.5 to 32.5 min. The ^{1}H-NMR and ^{13}C-NMR spectra are show in Fig. 1 and 2 and tabulated in Table 1.

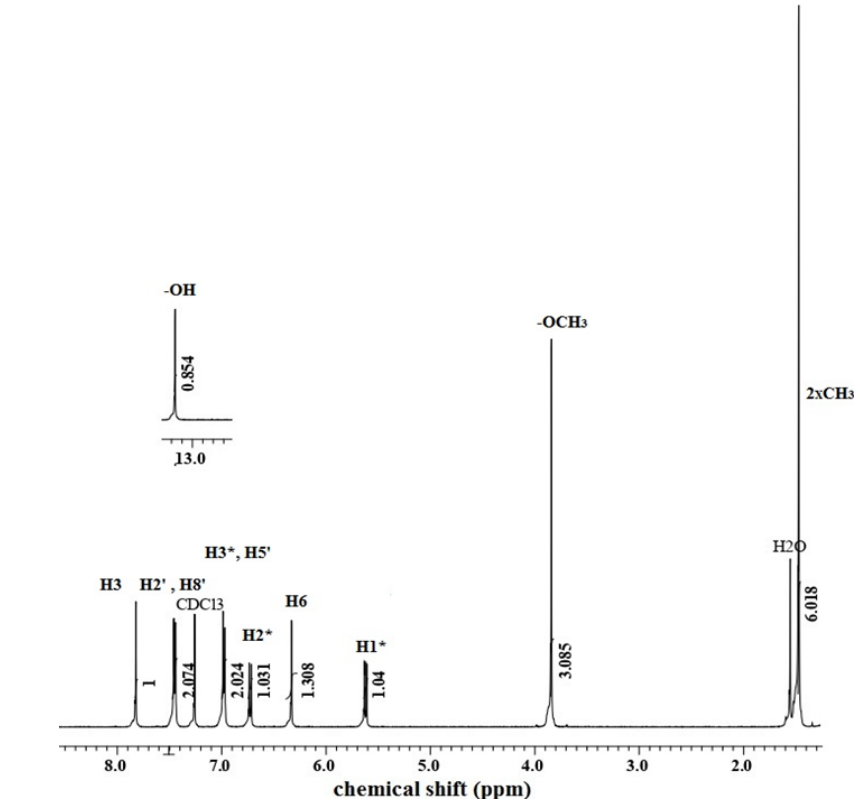

Fig 1. [1]H-NMR spectra of isolate of 5-hidroxy-2-(4-methoxy-phenyl)-8,8-dimethyl-8H-pyrano[2,3-f] chromen-4-one

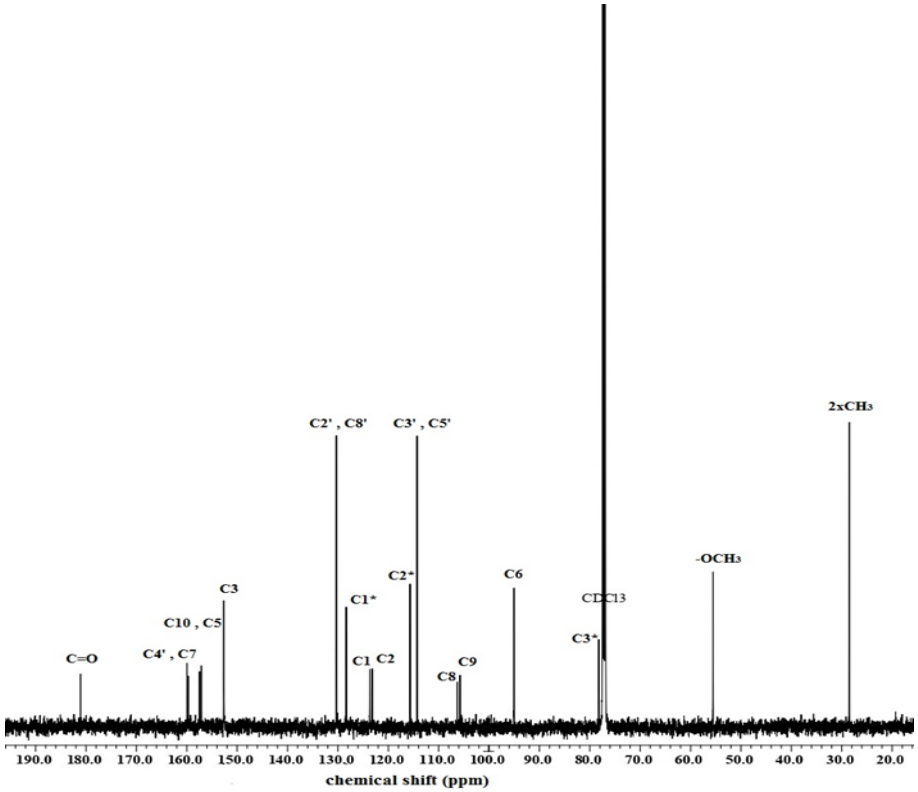

Fig 2. [13]C-NMR spectra of isolate of 5-hidroxy-2-(4-methoxy-phenyl)-8,8-dimethyl-8H-pyrano[2,3-f] chromen-4-one

Table 1. The summary result of NMR signal of 5-hidroxy-2-(4-methoxy-phenyl)-8,8-dimethyl-8H-pyrano[2,3-f] chromen-4-one

No Atom C	^{13}C-NMR (ppm)	^{1}H-NMR (ppm)	COSY	HMQC	HMBC
C2	123.19	-	-	-	-
C3	152.65	7.82 (1H, s)	-	C3	C4, C10, C1’
C4 (C=O)	181.09	-	-	-	-
C5	157.11	-	-	-	-
C6	95.04	6.33 (1H, s)	-	C6	C7, C9, C10
C7	159.68	-	-	-	-
C8	106.30	-	-	-	-
C9	105.75	-	-	-	-
C10	157.48	-	-	-	-
C1’	123.68	-	-	-	-
C2’/C6’	130.29	7.45 (2H, d, J = 8.45 Hz)	H3’/H5’	C2’/C6’	C1’, C4’, C6’
C3’/C5’	114.28	6.98 (2H, d, J = 8.45 Hz)	H2’/H6’	C3’/C6’	C1’, C4’, C2
C4’	159.95	-	-	-	-
C1”	128.34	5.62 (1H, d, J = 10.4 Hz)	H2”	C1”	C9, C3”
C2”	115.67	6.73 (1H, d, J = 9.7 Hz)	H1”	C2”	C7, C3”
C3”	78.22	-	-	-	-
-OCH_3	55.54	3.80 (3H, s)	-	OCH_3	C4’
-CH_3	28.47	1.47 (6H, s)	-	CH_3	C1”, C3”, -CH_3
OH	-	13.16 (1H, s)	-	-	C5, C9, C10
$CDCl_3$	77.20	7.25	-	$CDCl_3$	-

The data of FTIR spectra of isolate are shown functional group of aromatic (717.52 cm^{-1}), C-benzene (786.96 and 833.25 cm^{-1}), C-O ether (1180.44 and 1242.16 cm^{-1}), CH_3 (1458.18 cm^{-1}), C=O ketones (1743.65 cm^{-1}), C-H aliphatic (2839.22 and 2924.09 cm^{-1}), and OH (3487.30 cm^{-1}) [9]. FTIR spectra analysis results show that the isolate obtained is a class of flavonoid compounds, because of functional group of C=O ketones, C-O ether, and C-alcohol.

^{1}H-NMR spectrum (Fig. 1) showed 18 protons. Signals at δ 13.16 (1H, s) indicated the presence of hydroxy groups. The methoxy group was represented by a singlet signal at δ 3.80 (3H, s). Then, a signal at δ 1.47 (6H, s) indicated the presence of two equivalent methyl groups. The ^{1}H-NMR spectrum showed two proton doublets at δ 6.98 (2H, d, *J* = 8.45 Hz, H-3'/H-5') and 7.45 (2H, d, *J* = 8.45 Hz, H-2'/H-6'), as well as showed a substituted benzene B ring in the para position. Subsequently, an alkene proton signal appeared which coupling each other in the form of cis. The singlet proton signals at δ 6.33 (1H, s, H-6) and δ 7.82 (1H, s, H-3) were identified as a single proton on the benzene and alkene rings.

The ^{13}C-NMR spectrum (Fig. 2) showed 18 signals for 21 carbon consisting of 4 sp^3 and 17 sp^2 configurations. A signal at δ 181.09 denoted the presence of a carbonyl group, while the methyl group is identified at δ 28.47. Signals at δ 55.54 was a characteristic signal of methoxy groups. On DEPT the 78.22 signal proved the quaternary carbon (C-3") of the pyrano structure.

The ^{1}H-NMR and ^{13}C-NMR spectra can be described in detail with the help of the 2D-NMR (^{1}H-^{1}H COSY, HMQC, and HMBC) spectrum. All of NMR signal was summarize at Table 1. The ^{1}H-^{1}H COSY spectrum indicated a correlation between proton alkene pyrano (H-1" and H-2") in cis form. The HMQC spectrum indicated that the methyl protons at δ 1.47 correlate with the same carbon at δ 28.47, while the proton of the methoxy group was correlated with carbon at δ 55.54. The HMBC spectrum showed the long-range correlation between H-3 to C-10, C-carbonyl (C-4) and H-6 to C-7, C-10.

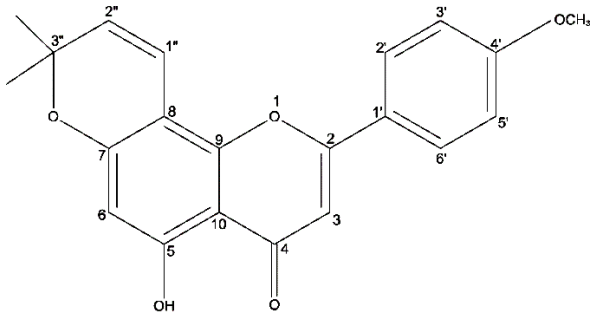

Fig 3. Structure of 5-hidroxy-2-(4-methoxy-phenyl)-8,8-dimethyl-8H-pyrano[2,3-f] chromen-4-one

Furthermore, long-range correlations were also shown between H-1" to C-9, C-3" and H-2" to C-7, C-3". The methoxy group on ring B bound to C-4 was identified by the correlation between proton methoxy with C-4'. The presence of OH groups in ring A bound to C-5 was indicated by the long-range correlation of proton OH with C-5, C-9, C-10. The proton H-6 is shown by the presence of long-range correlation with C-7, C-9, C-10. The presence of a correlation between the proton of the methyl groups in the pyrano ring was indicated by the long-range correlation with C-1" and C-3". While the presence of correlations between H-3' to C-2 and H-3 to C-1' revealed ring B bound in the form of flavonoid structure. Based on these values, it can be deduced that the compound is 5-hydroxy-2-(4-methoxyphenyl)-8,8-dimethyl-8H-pyrano[2,3-f] chromene-4-one. The chemical structure of this compound is shown in Fig. 3.

Antibacterial Activity

Isolates F1 has an antibacterial activity against *E. coli* and *S. aureus*. The clear zone was formed as a result of antibacterial compounds in isolates fraction that diffuses into the surface of the medium and inhibits the growth of bacteria A clear zone indicated the absence of bacterial growth.

Compound 5-hydroxy-2-(4-methoxy-phenyl)-8,8-dimethyl-8H-pyrano[2,3-f] chromen-4-one was a flavonoid that have an antibacterial activity. This flavonoid could act as an antibacterial which inhibits the growth of bacteria by destroying the bacterial cell wall permeability so that the bacterial cells will be stunted [10].

Antioxidant Activity

The test results showed that ethyl acetate fraction isolates of *F. variegata Blume* fruit have antioxidant activity. The positive results of antioxidant activity were caused by the ability of antioxidant compound that can react with DPPH and caused the DPPH discoloration. In methanol solution, the color of DPPH is purple and will turn into a yellow solution after reaction with an antioxidant.

DPPH is free radical that is unstable because there is an unpaired electron. Therefore, DPPH discoloration indicates the number of electrons captured by DPPH radical that can be interpreted as the antioxidant activity of a sample. DPPH discoloration indicates the binding of an electron atom N (nitrogen) in DPPH stable form [11]. Based on the result above, isolate flavonoid, 5-hydroxy-2-(4-methoxy-phenyl)-8.8-dimethyl-8H-pyrano [2,3-f] chromen-4-one have the antioxidant ability to transfer an electron to the free radical compounds [12].

■ CONCLUSION

Flavonoid compound of 5-hydroxy-2-(4-methoxy-phenyl)-8,8-dimethyl-8H-pyrano[2,3-f] chromen-4-one was successfully isolated from *F. variegata Blume*, and have the antibacterial activity against bacteria *E. coli* and *S. aureus* and have the antioxidant activity as well.

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