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HYDROCHALCONE COMPOUNDS FROM INDONESIAN MEDICINAL PLANT, "SIRIH HUTAN", *Piper aduncum* (*Piperaceae*)

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ABSTRACT

Isolation, purification and identification of two hydrochalcone compounds from Indonesian medicinal plant "Sirihhutan", *Piper aduncum* (*Piperaceae*) had been done. Isolation and purification of ethylacetate extract subjected to column chromatography (SiO_2; (i).*n*-hexane-ethylacetate = 10:1 ~ 1 : 1; ethylacetate (ii).*n*-hexane-ethylacetate = 8: 1 gave two pure compounds. Based on infra red, 1D & 2D-NMR, mass spectral data and comparison chemical shift of protons and carbons, the isolated compounds are 2',6'-dihydroxy-4'-methoxy dihydrochalcone and 2',6',4-trihydroxy-4'-methoxy dihydrochalcone with free radical scavenging inhibitionvalues are 21.77 % and 90.1 % respectively.

Keywords: Hydrochalcone; *Piper aduncum*; *Piperaceae*; 2',6'-dihydroxy-4'-methoxy dihydrochalcone; 2',6',4-trihydroxy-4'-methoxy dihydrochalcone

INTRODUCTION

This research is a continuation of chemical studies on Indonesian medicinal plants, especially on bioactive chemical compounds as antioxidant.[1-5] This research focused on hydrochalcone compounds from"SirihHutan" (localname), *Piper aduncum* L.(*Piperaceae*). Traditionally, this plant isused as a deodorant, antihaemorraghea, antivomit, anti-septic, antibacterial and antifungi[6]. Based on the literature, this plant has activities as antibacteria[6], insectiside[7], andantioxidant[8].

Isolation results of antioxidant bioactive compounds from ethylacetate fraction of *P.aduncum* L based on free radicals cavenging activity test usingDPPH (1,1-diphenylpycrilhidrazine) gave two hydrochalcone compounds, i.e.2',6'-dihydroxy-4'-methoxy dihydrochalcone and 2',6',4-trihydroxy-4'-methoxy dihydrochalcone.

Fig.-1: Sirihhutan, *Piper aduncum*(Piperaceae)

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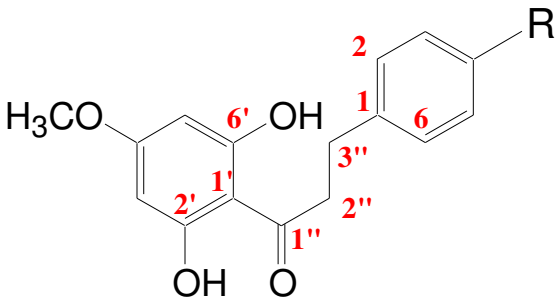

R = H, 2',6'-dihydroxy-4'-methoxy dihydrochalcone (**1**)
R = OH, 2',6',4-trihydroxy-4'-methoxy dihydrochalcone (Asebogenin) (**2**)

Fig.-2: Chemical Structures of the Isolated Compound from "Sirihhutan" *Piper aduncum*

EXPERIMENTAL

Materials

The leaves of *Piper aduncum* were collected from Samarinda forestry, East Kalimantan, Indonesia. The spectra of IR were taken with Hitachi 260-30 and JASCO FT-IR-5300 spectrophotometers. The ^{1}H- and ^{13}C-NMR spectra were measured with Jeol GX-500 spectrometer (500 MHz), whilemass spectra (MS) were measured with LCMS (Q Micro QQA 842 1.0).Column chromatography was carried out by using silica gel 60 (70-230 mesh ASTM, Merck) as absorbent. Thin layer chromatography (TLC) was conducted on precoated Kieselgel 60 $GF_{254/366}$ plate (0.2 mm, Merck). Spot on the TLC plates were detected byreagent spray 1% $Ce(SO_4)_2$/10% H_2SO_4 followed with heating at 110 ^{o}C.

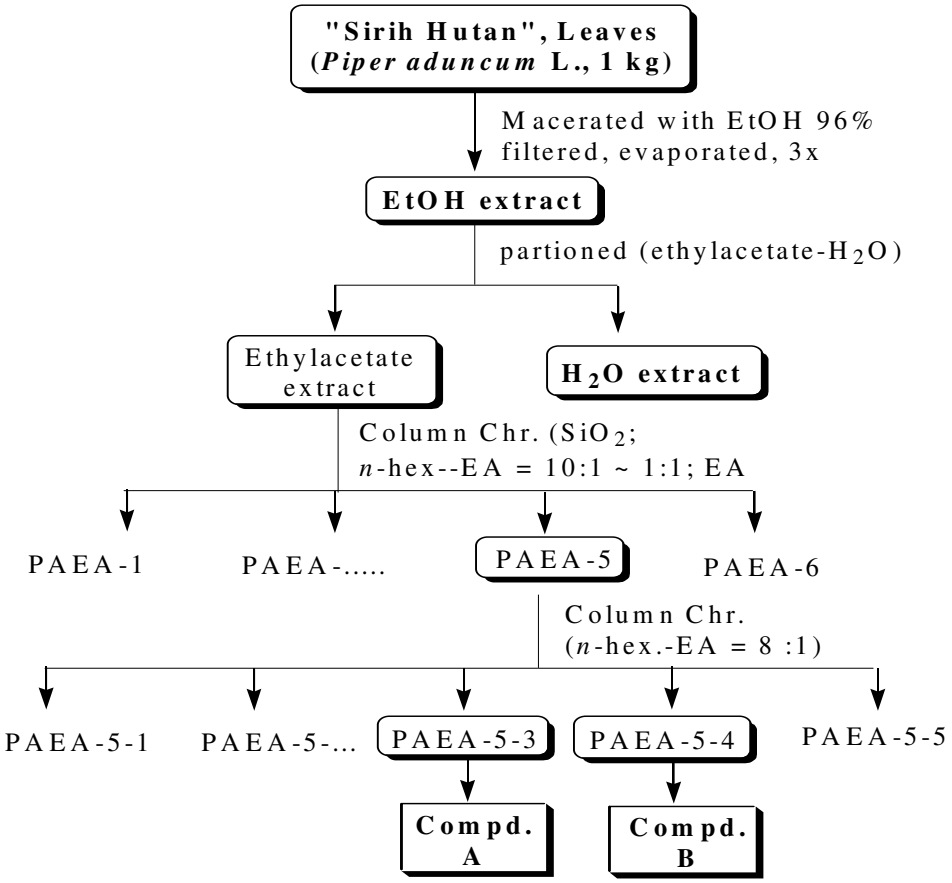

Fig.-3: Isolation Procedure for Hydrochalcone Compounds

Extraction, Isolation and Purification

The air-dried leaves (1 kg) of *P.aduncum* collected from Samarinda Forestry, East Kalimantan, Indonesia were extracted with ethanol 96% by maceration. The solvents were evaporated under reduced pressure to give ethanol extract (78 g; 7.8%). The ethanol extracts were partitionedwith ethylacetate-water = 1 :1 and evaporated to give water extract (45.1g; 4.51%) and ethylacetate extract (30.8 g; 3.07%). The ethylacetate extracts(10 g) were then subjected to column chromatography [SiO_2;n-hexane-ethylacetate = 10 : 1 ~ 1 : 1,ethylacetate and ethanol successively] to provide fractions. 1 (PAEA-1) ~ 6 (PAEA-6).

Fraction PAEA-5 wasseparated by column chromatography [SiO_2,*n*-hexane-ethylacetate =5 : 1] togive compound A (20,2 mg), B (35,1 mg) and other fractions.

RESULTS AND DISCUSSION

Compound A (**1**) was obtained in white powder form and has a molecular ion peak at *m/z*272 $(M)^+$init's mass spectrum by LC-MS.Infra Red (IR)spectrum of compound A showed absorbtion band at λ1650 cm^{-1} (carbonyl group) and 3265 cm^{-1} (hydroxyl group). The ^{1}H-NMR spectrum of compound **1** showed signals due to one methoxy group at δH 3.78 (s); two methylene groups at δH 3.02(t, J=8.5; 7.0 Hz); δH3.79 (d, J=8.5; 7.0 Hz). Furthermore, the chemical shift of proton on a benzene ring was observed at δH 5.93m(s); δH7.19 (m); δH7.26 (m).Distortionless enhancement by polarization transfer (DEPT) experiment oncompound**1** by ^{13}C-NMR spectroscopy disclosed the presence of one methoxy carbon, two methylene carbons, seven methane carbons, six quaternary carbons and a carbonyl carbon (Table-1). The ^{13}C-^{1}H COSY experiment revealed connectivity between respective protons and carbons. The plane structures of compound **1** were constructed byH-H COSY experiment of **1** estimated the presence of correlation between H-2, H-3, H-4, H-5 to H-6 and between H-2" to H-3". In the HMBC experiment, compound**1** was shown to have correlations between H-2" to C-1", C-3",C-1; H-3" to C-2", C-1", C-1, C-2, C-3; H-5' to C-6', C-1', C-4'; H-3' to C-2', C-1' (Fig.-4).

Basedon IR, NMR and mass spectral data and supported by comparison data onthe chemical shift of protonsand carbons by Masuoka *et al*., (1997),compound A (**1**) wasdetermined as 2',6'-dihydroxy-4'-methoxy dihydrochalcone.

Compound B(**2**) was an amorph powder form. It gave molecular ion peak at *m/z* 288 with molecule formula $C_{22}H_{32}O_5$ indicate the addition of hydroxyl groups to compound **1**. The IR spectrum of **2** showed absorption bands due to the hydroxyl group (3320 cm^{-1}), 1672 cm^{-1} and 1561 cm^{-1} due to C=O and C=C stretching. The proton and carbon NMR spectra showed no significant chemical shift difference between compound **1** and compound **2**, unless there is a chemical shift in the C-4 of δC 126,10 (d) on compound **1** to be δC 157.8 (s) on compound **2**.

Table-1: Chemical Shift (δ_H)of Compound **1** and **2** compared to2',6'-dihydroxy-4'-methoxy dihydrochalcone[8]

No	Isolated Compound (**1**) J in Hz	2',6'-dihydroxy-4'-methoxy dihydro chalcone[8]	Isolated Compound(**2**)	2',6',4-trihydroxy-4'-methoxy dihydrochalcone[8]
1	-	-	-	-
2	7.27 (m)	7.23 (m)	6.71 (d, J=2.0)	6.40 (d,J=2.0)
3	7.27 (m)	7.23 (m)	7.11 (d, J=2.0)	7.04 (d, J=2.0)
4	7.18 (t, J=2.21)	7.16 (t-like)	-	-
5	7.26 (m)	7.23 (m)	7.21 (d, J=2.0; 6.4)	7.04
6	7.19 (m)	7.23 (m)	6.00 (J=2.0)	6.40
1'	-	-	-	-
2'	-	-	-	-
3'	6.00 (s)	5.92 (J=13.2)	5.89 (s)	5.92 (s)
4'	-	-	-	-
5'	5.93 (s)	5.92 (J=13.2)	5.71 (s)	5.92 (s)
6'	-	-	-	-
1"	-	-	-	-
2"	3.39 (t, J=7.75; 7.80)	3.33 (m)	3.29 (m)	3.27 (m)

3”	3.02 (t,J=8.45; 7.1)	2.94 (m)	2.89 (m)	2.85 (m)
4’-OMe	3.78 (s)	3.75 (s)	3.79 (s)	3.75 (s)

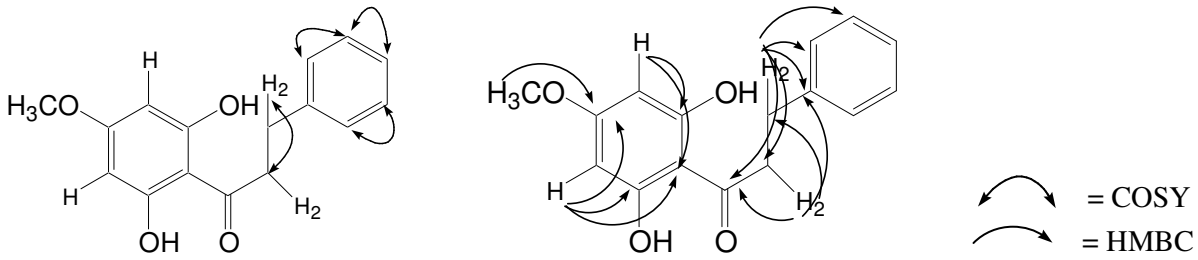

Fig.-4: H-H COSY Analysis (left) and HMBC Analysis (right) for Compound **1**

Tabel-2: Chemical Shift δC ^{13}C-NMR for Compound **1** and **2**

CarbonNumber	Isolated Compound (**1**)	Isolated Compound(**2**)	2',6',4-trihydroxy-4'-methoxy dihydrochalcone
1	141.79 (s)	134.7 (s)	135.1 (s)
2	128.56 (d)	131.1 (d)	132.2 (d)
3	129.40 (d)	116.4 (d)	116.6 (d)
4	126.10 (d)	157.2 (s)	157.8 (s)
5	129.40 (d)	116.4 (d)	116.6 (d)
6	128.56 (d)	131.1 (d)	132.2 (d)
1’	104.95 (s)	106.8 (s)	108.1 (s)
2’	165.71 (s)	166.3 (s)	167.8 (s)
3’	94.48 (d)	95.1 (d)	95.9 (d)
4’	165.71 (s)	168.2 (s)	169.1 (s)
5’	94.48 (d)	95.1 (d)	95.7 (d)
6’	165.71 (s)	166.3 (s)	168.2 (s)
1”	204.74 (s)	207.5 (s)	207.8 (s)
2”	45.76 (t)	48.2 (t)	48.4 (t)
3”	30.69 (t)	32.1 (t)	33.1 (t)
4’-OMe	55.65 (q)	56.6 (q)	56.8 (q)

Free Radical Scavenging Activity

The result of antioxidant activity using free radical scavenging activity method for all extracts(ethanol, ethylacetate and water), some fractions and pure compounds (**1,2**) obtained from column chromatography analysis can be seen in Table-3. Compound **1** (2',6'-dihydroxy-4'-methoxy dihydrochalcone) showed no antioxidant activity, while Compound **2** (2',6',4-trihydroxy-4'-methoxy dihydrochalcone) showed activity at 90.1 %.[8]

Table-3: Free Radical Scavenging Activities of all Extracts and Fractions

No.	Sample Name	Inhibition (%)	Level [14]*)
1	Ethanol extract	88.65	Active
2	Fr. Ethylacetate	89.42	Active
3	Fr. Water	96.73	Active
4	Fr. PAEA-1	7.24	No
5	Fr. PAEA-2	21.33	No
6	Fr. PAEA-3	34.0	No
7	Fr. PAEA-4	83.3	Active
8	Fr. PAEA-5	84.31	Active

9	Fr. PAEA-6	72.03	Active
10	Fr. PAEA-5-1	34.12	No
11	Fr. PAEA-5-2	27.35	No
12	Fr. PAEA-5-3 (Compound**1**)	21.77	No
13	Fr. PAEA-5-4 (Compound**2**)	90.1	Active
14	Fr. PAEA-5-5	17.56	No

*) :0 - 50 %= no active;
>50 % = active

CONCLUSION

Two hydrochalcone compounds from Indonesian medicinal plant "Sirih Hutan", *Piper aduncum*have been isolated and identified as 2',6'-dihydroxy-4'-methoxy dihydrochalcone and 2',6',4-trihydroxy-4'-methoxydihydrochalcone. One of them is 2',6',4-trihydroxy-4'-methoxy dihydrochalcone has free radical scavenging inhibition of 90.1 %,potentially as an antioxidant compound.

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