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Flavonoid Compounds From The Leaves Of *Kalanchoe Tomentosa* And Their Cytotoxic Activity Against P-388 Murine Leukemia Cell

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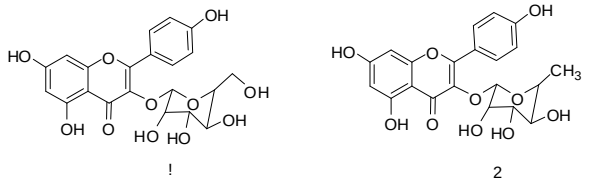
Abstract *Kalanchoe* plant, known as "sosor bebek" in Indonesia is a perennial herb and has succulent leaves. The plant is known in folklore and traditional medicine in Indonesia for the treatment of fever, abscesses, bruises, contused wounds, coughs and skin diseases. During the course of our continuing search for novel cytotoxic compounds, the methanolic extract of *Kalanchoe tomentosa* plants showed cytotoxic activity against P-388 murine leukemia cells. The methanolic extract of the fresh leaves of *K. tomentosa* was concentrated and extracted successively with *n*-hexane, ethyl acetate and *n*-butanol. The ethyl acetate extract exhibited strongest cytotoxic activity againts P-388 murine leukemia cells. By using the cytotoxic activity to follow the separation, the ethyl acetate fraction was separated by combination of column chromatography on silica gel and preparative TLC on silica gel GF_{254} to afford a kaempferol-3-*O*-glycosides (**1**) and kaempferol-3-*O*-rhamnoside (**2**). Compound **1** and **2** showed cytotoxic activity against P-388 murine leukemia cells with IC_{50} > 100µg/mL and IC_{50} 3.32 µg / mL.

Keywords: P-388 murine leukemia cells, kaempferol-3-*O*-glycosides, Kaempferol-3-*O*-rhamnoside, *Kalanchoe tomentosa*



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1. INTRODUCTION

Kalanchoe tomentosa belongs to the family Crassulaceae is a beatiful, perennial and succulent plant. Genus *Kalanchoe* consists of about 130 species of annual and perennial shrubs. *The* plant ussually occur in semi desert of Saudia Arabia, Yemen, CentarlAfrica, Madagascar, tropical areas of Asia, Australia, and tropical America. Ussually, it is cultivated as garden ornamental and sand gardens with a medium humidity[1)].

Kalanchoe plants are used as traditional medicines to cure headache, cough, chest pain, ulcer, and other skin deseases. They overcome fever, fix the irregular menstruation, heal wound and boil, not only in Indonesia but also almost everywhere in the world[2)]. Some researches reported that *Kalanchoe* plants contain bufadienolide[3,4)], triterpenoid[5)], and flavonoid[6,7)], and biological activities like antileismanial, antiinflamatory, cytotoksic, and inhibiting tumor cell growth4[)]. One of unknown *Kalanchoe* plants ethnopharmacologically is *Kalanchoe tomentosa*, especially its anticancer activity. Cancer is a disease that is becoming one of the major threats to health as it is the second cause of death after heart disease. In Indonesia reported increased cancer deaths each year ranging 1.4% in 1972 to 4.4% in 1992[8)]. In coping with cancer, various efforts had been whom did seek anticancer compounds from plants.

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In this research, the anticancer activity of *K. tomentosa* leave extract phenolic compound was tested, followed by isolation, structure determination.

2. EXPERIMENTAL

2.1 Materials

Plant

The leaves of *K. tomentosa* were collected from Lembang, West Bandung area, West Java, Indonesia, and identified at Herbarium Bogoriense, Biology Research Center of LIPI *Lembaga Ilmu Pengetahuan Indonesia* or LIPI (The Indonesian Institute of Sciences), Cibinong, Bogor, West Java, Indonesia.

Chemical

The chemicals needed were both the various technical solvents (redestiled), like *n*-heksane, methanol, aceton, and pro-analysis ones, like dichloromethane and chloroform. Silica GF_{254} was used in Thin Layer Chromatography (TLC), Silica-G60 (10-40μm) with the surface area (500 m^2/g) was used in vacuum liquid chromatography, and silica G60 (70-230 and 230-400 mesh) were used in the open column chromatography, and the solution of $AlCl_3$ (10% in ethanol) as stain-display reagent.

Equipments

The equipments used were glass wares common in organic chemistry laboratory, macerator, rotary evaporator R-200 Buchi with vacuum pump Vac V-500 Buchi and water heater B-490 Buchi, open chromatography column with various sizes, UV lamp Vilbert Luomart (λ 254 nm dan λ 365 nm), spectrophotometer FTIR Spectrum One Perkin Elmer, Spectrometer *Nuclear Magnetic Resonance* (NMR) JEOL JNM ECA-500 with TMS as internal standard.

2.2 Methods

Bioassay using murine leukemia cells P-388[9)]

P-388 cancer cells cultured in RPMI-1640 medium were given calf serum 5% and kanamycin (100 μg/mL). Cells (3 × 3 cells / well) were seeded into 96 well plates composed of 100 L growth medium per well and incubated in a humidifier 37^0C in 5% CO_2. Several variations of the compounds (10 μL) was added into the culture at the PER all after transplantation. On the third day of 20 μL solution of MTT (5 mg / mL) per well was added to each culture medium. After 4 hours of incubation, a solution of 100 mL of 10% SDS was added each HCl 0.01 N wells and formazan crystals in each well were dissolved by stirring with a pipette. After the solution was measured using an optical densiti mikroplat reader (MPR-A4i Tohso) or ELISA reader at a wavelength of 550 and 700 nm. The experiments were conducted by measuring triplo

Extraction and Isolation

The 18,3 kg of fresh K. tomentosa leave was grinded, extracted, and then concentrated. The 390.75 g of methanol extract obtained was dissolved in water and partitioned respectively using n-hexane and ethyl acetate, yielding in n-hexane extract (20 g) and ethyl acetate extract (10 g). The ethyl acetate extract was fractioned using liquid vacuum chromatography with gradient system using a variety of solvents including n-hexane,ethyl acetate and methanol, resulted in 8 combined fractions. The fraction combination was performed through the thin layer chromatography guiding under the UV lamp 254 nm with stain-displaying reagent 10% AlCl3 in ethanol. Out of the 8 combined fractions, the fraction 5 was further fractioned, obtained 6 combination wherein at 5 and 6 formed yellow precipitate as compound 1 (15 mg) and compound 2 (10 mg). Compounds 1 and 2 which show the pattern of the stain for multiple comparisons solvent was then characterized using spectroscopy

Senyawa (1) Kaempferol-3-O-glicoside (astragalin) : yellow powder (5 mg); UV (MeOH) λ_{max}(nm) (log ε) : 273,333;IR (KBr) V_{max} (cm^{-1}): 3243, 1711, 1660, 1182-1081. Spektrum ^{1}H NMR (400 MHz, aseton-d_6) (ppm) : see table 1.Spektrum ^{13}C-NMR (100 MHz, aseton-d_6) (ppm): see table 1.Data HR-EIMS *m/z* $[M]^+$ 432 calculation for $C_{21}H_{20}O_{10}$.

Compound (2) Kaempferol-3-O-rhamnoside (afzelin) : yellow powder (10 mg); UV (MeOH) λ_{max}(nm) (log ε) : 341, 264 .IR (KBr) V_{max} (cm^{-1}): 3278, 2900, 1655, 1470, 1062. ^{1}H NMR spectra (400 MHz, aceton-d_6) (ppm) : see table 1. ^{13}C-NMR spectra (100 MHz, aseton-d_6) (ppm): see table 1. Data HR-EIMS m / z [M] + 431 (calculation of [M] + for $C_{21}H_{20}O_{10}$

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3. RESULT AND DISCUSSION

The ethyl acetate was fractionated using various methods of chromatography, a yellow precipitate was obtained which was identified as kaempferol by comparison of the NMR spectral data. Same aglycone obtained for both compounds 1 and 2.

Compound 1 has hydroxy group of glycoside at δH 3.43 (d, 1H); 3,48 (m, 1H); 3.73(dd, 1H; 5.5; 11.2 Hz), whereas in compound 2 showed the prsence of an anomeric proton as a doublet at 5.32 suggesting a sugar residu in its structure which was identified as L-rhamnosyl moiety on the basis of acid hydrolysis

Table 1 H and 13 C NMR chemical shift values for compounds 1 and 2 in CD3OD

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
2	-	156.3747	-	158,6
3	-	133.2633	-	136,3
4	-	177.5688	-	179,7
5	-	161.3155	-	163,3
6	6.30 (d, 1H, J=2.4 Hz)	98.8014	6,19 (1H; *d*; 2)	99,9
7	-	164.2247	-	165,9
8	6.54 (d, 1H, J=2.4 Hz)	93.7651	6,35 (1H, *d*; 2)	94,9
9	-	156.4796	-	159,4
10	-	104.1142	-	105,9
1’	-	121.0066	-	122,7
2’/6’	6.893 (d, 2H, J = 9.0 Hz)	131.0123	7,75 (1H, *dd*, 8,9)	132
3’/5’	8.064 (d, 2H, J= 9.0 Hz	115.2168	6,93 (1H, *dd*, 9,0)	116,6
4’	-	160.0374	-	161,7
Sugar				
1”	5.269 (d, 1H, J = 7.8 Hz)	100.9284	5,32 (1H, *d*, 2)	103,6
2”	3.179 - 3.701 (m, 5H glucoside)	74.3070	4,22(1H, *dd*, 2; 3,25)	72,1
3”	-	76.4912	3,71 (1H, *dd*, 3,25; 9)	72,2
4”	-	69.9670	3,33 (1H,	73,3
5”	-	77.5881	3,32 (1H, *m*)	72
6”	-	60.9152	0,93 (3H, *d*, 5,7)	17,8

Anticancer activity (murine leukemia cells P-388 to the risk) showed potent anticancer compounds with IC_{50} > 100μg / mL and IC_{50} 3.32 μg / mL. This value indicates that the compound (2) is very active as anticancer flavonoids. According to Alley et al[9]. strong anticancer activity is expressed as

1 IC_{50} 5 μg/mL = very active;
2 IC_{50} of 5-10 μg / mL = active;
3 IC_{50} 11-30 μg / mL = moderate; and
4 IC_{50} > 30 μg / mL = not active

The discovery of these compounds on the type of *Kalanchoe tomentosa* was reported for the first time in this research

4. CONCLUSION

From the results of the isolation of ethyl acetate fraction kalanchoe tomentosa plants produce compounds of the flavonoid group Kaempferol 3-Oa-glycoside (1) and Kaempferol 3-Oa-ramnosida (2). Compound **1** and **2** showed cytotoxic activity against P-388 murine leukemia cells with IC_{50} > 100μg/mL and IC_{50} 3.32 μg / mL

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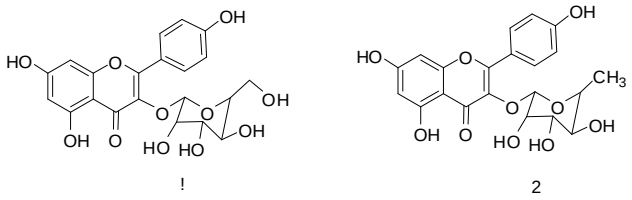

Fig. 1. The chemical structure of Kaempferol 3-Oa-glycoside (1) and Kaempferol 3-Oa-ramnosida (2)

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