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# 3',4'-Dimethoxy Quercetin, a Flavonol Compound Isolated from *Kalanchoe pinnata*

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## **ARTICLE INFO**

#### ABSTRACT

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Kalanchoe pinnata (Lam.) Pers., flavonol, 3',4'dimethoxy quercetin

## INTRODUCTION

Kalanchoe is a plant genus widely distributed in tropical and subtropical countries (Beckett, 1990), has been used in Indonesian folk medicine to treat infections, rheumatism, cough, fever, inflammation, wounds, boils, arthritis, gastric ulcer, dysentery, cholera, whitlow, headaches (Siddiqui et al., 1989; Hutapea, 1994; Akinpelu, 2000; Kuo et al., 2008; Shivananda et al., 2010), and their extracts also reported potential as antifungal (Misra and Dixit, 1979), antiinflammatory (Pal et al., 1991; Afzal et al., 2012), antiulcer (Pal et al., 1999), antimicrobial (Akinpelu, 2000), insecticidal (Supratman et al., 2000 and 2001a), anti-tumor (Supratman et al., 2001b), antihypertensive (Ojewole, 2002), antihyhepatoprotective (Yadav and Dixit, 2003), analgesic (Nguelefack et al., 2006), anti-leishmanial (Muzitano et al., 2006), immunomodulatory (Cruz et al., 2008). Many chemical compound group constituents had been isolated from Kalanchoe genus, such as triterpenoids, sterol, phenanthrenes (Gaind et al., 1976; Siddiqui et al., 1989), bufadienolides (Supratman et al., 2000 and 2001a; Wu et al., 2006; Kuo et al., 2008), flavonoids (Liu et al., 1989; Singab et al., 2011).

Megawati

Research Center for Chemistry, Indonesian Institute of Sciences, Kawasan PUSPIPTEK Serpong, Banten, Indonesia. *Kalanchoe pinnata* (Lam.) Pers. also known as cocor bebek (Indonesia), is one of the *Kalanchoe* species. This paper describe about extraction, fractionation, purification and structure elucidation of phenolic compound content from the methanol extract of *K. pinnata* leaf as part of our research study about bioactive compounds from *K. pinnata*.

## MATERIAL AND METHODS

From methanol extract of Kalanchoe pinnata (Lam.) Pers. leaves, a flavonol compound has been isolated.

Fractionation of the methanol extract with ethyl acetate, followed by ethyl acetate fraction purification

using column chromatography method with ethyl acetate : n-hexane as mobile phase gave a yellow crystal

compound (A). Further analysis using 1D- and 2D-nuclear magnetic resonance (NMR), confirmed with

## General

mass spectrometer (LC-MS), compound A identified as 3',4'-dimethoxy quercetin.

The infra-red spectrum was recorded with Shimadzu Prestige-21 Instrument, mass spectrum recorded using a Mariner Biospectrometry-Finnigan instrument, and 1D- and 2D-NMR spectra was obtained with a JEOL JNM-ECA 500 spectrometer using TMS as internal standard. Chromatographic separation process carried out using silica gel (Kieselgel 60, Merck 1.07734). Purity confirmation carried out using Silica gel 60  $F_{254}$  (Merck 1.05554) with 10%  $H_2SO_4$  in ethanol as compound detection reagent.

#### Plant material

Kalanchoe pinnata leaf was collected from Bogor regions, West Java, Indonesia, and determined at Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia.

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## **Extraction and isolation**

Dried K. pinnata leaves (1.8 kg) was extracted with 5 L methanol (MeOH) 24 hours at room temperature for three times. evaporated using rotary evaporator. 100 gr MeOH extract with *n*-hexane, ethyl acetate partitioned (EtOAc) and dichloromethane (DCM), successively to obtained n-hexane extracts (20.77 gr), EtOAc extracts (4.40 gr) and DCM extracts (0.04 gr). The EtOAc soluble fraction was silica gel column chromatographed, eluted successively with a gradient of *n*-hexane-EtOAc solvent to obtained 9 fraction (F1-F9). F4 further chromatographed using column of silica gel, eluted successively with a gradient of *n*-hexane-EtOAc solvent to give yellow crystal compound (A). Structure elucidation of the compound A, conducted base on spectroscopic result data obtained from infrared, mass spectroscopy and 1D- and 2D-NMR analysis.

## **RESULT AND DISCUSSION**

Compound A (Fig. 1), a yellow crystal obtained from two step processes of silica gel column chromatography of the EtOAc fraction for the first silica gel column chromatography and F4 for the second process, eluted successively with a gradient nhexane:EtOAc solvent.

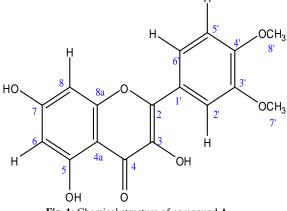


Fig. 1: Chemical structure of compound A

Compound A: yellow crystal (20 mg), which appear as yellow spot under UV-light. NMR data: <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 500 MHz); ( $\delta$  ppm): 6.30 (1H, *s*)(H-8), 6.73 (1H, *s*)(H-6), 7.66 (1H, *s*)(H2'), 7.04 (1H, *d*, *J*= 9.16 Hz)(H5'), 7.65 (1H, *d*, *J*= 9.16 Hz)(H6'), 3.97 (3H, *s*)(-OCH<sub>3</sub>) (H7'), 4.01 (3H, *s*)(-OCH<sub>3</sub>)(H8'). <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz); ( $\delta$  ppm): 157.7 (C-2), 129.0 (C3), 183.3 (C4(C=O)), 105.0 (C4a), 164.5 (C5(C-OH)), 104.4 (C6), 158.3 (C7(C-OH)), 99.7 (C8), 158.0 (C8a), 123.7 (C1'), 116.6 (C2'), 151.5 (C3'(C-OCH<sub>3</sub>)), 148.9 (C4'(C-OCH<sub>3</sub>)), 110.4 (C5'), 121.4 (C6'), 61.9 (C7'(-OCH<sub>3</sub>)), 56.6 (C8'(-OCH<sub>3</sub>)).

Based on <sup>1</sup>H-NMR data showed that compound **A** has five aromatic protons between  $\delta_{\rm H}$  6.30 - 7.65 ppm (consist of three singlet aromatic proton ( $\delta_{\rm H}$  6.30, 6.73, and 7.66 ppm) and two doublet aromatic proton ( $\delta_{\rm H}$  7.04 and 7.65 ppm)) and two singlet methyl from methoxy groups at  $\delta_{\rm H}$  3.97 and 4.01 ppm. <sup>13</sup>C-NMR showed that compound **A** has 17 atom carbons, included two carbon from methoxy groups at  $\delta_{\rm C}$  61.9 and 56.6 ppm, five methine carbon ( $\delta_{\rm C}$  99.7, 104.4, 110.4, 116.6, and 121.4 ppm), and ten quaternary carbon ( $\delta_{\rm C}$  105.0, 123.7, 129.0, 148.9, 151.5, 157.7, 158.0, 158.3, 164.5, and 183.3 ppm).

Based on the HMBC correlation data showed that H8 ( $\delta$  6.30 ppm (1H, *s*)) correlated with C4a ( $\delta$  105.0 ppm), H6 ( $\delta$  6.73 ppm (1H, *s*)) with C5 ( $\delta$  164.5 ppm), H2' ( $\delta$  7.66 ppm (1H, *s*) with C6' ( $\delta$  121.4 ppm), H5' (1H, *d*, *J*= 9.16 Hz) correlated with two carbon at C1' ( $\delta$  123.7 ppm) and C4' ( $\delta$  148.9 ppm) positions, H6' ( $\delta$  7.65 ppm (1H, *d*, *J*= 9.16 Hz)) also has correlation with two carbon at C5' ( $\delta$  110.4 ppm) and C4' ( $\delta$  148.9 ppm). Meanwhile, methoxy proton peak at  $\delta$ H 4.01 ppm ( $\delta$ C 56.6 ppm) showed a correlation with C4' ( $\delta$  148.98 ppm) (Fig.**2**).

Mass spectroscopy measurements showed that compound A has a molecule weight  $329.97 \approx 330 \text{ m/z} (330,97 = \text{M}+\text{H})$ .

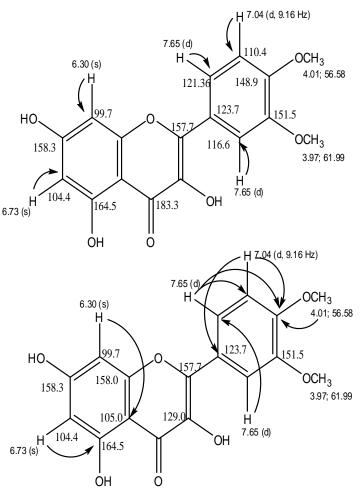


Fig. 2: HMQC and HMBC correlation of compound A.

#### CONCLUSION

From the ethyl acetate fraction (F4) of methanol extract of *Kalanchoe pinnata* leaves, processed through two steps of silica gel column chromatography, has been isolated and purified a yellow crystal compound (compound **A**). Based on 1D- and 2D- NMR data confirmed by mass spectroscopy data, it can be concluded that compound A is a flavonol compound named 3',4'-dimethoxy quercetin.

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