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

Akhmad Darmawan, Megawati* and Sofa Fajriah
Research Center for Chemistry, Indonesian Institute of Sciences, Kawasan PUSPIPTEK Serpong, Banten, Indonesia

ARTICLE INFO

Article history:
Received on: 19/12/2012
Revised on: 09/01/2013
Accepted on: 19/01/2013
Available online: 28/01/2013

Key words:
Kalanchoe pinnata (Lam.) Pers., flavonol, 3',4'-dimethoxy quercetin

ABSTRACT

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 mass spectrometer (LC-MS), compound A identified as 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; Ażfal 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), antihyperhepatoprotective (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 (Gaign 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).

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

General
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 F254 (Merck 1.05554) with 10% H2SO4 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.
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 partitioned with n-hexane, ethyl acetate (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 n-hexane-EtOAc solvent.

![Fig. 1: Chemical structure of compound A](image)

Compound A: yellow crystal (20 mg), which appear as yellow spot under UV-light, NMR data: 1H-NMR (CD3OD, 500 MHz); (δ 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)(-OCH3) (H7'), 4.01 (3H, s)(-OCH3)(H8'). 13C-NMR (CD3OD, 125 MHz); (δ ppm): 157.7 (C-2), 129.0 (C3), 183.3 (C4-C=O), 105.0 (C4a), 164.5 (C5-C=O), 104.4 (C6), 158.3 (C7-C=O), 99.7 (C8), 150.0 (C8a), 123.7 (C1'), 116.6 (C2'), 151.5 (C3'(C-OCH3)), 148.9 (C4'(C-OCH3)), 110.4 (C5'), 121.4 (C6'), 61.9 (C7'(C-OCH3)), 56.6 (C8'(C-OCH3)).

Based on 1H-NMR data showed that compound A has five aromatic protons between δH 6.30 - 7.65 ppm (consist of three singlet aromatic proton (δH 6.30, 6.73, and 7.66 ppm) and two doublet aromatic proton (δH 7.04 and 7.65 ppm) and two singlet methyl from methoxy groups at δH 3.97 and 4.01 ppm. 13C-NMR showed that compound A has 17 atom carbons, included two carbon from methoxy groups at δC 61.9 and 56.6 ppm, five methine carbon (δC 99.7, 104.4, 110.4, 116.6, and 121.4 ppm), and ten quaternary carbon (δC 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 (δ 6.30 ppm (1H, s)) correlated with C4a (δ 105.0 ppm), H6 (δ 6.73 ppm (1H, s)) with C5 (δ 164.5 ppm), H2' (δ 7.66 ppm (1H, s) with C6' (δ 121.4 ppm), H5' (1H, d, J= 9.16 Hz) correlated with two carbon at C1' (δ 123.7 ppm) and C4' (δ 148.9 ppm) positions, H6' (δ 7.65 ppm (1H, d, J= 9.16 Hz)) also has correlation with two carbon at C5' (δ 110.4 ppm) and C4' (δ 148.9 ppm). Meanwhile, methoxy proton peak at δH 4.01 ppm (δC 56.6 ppm) showed a correlation with C4' (δ 148.98 ppm) (Fig.2).

Mass spectroscopy measurements showed that compound A has a molecule weight 329.97 = 330 m/z (330.97 = M+H).

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.

ACKNOWLEDGEMENT

Authors convey their gratitude to Prof. Dr. Muhammad Hanafi and Puspa Dewi N.L. M.Eng from Research Center for Chemistry, Indonesian Institute of Sciences, for NMR and MS measurements, and Mr. Ismail Rahman from Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, for plant determination.

REFERENCES


How to cite this article: