Phenolic Compounds from the Leaves of Kalanchoe blossfeldiana (Crassulaceae) Plant

Yenny Febriani Yun\textsuperscript{a,b}, Lilis Siti Aisyah\textsuperscript{a,b}, Tri Reksa Saputra\textsuperscript{a}, Arif Rahman Hakim\textsuperscript{a}, Tati Herlina, Euis Julaeha\textsuperscript{a}, Achmad Zainuddin\textsuperscript{a}, Unang Supratman\textsuperscript{a}

Abstract

Kalanchoe blossfeldiana (Crassulaceae) is a succulent plant belonging to the genus Kalanchoe. Various species of Kalanchoe plants have been widely used as raw material for traditional medicine and also as an ornamental plant. This study is a continuation searching for secondary metabolites from Kalanchoe Indonesia plants. Fresh K. blossfeldiana leaves 5.7 kg was extracted with methanol at room temperature to obtain a concentrated extract as much as 155.74 g. Concentrated methanol extract was dissolved in water and then partitioned successively with hexane and ethyl acetate. Ethylacetate extract (20 g) was separated by a combination of chromatography on silica and ODS and produced compound 1 (7.1 mg) in the form of a yellow solid, compound 2 (4 mg), and compound 3 (15.4 mg) in the form of a white solid. The chemical structures of compounds 1, 2, and 3, were determined by UV spectroscopy, IR, MS, 1H-NMR, 13C-NMR spectroscopy and comparison of data obtained from the literature and identified as phenolic compounds, namely 3,3’,4’,5,7 pentahydroksi flavonorquercetin (1), epigallocatechingallate (2), and gallic acid (3).

Keywords: Kalanchoe blossfeldiana, phenolic, 3,3’,4’,5,7 pentahydroksi flavon, epigallocatechingallate, gallic acid

\* Natural Product Chemistry Group, Department of Chemistry, The Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung-Sumedang Km 21, Jatinangor, Sumedang 45363
\textsuperscript{b} Department of Chemistry, The Faculty of Mathematics and Natural Sciences, Jenderal Achmad Yani University, Cimahi 40528

Corresponding author email addresses: *yennyfy@gmail.com, **u_supratman@ unpad.ac.id

Introduction

Indonesia has high plant biodiversity and its ecosystems (Walujo, 2011). Some of the plants mostly used by Indonesian society, especially as herbal medicines, are from the genus Kalanchoe. Usually they are used as ornamental plants, with the characteristics of thick and watery leaves. These plants are very popular because of easy propagation, low water needer, and the flower colours. From the point of ethnopharmacology 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 (Quazi et al., 2011). Some researches reported that Kalanchoe plants contain bufadienolide (Supratman et al., 2000; Supratman et al., 2001), triterpenoid (Gaind and Gupta, 1972), and flavonoid (Muzitano et al., 2006; Okwu and Nnamdi, 2011), and biological activities like antileismanial, antiinflammatroy, cytotoxic, and inhibiting tumor cell growth (Supratman et al., 2001; Costa, 2006; Biswas et al., 2011). This study is a continuation searching for secondary metabolites from Kalanchoe Indonesia plants, specially Kalanchoe blossfeldiana (Crassulaceae).

Methodology

Materials

Plant

The leaves of K. blossfeldiana were collected from Lembang, Western Bandung area, West Java, Indonesia, and identified at Herbarium Bogoriense, Biology Research Centre 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 (redistilled), i.e n-hexane, ethyl acetate, methanol, acetone, andpro-analysis i.e. dichloromethane and chloroform. Silica GF\textsubscript{254} was used in Thin Layer Chromatography (TLC), Silica-
G60(10-40µm) with the surface area (500 m²/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 AlCl₃ (10% in ethanol) as stain-display reagent.

### Equipments

The equipments used were glass wares common in organic chemistry laboratory, macerar, 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, spectrophotometer FTIR Spectrum One Perkin Elmer, Spectrometer Nuclear Magnetic Resonance (NMR) JEOL JNM ECA-500 with TMS as internal standard, Preparative HPLC.

### Methods

#### Extraction and Isolation

The 18 kg of fresh K. blossfeldiana leaves was ground, extracted, and then concentrated. The 385.83 g of methanol extract obtained was dissolved in water and partitioned respectively using n-hexane and ethyl acetate, yielding in n-hexane extract (32 g) and ethyl acetate extract (25 g). The ethyl acetate extract was fractioned using liquid vacuum chromatography, resulted in 7 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 7 combined fractions, the fraction 5 was further fractioned. Fraction 5 was purified using column chromatography using silica G60 with the eluent methanol:water (4:6) which resulted in white solid isolate of 4 mg (2).

### Results and Discussion

The data of UV showed that the compound (1) has the absorbance at the region λmax 370.8 nm (band 1) and 255.4 nm (band 2) which are the characteristics of flavonoid compound. Band 1 indicated the absorbance which was related to the resonance of sinamoil group that involved ring B. Band 2 indicated the absorbance which was related to the resonance of benzoi group that involved ring A of flavonoid. The addition of AlCl₃ caused the shifting is from 370.8 to 340.0 nm, and from 255.4 to 268.4. The batochromic shift took place, with the decrease of the intensity of band 1 which was caused by the formation of chelat between AlCl₃ and the ring B.

The data of IR showed the absorbance of wide band at 3400 cm⁻¹ which showed the existence of hydroxyl group (the spread of O-H), 2000-1750 cm⁻¹ (overtone aromatic), 1650 cm⁻¹ (the spread of C=O carbonil), 1600-1400 cm⁻¹ (the spread of C=C aromatic), 1092 cm⁻¹ (the spread of C-O ether) and 800-750 cm⁻¹ (distributed benzene).

The data of 1H-NMR showed that the compound (1) has 15 carbon signals, which consist of 1 carbonol at δc 176.55 ppm, 12 aromatic carbon atom, which appeared in the signal range δc 100 – 160 ppm, and 2 shielded aromatic carbon atom, which appeared at the signals δc 94.48 ppm and 99.09 ppm, because they were in close range with carbon group bonded by hydroxyl group.

The data of 1H-NMR on the compound (1) showed it consisted of 5 protons which were bonded at Csp², which could be calculated or coupling constant. This J value (1.95) showed that both H6 and H8 had meta position. The locations of H2' dan H6' were in meta in aromatic system. The data 1H-NMR on the compound (1) showed that the compound (1) has 15 carbon signals, which consist of 1 carbonol at δc 176.55 ppm, 12 aromatic carbon atom, which appeared in the signal range δc 100 – 160 ppm, and 2 shielded aromatic carbon atom, which appeared at the signals δc 94.48 ppm and 99.09 ppm, because they were in close range with carbon group bonded by hydroxyl group.

The data of 1H-NMR on the compound (1) showed that the compound (1) has 15 carbon signals, which consist of 1 carbonol at δc 176.55 ppm, 12 aromatic carbon atom, which appeared in the signal range δc 100 – 160 ppm, and 2 shielded aromatic carbon atom, which appeared at the signals δc 94.48 ppm and 99.09 ppm, because they were in close range with carbon group bonded by hydroxyl group.

From the data of UV, IR, NMR and MS, it could be predicted that the compound (1) had the molecular formula C₁₆H₁₀O₁₉. Knowing the predicted formula, the DBE value was determined 11, with 8 double bondings and 3 cycles. It was predicted that the structure of the compound (1) was the 3,3',4',5'7 pentahydroxyflavon or known as Quercetin.
The compound (2) were identified by 1H-NMR, 13C-NMR, and comparison with literature values as epigallocatechin gallate (EGCG) (4 mg)(Peres et al., 2010; Eldahshan, 2011; Fu et al., 2011): the white solid, 1H-NMR (500 MHz, MeOH-d4) δ: 2.88 (eq. H, dd, J = 16.4, 3.5 Hz, H-4), 3.02 (ax. H, dd, J = 16.4, 4.4 Hz, H-4), 5.05 (1H, d, J = 7.5 Hz, H-2), 5.56 (1H, m, H-3), 5.95 (1H, d, J = 1.8 Hz, H-6), 5.97 (1H, d, J = 1.8 Hz, H-8), 6.75 (2H, s, H-2’ & H-6’), 7.00 (2H, s, H-2” & H6”), 13C-NMR (500 MHz, MeOH-d4) δ: 7.05 (1 H, s, H-2), 96.00 (C-3 & C-5), 105.38 (C-2’ & C-4’), 110.26 (C-2” & C-6”), 121.46 (C-1”), 130.64 (C-6”), 132.24 (C-4”), 139.98 (C-3’ & C-5’), 146.60 (C-3’’ & C5”), 149.00 (C-5), 157.38 (C-9), 158.00 (C-7), 167.32 (C-7” & C=O).

The compound (3) was identified by UV,1H-NMR, 13C-NMR, and compared with literature values as gallic acid (5 mg) (Binutu and Cordell,2000; Saxena et al., 2008; Liu et al., 2008; Eldahshan, 2011): the white amorphous solid, \( \lambda_{\text{max}}^{(\text{MeOH})} \) 271 nm, 1H-NMR (500 MHz, MeOH-d4) δ: 7.05 (1 H, s, H-2, 6), 13C-NMR (500 MHz, MeOH-d4) δ: 122.12 (C-1), 110.40 (C-2’ & C-4’), 146.48 (C-3 & C-5), 139.66 (C-4), and 170.55 (C-7).

**Figure 1.** The chemical structure of quercetin (1), epigallocatechingallate(2), and gallic acid(3).

**Conclusions**

The chemical structures of compounds (1), (2), and (3), determined by UV spectroscopy, IR, MS, 1H-NMR, 13C-NMR spectroscopy and compared with data obtained from the literature and identified as phenolic compounds, namely \( 3,3’,4’,5,7 \)pentahydroxyflavonorquercetin (1), epigallocatechingallate(2), and gallic acid(3).

**Acknowledgments**

We thank SimilitabmasDikti for funding this research in the program Desentralization Grant, scheme Competitive Grant 2013-1014. We also thank Herbarium Bogor, Indonesia, Biology Research Centre of Lembaga Ilmu Pengetahuan Indonesia or LIPI (The Indonesian Institute of Sciences), Cibinong, Bogor, West Java, Indonesia, and Chemistry Research Centre of LIPI Serpong, Tangerang for their support in measuring the spectra NMR. The staff of Medical Pharmacokinetic Laboratory of Padjadjaran University, thank you for measuring the MS.

**References**


flavonoids from *Kalanchoe pinnata*, Phytochemistry, 67: 2071-2077;


Walujo, E. B. (2011). Keanekaragaman Hayati Untuk Pangan, Herbarium Bogoriense, Research Centre for Biology Indonesian Institute of Sciences Presented at the National Science Congress X, Jakarta, 8 – 10 November;