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Flavonoids from the Fresh Leaves of *Kalanchoe tomentosa* (Crassulaceae)

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Abstract: Flavonoids compounds such as kaempferol (**1**), kaempferol-3-*O*-β-D-glucopyranoside or astragalin (**2**) and kaempferol-3-*O*-α-L-rhamnoside or afzelin (**3**) have been isolated from the fresh leaves of *Kalanchoe tomentosa* (Crassulaceae). The chemical structure of isolated compounds **1-3** were identified by spectroscopic evidences and comparison with those compound previously reported. Compounds **1-3** showed cytotoxic activity against P-388 murine leukimia cells with IC_{50} values of 51.8, > 100 and 3.32 μg/mL, respectively.

Keywords: Crassulaceae, cytotoxic activity, flavonoids, *Kalanchoe tomentosa*, P-388 murine leukimia cells.

INTRODUCTION

Kalanchoe tomentosa (Crassulaceae) is a perennial, succulent medicinal herb which grown in high terrain and indigenous to low altitude of Indonesia [1]. The leaves of *K. tomentosa* are used in Indonesian folk medicine for the treatment of fever, infections, rheumatism and skin diseases [2]. The genus of *Kalanchoe* reported to contain bufadienolides [3-5], triterpenoids [6] and flavonoids [7-9] which possess multiplebiological activities such as blocking human lymphocyte proliferation [6,10], cytotoxic agents [11], insecticidal properties [5, 12] and inhibiting cancer cell growth [13, 14]. However, information about phytochemical constituents of *K. tomentosa* is unavailable. Our preliminary screening for novel cytotoxic agents from Indonesian *Kalanchoe* plants, we found that the methanolic extract of *K. tomentosa* exhibited significant cytotoxic effect against P-388 murine leukimia cells. In this communication, the isolation and structure identification of flavonoids (**1-3**) along with their cytotoxic activity against P-388 murine leukimia cells will be described.

EXPERIMENTAL

General Experimental Procedures

Ultra-Violet spectra were recorded in methanol on Jasco UV-1575 spectrophotometer. The IR spectra were measured on a Perkin Elmer spectrum-100 FT-IR in KBr. Mass spectra were obtained with a Water, Qtof HR-MS XEV^{otm} mass spectrometer. NMR spectra were recorded with a JEOL JNM A-500 spectrometer using tetra methyl silane (TMS) as an internal standard. Chromatographic separation was done on silica gel 60 (Merck). PTLC glass plates were precoated with silica gel GF_{254} (Merck, 0.25 mm). TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm) and detection was achieved with 10% H_2SO_4 in ethanol followed by heating.

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Plant Material

The fresh leaves of *K. tomentosa* were collected from Lembang Discrict, West Bandung, Indonesia in May, 2011. The plant was identified in Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen (No. B0-129211) was deposited at the herbarium.

Extraction and Isolation

Fresh grounded leaves (20 Kg) of *K. tomentosa* were extracted with MeOH at room temperature. The MeOH extract was evaporated under reduced pressure to yield a dark brown residue (360 g). The MeOH extract was dissolved in water and partitioned succesively with *n*-hexane, EtOAc and *n*-butanol. Evaporation of each solvents resulted in the crude extract of *n*-hexane (30.5 g), EtOAc (64.5 g) and *n*-butanol (43.5 g), respectively. The *n*-hexane, ethyl acetate and *n*-butanol extracts exhibited a cytotoxic activity against P-388 murine leukimia cells with IC_{50} values of 56.5, 24.4 and 45.2 μg/mL, respectively. A portion of the EtOAc extract (50 g) was subjected to vacuum liquid chromatography on silica gel G60 using gradient elution of *n*-hexane-EtOAc-MeOH to afford 15 fractions (A01-A015). Fraction A05 (3.5 g) was further subjected to column chromatography on silica gel (70-230 mesh) using mixture of *n*-hexane-EtOAc (10:0-5:1) as eluting solvents to afford 10 fractions (B01-B10). Fraction B04 (230 mg) was subjected to flash column chromatography on silica gel (230-400 mesh), eluted with $CHCl_3$-MeOH (9:1), to give **1** (12.4 mg). Fraction A06-A07 was combined (4.2 g) and subjected