



## Elucidation structure of coumarin from stem *Polyalthia longifolia*

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### ABSTRACT

One of coumarine compound has been isolated from the stem *Polyalthia longifolia*. The compound was purified by column chromatography with silica gel as absorbent. The purity of the isolated has been tested by TLC, melting point and gas chromatography. The structure of the compound was identified by means of spectroscopic include Ultraviolet Spectroscopy, Infrared Spectroscopy,  $^1\text{H}$  and  $^{13}\text{C}$  NMR and Mass spectroscopy. All data obtained indicate that the resulted compound is a coumarin compound with name 7-N, N-diethylamino-4- methyl coumarin.

**Keywords:** *Polyalthia longifolia*, 7-N,N- diethyl amino-4- methyl-coumarin.

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### INTRODUCTION

Indonesia is a tropical country, the vegetation is a giant chemical plants. Because they 17 % of all species on the surface of the earth there in Indonesia. So that Indonesia has an incredible biodiversity (1).

Materials contained in the herbs have been used as drugs that are pharmacologically derived from the content of secondary metabolites such as terpenoids, steroids, alkaloids, flavonoids, coumarin, kromon, anthocyanins, phenolic and phenyl propane and others (2).

Besides, it has also been reported that the results of research in computational chemistry, especially the phenolic compounds, coumarin, kromon, flavones and isokoumarin, and terpenoids there are several secondary metabolites that can contribute to fighting tumors (anti-tumor). Given the high demand for drugs increases and drugs of also increasing the use of traditional medicines derived from plants, the research direction that needs to be improved to provide a scientific explanation of the active component contained by plants and explanation of the bioactivity (3).

*Polyalthia longifolia* is a family Annonaceae. More than 1600 species are found in the tropic and subtropic region. This plant is originally from India and Sri Lanka. In Indonesia *Polyalthia longifolia* is used as a shade the street.

From the research that has been done is found that *Polyalthia longifolia* is a plant that contains a chemical compound that is very good as an antioxidant (5, 10)

In research that has been conducted against the methanol extract of leaves *Polyalthia longifolia* has shown strong antioxidant activity using the method of ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1- picryl hydrazil (DPPH), hydroxyl radical, radical nitric oxide (NO). Extract leaves *Polyalthia longifolia* has showed remarkable antioxidant in vitro. (4)

Research of leaves *Polyalthia longifolia* has successfully revealed three structures namely clerodane diterpenoid kolavenat acid, polialtialdoat acid and alpha hydroxy kleroda-16 3.13 (14) z-dien-15, 16-olide, where all three of these compounds have been tested as an anti-leukemia HL bioactivity -60 cell. Polialtialdoat acid compounds and

16-alpha-hydroxy kleroda 3.13 (14) z-dien-15, 16-olide have shown activity as antileukemia HL-60 cells. (13).

## EXPERIMENTAL SECTION

### Plant material

*Polyalthia longifolia* stem was compiled in Avril 2016 in Kolok village Municipality Sawah Lunto, West Sumatra, Indonesia. It was identified in Herbarium of Biology Department, Andalas University, Padang, Indonesia.

### Chemical material

n-hexane, ethyl acetate, methanol, filter paper, silica gel 60 (230-400 mesh) from Merck company. All chemicals in use were in high grade, NaOH, P<sub>2</sub>O<sub>5</sub>, and blue silica gel, dichloromethane, distilled water, and sephadex LH-20.

### Instruments

The general glassware in organic laboratory, rotary evaporator Heidolp WB 2000, oven, melting point apparatus (John Fisher) and vacuum desiccator.

<sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded in CDCl<sub>3</sub> solvent using JEOL-ECA 400 spectrometer with tetramethylsilane (TMS) as internal standard. MS spectra were recorded on Jeol JMS-700/GI mass spectrometer.

IR spectra were recorded on JASCOFT/IR-460. Plus spectrometer column chromatography (CC) was performed on silica gel e60 N, spherical, neutral, Kanto Chemical Co INC and sephadex LH-20LH-20 (GE Health care, Japan). UV spectra on a Spectrometer UV Secoman S 1000 PC and Rotary evaporator Heindilp WB 2000.

Thin Layer chromatography (TLC) was performed on silica gel 60 F254 for analytical chromatography (200 mikrometer layer thickness (Merck). Preparative thin layer (PTLC) was performed on silica gel 60F254 (1 mm layer Thickness, merck). All the chemical used in study were purchased from Merck.

### Procedure

Stem powder of *Polyalthia longifolia* (3000 grams) was extracted by maceration method using n-hexane, ethyl acetate, and methanol successively. Ethyl acetate extract (10 g) was purified with column chromatography (400 g) of silica gel as the absorbent (230-400 mesh). It was then eluted with increasing polarity using n-hexane 100% to ethyl acetate 100%. Each fraction was monitored with TLC, the same R<sub>f</sub> were combined to yield fraction F1, F2, F3, F4, F5, F6, F7, F8, F9 and F10. (11)

Fraction F4, positive to contain coumarin but not pure, was repurified with recolumn chromatography using sephadex LH-20, and methanol as eluent. This process yielded white crystals (50 mgr) positive as coumarin, gave one spot on TLC and had narrow range of melting point. Spectrophotometer UV, IR and <sup>1</sup>H NMR, <sup>13</sup>C NMR, mass were also carried out to determine the structure.

## RESULTS AND DISCUSSION

The compounds yielded from ethyl acetate extract of *Polyalthia longifolia* stem was tested by thin layer chromatography and gave one single spot at R<sub>f</sub> 0.6 (n-hexane: ethyl acetate = 4: 6), the melting point 71-72°C, and showed a single peak at R<sub>t</sub> 24.450 through gas chromatography. These data indicate that the isolated compound is pure.

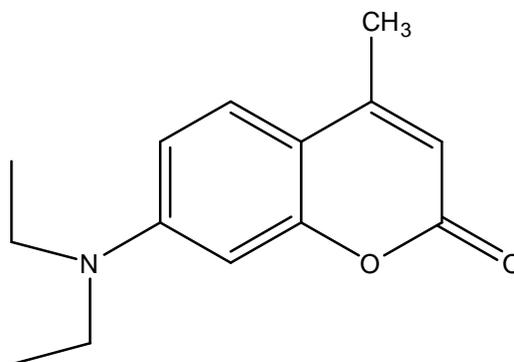
UV spectra gave absorption at λ<sub>max</sub> (MeOH) 209; 243; and 374 nm, which is suitable for the presence conjugated double bond. Comparing with the previous isolated coumarin compound, they have the same similarities.

IR spectrum gave absorption at wave number 651; 803; 1073; 1140; 1272; 1349; 1412; 1589; 1694; 2002; 2680; 2972; and 3636 cm<sup>-1</sup>. Absorption at 1589 cm<sup>-1</sup> showed (C = C) aromatic. Absorption at 1694 and 1140 cm<sup>-1</sup> indicated the C = O and C (O) -O strain, respectively. The absorption at 3646 cm<sup>-1</sup> showed the presence of CN amine group attached to coumarin compound.

<sup>1</sup>H-NMR spectra (500MHz, CD<sub>3</sub>OD): provides peaks at chemical shifts δ 7.523 (d, J = 6.5 Hz, 1H); 6.733 (d, J = 13 Hz, 1H); 6.498 (s, 1H), 5.914 (s, 1H); 3.467 (q, J = 7.0 Hz, 4H; 2CH<sub>2</sub>); 2.75 (s, 1CH<sub>3</sub>, 3H); 1.203 ppm (t, J = 7 Hz, 2 CH<sub>3</sub>, 6H). Two methyl groups appeared at the same chemical shift due to the same magnetic and physical nature. The same feature for two methylene groups. This means there are two groups C<sub>2</sub>H<sub>5</sub> attached to the atom which is N. In addition the data also shows that a single CH<sub>3</sub> group. This means the isolated compounds has 17 hydrogen atoms.

$^{13}\text{C}$ -NMR spectra (125MHz,  $\text{CD}_3\text{OD}$ ) showed peaks at 164.968; 157.423; 156.473; 152.595; 127.264; 110.457; 110.284; 108.499; 98.209; 45.742; 18.597, 12.886 ppm. Based on these data, it indicated that the isolated compounds has 14 carbon atoms, three primary carbon atoms, two secondary carbon atoms, four tertiary carbon atoms and five quaternary carbon atoms.

Mass spectra exhibited peaks at 231.1 ( $\text{M}^+$ ); 216.1 ( $\text{M}-\text{CH}_3$ ); 209.1; 188.1 ( $\text{M}-\text{CH}_3$  and  $-\text{C}_2\text{H}_5$ ); 209.1; 159.0; 131.0; 103.0; 77.0; and 51.0. All the above data indicate isolated compounds as 7-N, N-diethyl amino-4-methyl-coumarin like structure below.



### CONCLUSION

Based on data analysis of UV, IR,  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR and mass spectroscopy, the isolated compounds from ethyl acetate fraction of *Polyalthia longifolia* stem is a coumarin with name of 7-N,N-diethyl amino-4-methyl coumarin.

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