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## Structure elucidation and antioxidant activity of anthraquinone derivate from *Cassia alata* Linn

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### ABSTRACT

*Cassia alata* Linn is an Indonesian tropical plant that are well spread in tropical regions such as in the countries of Asia, America and Africa. This plant has traditionally been used as an anthelmintic, thrush, laxative, anti-parasitic, herpes, syphilis, scabies and other skin diseases. The objective of this research was to isolate the anthraquinone compound from the leaves of the plant. Extraction was done by soxletation method, while purification was done by vacuum liquid chromatography with silica gel as stationary phase and gradually eluted using Step Gradient Polarity (SGP) method by using the solvent n-hexane, ethyl acetate and methanol. Structure elucidation done by ultraviolet, infrared and <sup>1</sup>H-NMR spectroscopy. Compound was isolated from ethyl acetate fraction as an orange powder as much as 8 mg. The isolated compounds were anthraquinone derivate. Testing the antioxidant activity of the ethyl acetate fraction shown  $IC_{50} = 310 \mu\text{g}/\text{mL}$  and classified as active antioxidants.

**Keywords:** *Cassia alata* Linn, anthraquinone, antioxidants

### INTRODUCTION

*Cassia alata*. Linn is an American tropical plant that widely distributed in some regions such as Africa, Asia and including Indonesia. This plant usually grows to 1,400 m in height that live in low and also high attitudes area. In Indonesia the leaves of *Cassia alata*. Linn have been traditionally used as anti-parasite, anthelmintic, laxative, herpes, syphilis, scabies, ringworm, etc. [1,2]

Baharun has reported there were seven flavonoids from *Cassia fistula* such as Catechin, Epicatechin, ProsiandininB2, Rhamnetin-3-O- Gentibosa, Epiatzeachin, Quercetin and Kaempferol.[3]

Another species that also has reported are *Cassia garettiana*, *Cassia nigrican* and *Cassia sophera*. Antrone-C-Glikosid is Xanthone compound that occur to *Cassia garettiana* and used as anti-tumor[4]. Where as, Quinone and Rhein have be found in isolated *Cassia nigrican species*[5]. Then, triterpenoid glycosides that is Cyclosophoside A has been reporte doccur to *Cassia sopher*[6,7].

The aim of this work was to isolate the anthraquinone compounds from the leaves of *Cassia alata* Linn.

### MATERIALS AND METHODS

#### Chemical material

n-hexane, ethyl acetate, chloroform, dichloromethane, sulphuric acid, sodium hydroxide, methanol, filter paper, acetic anhydride. silica gel 60 (230-400 mesh) from Merck company. All chemicals in use were in high grade.

### Instruments

The general glassware in organic laboratory, rotary evaporator Heidolph WB 2000, oven, melting point apparatus (John Fisher ) and vacuum desiccator. <sup>1</sup>H-NMR spectra was recorded in CDCl<sub>3</sub> solvent using JEOL-ECA 400 spectrophotometer with tetramethylsilane (TMS) as internal standard. Column Chromatography (CC) were carried out on silica gel 60 F254 (Merck). Thin Layer Chromatography (TLC) was performed on silica gel 60 F254 for analytical chromatography (200 μm layer thickness; Merc). UV spectrum were measured with a UV-160A spectrophotometer (Shimadzu) and Rotary evaporator Heidolph WB 2000.

### Procedure

#### a. Isolation of anthraquinone compound

Leaves powdered of *Cassia alata*. Linni (150 g) was extracted by soxletation method using n-hexane, ethyl acetate, and methanol successively. Ethyl acetate extract (7 g) was purified with column chromatography (400 g) of silica gel as an 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 eight fractions (F1-F7). Fraction F III was purified with re-column chromatography, this process yield an orange solid substance (8 mg). Isolated compound structure was determined by using spectroscopy UV, IR and <sup>1</sup>H-NMR.

#### b. Antioxidant Test of Ethyl Acetate Fraction

Antioxidant test of ethyl acetate was done by DPPH method with various concentration 800, 600, 400, 200, 100, μg/mL. 0.2 mL of each sample and control (methanol) reacted with 3.8 mL DPPH for 30 minutes without any ligand the absorbance was measured by spectroscopy UV-Vis.

## RESULTS AND DISCUSSION

Anthraquinone test was done with Brontrager Test. Isolated compound was dissolved in chloroform and reacted with NaOH, red colour formed in aqueous layer indicated (+) anthraquinone.<sup>[8]</sup>

The UV spectrum showed maximal absorptions at λ<sub>max</sub> 429, 285, 254, and 225 nm. Interpretation of UV spectrum at λ<sub>max</sub> 225, 285 and 254 nm shown the electron transition from orbital π → π\* (C=C-C=C) along λ<sub>max</sub> 429 nm shown an electron transition from orbital n → π\* (C=C-C=O). The UV spectrum was assumed the isolated compound has double bond conjugated so that indicated the existences of aromatic ring and hydroxyl substituent.<sup>[9]</sup>

While the IR spectrum of isolated compound exhibited adsorption bands of hydroxyl group (ν 3450 cm<sup>-1</sup>), and C=O group, characteristic of quinone group (ν 1634.38 cm<sup>-1</sup>). Another specific group were known by a Group -CH<sub>2</sub>- stretching (ν<sub>max</sub> 2930 cm<sup>-1</sup>), Group -CH<sub>2</sub>- bending (ν<sub>max</sub> 1459 cm<sup>-1</sup>), Group C-O stretching alcohol (ν<sub>max</sub> 1043 cm<sup>-1</sup>)<sup>[10]</sup>.

The <sup>1</sup>H-NMR spectrum (Figure 1) shown there were 6 signals with integration 2.2; 1.2; 1.0; 1.1; 1.0; 1.0 that suited 7 protons. Seven protons divided in to two protons group which are two proton sat chemical shift 4.7 ppm (C-CH<sub>2</sub>-O) with integration 2.2 and 5 aromatic protons at chemical shift 7.3 – 7.9 ppm with integration each other 1.2; 1.0; 1.1; 1.0 and 1.0. Signal shift of aromatic proton at chemical shift 7.3-7.9 ppm indicated multiplicity of 5 aromatic protons. The five aromatic protons are 7.31 ppm (1 H, d, J = 8 Hz), 7.35 ppm(1 H, s); 7.69 ppm (1 H, t, J = 8 Hz); 7.80 ppm (1 H, s); 7.84 ppm (1H, d, J = 8 Hz). Expanded aromatic proton signals are shown in Figure 1.

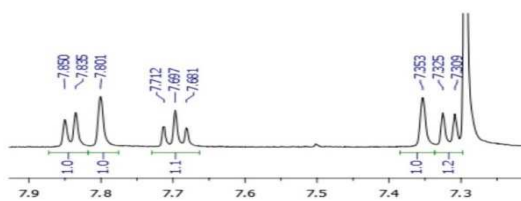


Figure 1. <sup>1</sup>H-NMR aromatic proton signal 7.3 – 7.9ppm

Based on the aromatic proton signals above, there were three protons that have same coupling constant at chemical shift 7.02 ppm; 7.89 ppm; and 7.4 ppm about 8 Hz. This indicated the three protons are in one aromatic ring (ring A) which ortho position. Based on the explanation, partial of isolated compound structure expected is anthraquinone structure (ring A) described by Figure 2.

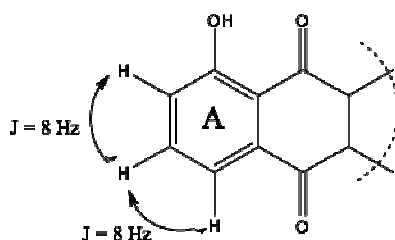


Figure 2. Partial structure (ring A) of isolated compound

The proton signal at chemical shift 4.7 ppm (2H) shown there was benzylic proton which bonding with oxygen. The isolated compound has proton signal string B are 7.35 ppm and 7.80 ppm which have singlet multiplicity each other or there was no coupling constant ( $J = 0$ ). Therefore, both protons in ring B should have the orientation like in Figure 3.

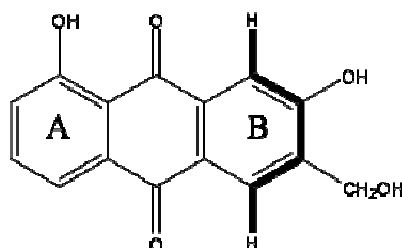


Figure 3. Structure of isolated compound

However, the structure of isolated compound is initial assumption. For more accurate, the result have to be completed with Mass Spectroscopy data,  $^{13}\text{C-NMR}$ , COSY, and NMR 2D .

The  $\text{IC}_{50}$  value of isolated compound is 310  $\mu\text{g/mL}$ . Based on antioxidant intensity classification by Jun. *et al* (2003) on this table, the isolated compound have weak ability to maintain the radical effect [11].

Tabel 1. Antioxidant ability of extract based on the value  $\text{IC}_{50}$

Intensity	Value $\text{IC}_{50}$ ( $\mu\text{g/mL}$ )
Most reactive	<50
Active	50-100
Medium	101-250
Weak	250-500
Non active	>500

## CONCLUSION

The isolated compound has shown the positive result occur to Anthraquinone by Brontriger's Test. Elucidation of structure has done by using Ultraviolet, infrared and  $^1\text{HNMR}$  spectroscopies, the structure of isolated compound was confirmed as anthraquinone derivate. The result of antioxidant activity by using DPPH, crude extract (fraction) of ethyl acetate from *Cassia alata* Linn leaves has weak ability to maintain the free radical effect with value  $\text{IC}_{50} = 310 \mu\text{g/mL}$ .

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