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Research Article

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Structure Elucidation of Anthraquinon Derivate from *Cassia Alata Linn* and Antioxidant Activity

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ABSTRACT

Cassia alata Linnis 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 reearch was to isolate the anthraquinone compound from the leaves of the plant. Extraction was done by soxhletation method, while purification was done by vacuum liquid chromatography with silica gel 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 1H-NMR spectroscopy. Compound was isolated from ethyl acetate fraction as an orange powder as much as 8 mg. The isolated compound is anthraquinone derivate. Testing the antioxidant activity of the ethyl acetate fraction shown IC_{50} *= 310 µg/ 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 inlowland and also highland. In Indonesia the leaves of C. Alata have been traditionally used as anti-parasite, anthelmintic, laxative, herpes, syphilis, scabies, ringworm, etc. [1,2]. Bahorun has reported there were seven flavonoids from *Cassia Fistula*such as catechin,epicatechin, Prosianidin B2, Rhamnetin-3-O- Gentibosa, Epiafzeachin, Quercetin and Kaempferol [3]. Another species that also has reported are *Cassiagarettiana*, *Cassia nigrican* and *Cassia sophera*. Antrone-C-Glikosida is xanthone compound that occur to *Cassiagarettiana*and used as anti-tumor [4]. Whereas, quinone and Rhein have be found in isolated *Cassia nigrican species* [5]. Then, triterpenoid glycosidesthat is Cyclosophoside A has been reported occur to *Cassia sopher* [6,7].
The aim of this work was to isolate the anthraquinone compounds from the leaves of *C. alata* Linn.

EXPERIMENTAL SECTION

Chemical material

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

Instruments

The general glassware in organic laboratory, rotary evaporator Heidolp WB 2000, oven,, melting point apparatus (John Fisher) and vacum desicator. 1H-NMR spectra was recorded in $CDCl_3$ solvent using JEOL-ECA 400 spectrometer 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 was measured with a UV-160A spectrophotometer (Shimadzu) and Rotary evaporator Heindolp WB 2000.

Procedure

Isolation of anthraquinon compound:

Leaves powdered of *Cassia alata. Linni* (150 g) was extracted by soxhletation 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 Rf were combined to yield fraction eigh fractions (F1-F7). Fraction F III was purified with recolumn chromatography, this process yielded orange solid (8 mg).
Isolated compound was determined the structure by using spectrochopy UV, IR and1HNMR.

Antioxidant test of ethyl acetate fraction: Antioxidant test of ethyl acetate was done by DPPH methode with various concentration 800, 600, 400, 200, 100, µg/mL. 0,2 mL of each sample and control (metahnol) reacted with 3,8 mL DPPH for 30 minutes wthiout any ligth and the absorbancy was measured by spectroscopy UV-Vis.

RESULTS AND DISCUSSION

Anthraquinone test was done with brontrager test. Isolated compound was dissolved in chloroformand reacted with NaOH, red colour formed in aquoues layer indicated (+) anthraquinone [8]. The UV spectrum showed maximal absoptions at λ_{maks} 429, 285, 254, and 225 nm. Interpretation of UV spectrum at λ_{maks} 225, 285 and 254 nm shown the electeron transition from orbital $\pi \rightarrow \pi^*$ (C=C-C= C) along λ_{maks} 429 nm shown electron transition from orbital n $\rightarrow \pi^*$ (C=C-C=O). The UV spectrum was assummed the isolated compound has double bond conjugated so that indicated the axistances of aromatis ring and hydroxyl subtituent [9]. While the IR spectrum of islated compound exhibited adsorption bands of hydroxl group (ν 3450 cm^{-1}), and *–C*=O group, characteristic of quionone group *(ν 1634.38 cm-1).* Another specific group were known by a Group *–CH_2-* stretching *(ν_{maks} 2930 cm^{-1})* , *Group -CH_2-* bending *(ν_{maks} 1459 cm^{-1}), Group C-O* stretching alkohol *(ν_{maks} 1043 cm^{-1})* [10]. The ^{1}H-NMR spectrum (Figure 1) shown there were 6 signals with intregation 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 protons at chemical shift 4.7 ppm (C-CH_2-O) with intregation 2.2 and 5 aromatis protons at chemical shift 7.3 – 7.9 ppm with integration each other 1.2; 1.0; 1.1; 1.0 dan 1.0. Signal shift of aromatis proton at chemical shift 7.3-7.9 ppm indicated multiplicity of 5 aromatis protons. The five aromatis 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 aromatis proton signal are shown Figure 1.

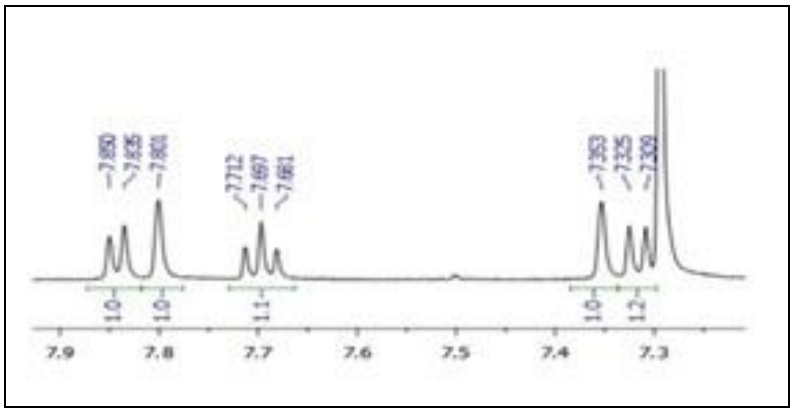

Figure 1: H-NMR aromatis proton signal 7.3-7.9 ppm

Based on the aromatis 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 aromatis 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.

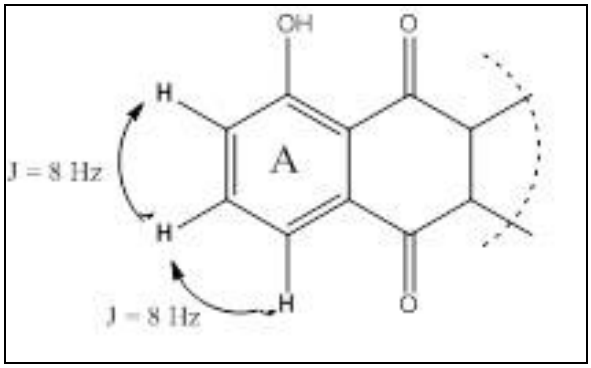

Figure 2: Partial structure (ring A) of isolated compound

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

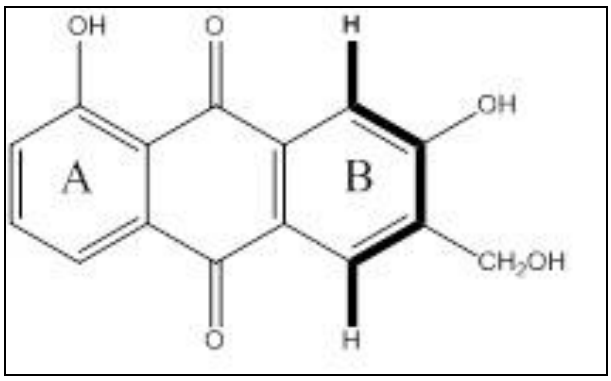

Figure 3: Structructure of isolated compound

However, the structure of isolated compound is initial assumption. For more accurate, the result have to be completed with Mass Spectroscopy data, 13C-NMR, COSY, and NMR 2D. The IC_{50} value of isolated compound is 310 µg/mL. Based on antioxidant intensity classification by Jun.et al (2003) on this table, the isolated compound has weak ability to maintain the radical effect [11].

Table 1: Antioxidant ability of extract based on the value IC_{50}

Intensity	Value IC50 (µg/mL)
Most reactive	<50
Active	50-100
Medium	101-250
Weak	250-500
Non active	>500

CONCLUSIONS

The isolated compound has shown the positive result occur to Anthraquinone by Brontriger's test. Elucidation of structure has done by using spectroscopy Ultraviolet, infrared and 1HNMR, 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 maintaince the free radical effect with value IC_{50} = 310 µg/mL.

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