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Research Article

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Isolation and structure elutidation of coumarin derivate from fruit seeds of *Clausena excavata*

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ABSTRACT

This study was conducted to obtain the structure of coumarin compound by extracting the seeds from the fruit powder Clausena excavata with dichloromethane and then separated by column chromatography. Fractions obtained from the separation process then purified by preparative TLC and isolated compound were obtained as 25.67 mgrams. Elutidation structure of isolated compound performed using UV spectrophotometer, IR, 1H-NMR, 13C-NMR, 2D-NMR (HMBC and HMQC), GC-MS, and also through a literature search was proposed, the isolated coumarin derivatives with the name: 7-hydroxy-6-methyl-coumarin.

INTRODUCTION

Clausena excavata is one of the plant species of the genus *Clausena.* Regional spread of this plant include Aceh (Temung), Minangkabau (Sicerek) , Malay (Tikusan) , Sundanese (Ki Bajetah) , Central Java (Tikusan) , Malaysia (Cherek black) and Thailand (San Soak) . Some parts of this plant has been known as a drug in the endemic area , such as in Java used as worm juice to treat pain , cough , and for body warmers [1] ; leaves and bark of this plant as a herbal medicine for dysentery and urethral infections in yunnan [2] , in Thailand , extracts of wood used as a cancer treatment [3]. The ability of a drug, which is owned by the plant *Clausena excavata* is because it contains many phenolic compound such as furanocoumarin, flavonoids, and carbazole alkaloids [4]. Based on literature data, coumarin compound that have been isolated from *Clausena excavata,* including at the leaves and twigs [1], leaves and bark [2], and leaf [5],whereas from fruit seeds has not been studied . Based on these data the authors perform the isolation and structure elucidation of coumarin compound from the fruit seeds of *Clausena excavata* .

Figure 1a. Picture of *Clausena excavata* [6]

1b. Picture of fruid seeds of *Clausena excavata*

EXPERIMENTAL SECTION

Plant Material

Clausena excavata plants taken from the natives in the village of first East Alai road, Parak Kopi, Northern District of Padang. Plants obtained *Clausena excavata* identified in the Herbarium Department of Biological Science, University of Andalas.

Chemicals

Ethyl acetate, n-hexane, dichloromethane, filter paper, silica gel 60 (0.063 to 0.200 mm) were obtainedfrom Merck , Germany. All other chemicals used in this study were analytical grade.

Intrumentations

The equipment used is of glassware commonly used to study organic chemistry of natural products, a set of tools solvent distillation, rotary evaporator Heidolp WB, 2000, oven, TLC plate (silica gel 60 F254, Merck KGaA Darmstadt Germany), melting point apparatus (John Fisher), UV-Vis spectrophotometer series 1700, Parkin Elmer 1600 IR spectrophotometer series, GC-MS QP 2010 plus Shimadzu, UV lamps (GL-58 UV 254 and 365 nm), JEOL spectrophotometer 400 MHz 1H-JNM-ECS400 NMR, 13C-NMR, and 2D-NMR (HMQC and HMBC).

Procedures

1. Extraction, isolation and purification of Coumarin from extract of *Clausena excavata*

Clausena excavata fruit seeds powder (854.11 g) was extracted with n-hexane, and dichloromethane and ethyl acetate. The fraction of N-hexane, dichloromethane and ethyl acetate was obtained as 2.68 g (0.314%), 22.67 grams (2.65%), and 7.25 g (0.85%). Furthermore, for the separation and purification carried out on dichloromethane fraction by column chromatography and preparative TLC technique. Eluent system, dichloromethane: ethyl acetate 7:3 was used with isolates obtained as 25.67 mgrams. Residue obtained was then re-crystallized for final purification.

2. UV-Visual Spectrophotometer

Ultraviolet Spectra of the isolate compund were recorded using Shimadzu UV-Vis Spectrometer, Model 1700, and chloroform was used as solvent for the dilution of sample as well as blank. The λmax value of test sample was recorded.[7].

3. Melting Point Analysis

The melting point of the isolate compound was analyzed by using melting point apparatus (John Fisher), where short of distance melting point reading will indicate the level of purity of the isolated compound

4. FT-IR Spectra

Infrared spectra of theisolated compound were recorded in the range of 4000 – 400 cm^{-1} using potassium bromide on Parkin Elmer 1600 IR spectrophotometer. The KBr pellet technique was used for this study.

5. Gas Chromatography and Mass Spectroscopy Analysis

The qualitative and compositions of the isolate compound were studied by GC-MS on a GC-MS QP2010 plus GC mass spectrometer. Components were separated on Rtx -5MS quartz capillary column (60m x 0.25mm) with crossbond R 5% diphenyl/95% dimethyl polysiloxane stationary phase.Compound were identified by comparison of mass spectra with those in the Wiley and NIST Libraries.

6. Nuclear Magnetic Resonance (NMR) Spectra

JEOL spectrophotometer 400 MHz 1H-JNM-ECS400 NMR, 13C-NMR, and 2D-NMR (HMQC and HMBC) were used for 1H NMR and 13C NMR spectra recorded.The chemical shifts are reported inδ (ppm) downfield from tri-methyl silane (TMS), which was used as internal standard. Deuterated chloroform(CDCl3) was used as solvent for this study

RESULTS AND DISCUSSION

Coumarin derivatives that have been isolated from the dichloromethane fraction is 7-hydroxy-6-methyl-coumarin. This compound was purified by preparative thin layer chromatography and determination of the structure of the spectral method and compared with data from the literature search.

Isolated compoundshowed white needle-shaped crystals having a melting point of 184-1850C and a molecular weight of 176. It is known from the molecular ion(m/z). From these data predicted this compound has the molecular

formula C10H8O3. The main fragmentation of the molecular weight from isolated compound showed a molecular ion m / z was 148 with a reduction in CO molecules. Figure 2 Show that following fragmentation pattern of compound isolated:

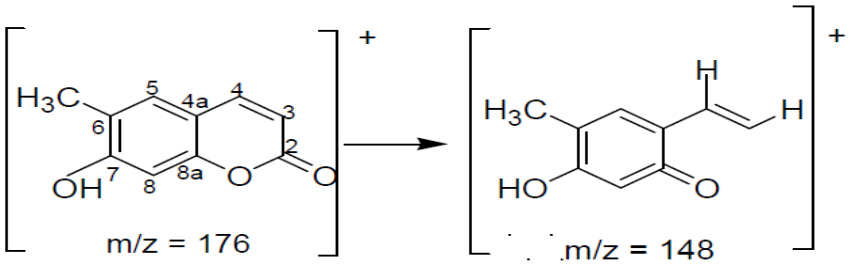
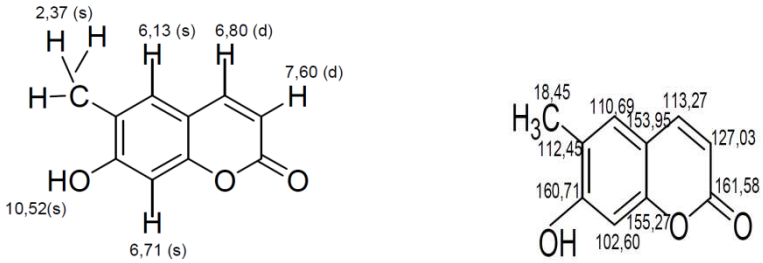


Figure 2. The main fragmentation of the molecular weight from isolated compound

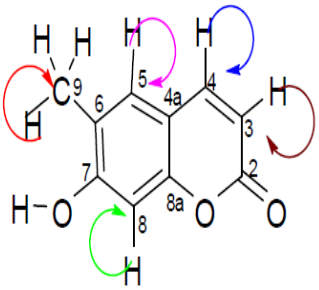
Isolated compound have the following characteristics: the measurement of UV (MeOH) λmax (log ε): 322.37 nm which indicates a group dienon conjugate (coumarin skeleton); IR (KBr) υmax: 3157.86 (OH), 1679.69 (C = O), 1593.88 and 1448.28 (C = C and CC of aromatic), 1385.6 (CH), 1270.86 (OC = O), 1236.15 cm-1 (C-OH); 1H-NMR (chloroform-D6, 400 MHz) δ: 10.52 (1H, s, OH), 6.71 (1H, s, H-8), 6.13 (1H, s, H-5), 6.80 (1H, d, H-4), 7.60 (1H, d, H-3), 2.37 ppm (3H, s,-CH3) (Figure 3a), 13C-NMR (chloroform-d6, 400 MHz) δ: 161.58 (C-2), 160.71 (C-7), 155.27 (C-8a), 153.95 (C-4a), 102.60 (C-8), 112.45 (C-6); 110, 69 (C-5), 127.03 (C-3), 113.27 (C-4), 18.45 (CH3) (Figure 3b)



3a **3b**

Figure 3a. 1H-NMR interpretation of isolated compound, 3b. 13C-NMR interpretation of isolated compound

To support the proposed structural formula of the isolated compound were followed by measuring the two-dimensional HMQC. HMQC spectral data of the isolated compound showed that each carbon (C)in lactone ring that binds one Hydrogen (H). 1H binding to C-3 (δ = 7.60 ppm, d), and C-4 (δ = 6.80 ppm, d) then on the aromatic ring was found that 1Hbinding to C-5 (δ = 6.13 ppm, s) and C-8 (δ = 6.71 ppm, s) (Figure 4) and HMBC spectral data of isolated compound showed correlation of protons H-9 with C-5 and C-6; correlation protons H-4 to C-8 and C-6; correlation protons H-5 to C-6, and the correlation of protons H-8 with C-6 (Figure 5).



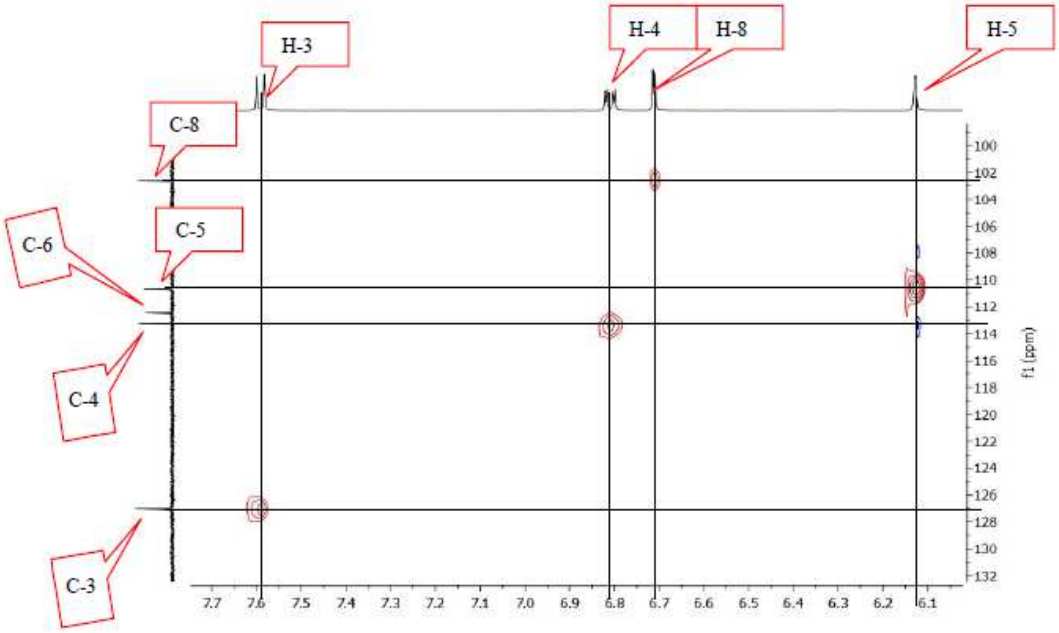
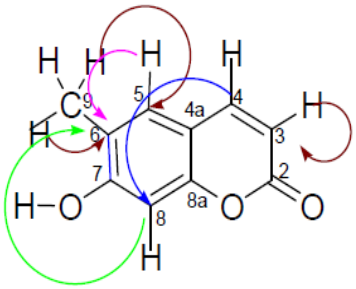


Figure 4. Picture of HMQC Spectrum and HMQC interpretation of isolated compound



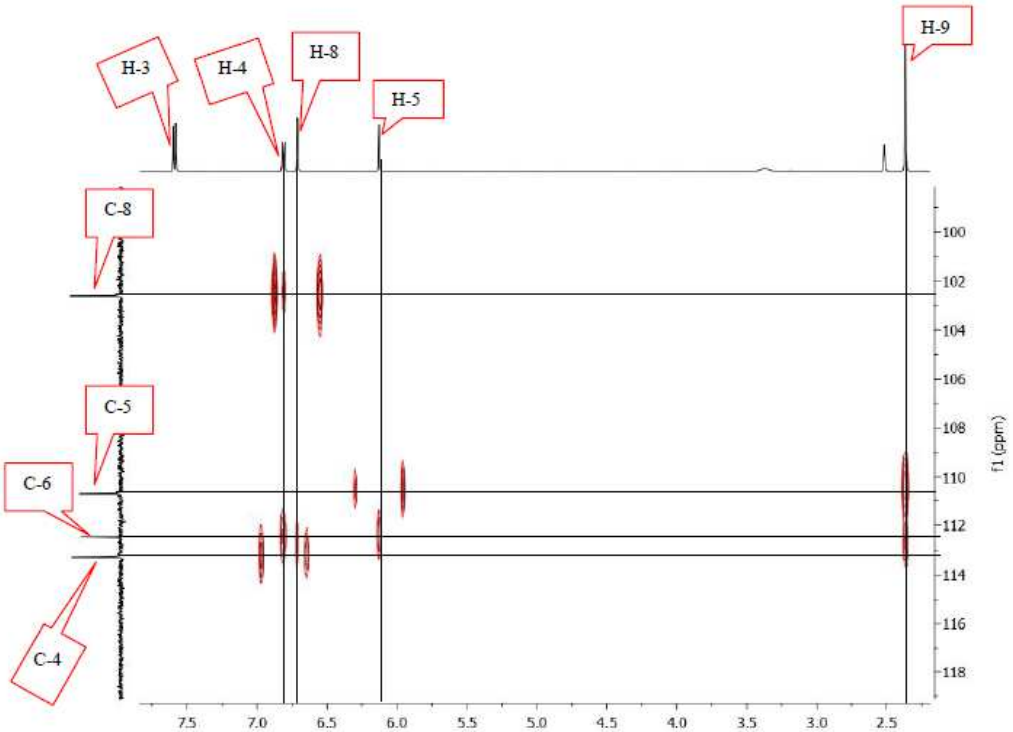


Figure 5. HMBC Spectrum and HMBC interpretation of isolated compound

CONCLUSION

Based on the analysis of UV, IR, GC-MS, 13C-NMR, 1H-NMR, 2D-NMR (HMQC and HMBC), the isolated compound from the fruit seeds of *Clausena excavata* in dichloromethane fraction is a derivative of coumarin compound with the name :7-hydroxy-6-methyl-coumarin with structural formulaas figure 6.

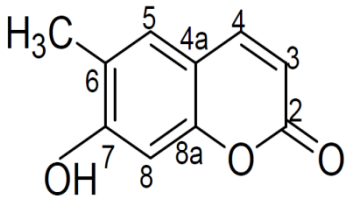


Figure 6. structural formula of 7-hydroxy-6-methyl-coumarin

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