

Antioxidative Activity of Xanthone from *Garcinia benthami* Pierre Leaves

Puteri Amelia^{1,2}, Berna Elya², Muhammad Hanafi³

¹Department of Pharmacy, Faculty of Medicines and Health Sciences, Islamic State University Syarif Hidayatullah Jakarta, Indonesia

²Department of Pharmacy, Universitas Indonesia, Indonesia

³Indonesian Institute of Sciences, Research Center for Chemistry, Indonesia

Abstract: A xanthone, 1,3,6,7-tetrahydroxy-xanthone (compound (1)) had been isolated from *Garcinia benthami* Pierre. The isolation was conducted through the chromatography technique and spectroscopy methods by spectroscopy: mass spectrometry (LC-MS), infra red (IR), UV, ¹H-NMR, ¹³C-NMR (1D and 2D-NMR). Antioxidant activity test by using DPPH (1,1-diphenyl-2-picrylhydrazyl) method from the isolated compounds showed antioxidant DPPH radical scavenging with IC₅₀ 8.01 µg/mL.

Keywords: Xanthone, *Garcinia benthami* Pierre, chromatography, antioxidant, DPPH.

Introduction

Garcinia belong to Clusiaceae family have a large contribution for health, because this family content some potential bioactive compounds among as antioxidants. Chemical contain from some species of *Garcinia* have reported as xanthenes, terpenoids, coumarins and flavonoids. *Garcinia benthami* Pierre, is a large tree with dark green foliage and scented flowers, which is suitable for cultivation in bee-raising gardens. Its white resinous sap becomes black when exposed to sunlight and the fruits are edible¹. The leaves of *Garcinia benthami* Pierre were collected in Bogor, Indonesia, in August 2010.

A number of benzophenones and triterpenoids have been isolated from *Garcinia benthami* Pierre. Two new benzophenones (ismailbenzophenone and hilmibenzophenone) were isolated from acetone extract of the stem bark². A new benzophenone, salimbenzophenone, was isolated from the stem bark³. and two triperpenoids compounds, (friedelin and 3β-hydroxy-lanosta-9(11),24-dien-26-oic acid) were isolated from the n-hexane extract of the stem bark of *Garcinia benthami* Pierre collected in Indonesia⁴. Friedolanostane, friedocycloartane and benzophenone constituents of the bark and leaves of *Garcinia benthami* Pierre was isolated from the stem bark collected in Viet Nam⁵. We now report the xanthone of the leaves from the species collected in Indonesia.

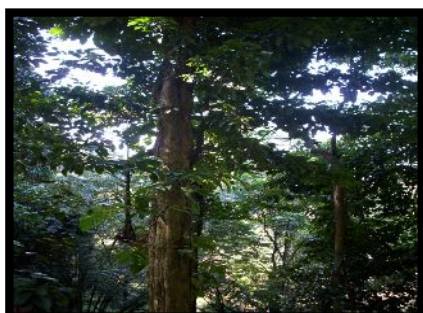


Figure 1.a. *Garcinia benthami* Pierre Plant



Figure 1.b. *Garcinia benthami* Pierre leaves

Experimental

Materials

Garcinia benthami Pierre leaves was obtained from Bogor, and determined in Center for Plant Conservation – Bogor Botanical Gardens, Indonesia.

Chemicals

n-hexane, ethyl acetate, methanol, filter paper, silica gel 60 (230-400 mesh), obtained from Merck Germany, sephadex LH-20. All other chemicals used in this study were analytical grade.

Instrumentation

The equipment used is glassware commonly used for 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 (STUART SMP 10), FT-IR Spectrometer (Prestige-21 Shimadzu), UV-Vis spectrophotometer (Shimadzu PharmaSpec UV-170), spectrophotometer NMR (¹H-NMR/500MHz), (¹³C-NMR/125 MHz) Agilent 500MHz.

Procedure

Extraction, isolation and purification of 1,3,6,7- tetrahydroxyxanthone (compound (1))

Powder of *Garcinia benthami* Pierre leaves as much 1500 grams was extracted by using stratified maceration method. This method chosen because it is a cold extraction method which does not damage the chemical compounds and also more simple and easy to do. Samples were extracted by using n-hexane, and then the residue was extracted again by using ethyl acetate, and the last residue was extracted by using methanol.

The filtrate was evaporated by using a rotary evaporator to obtain a viscous extract, yielding 47.06 g n-hexane extract, 34.01 g ethyl acetate extract, and 43.71 g methanol extract. A portion (20 g) of the methanol extract was fractionated by VLC eluted with n-hexane - ethyl acetate - methanol mixtures of increasing polarity and give fifteen fractions (Fr 1-15). Fraction 8 (Fr 8) was further fractionated by column chromatography eluted with ethyl acetate – methanol (4:6) and purified by column chromatography stationary phases sephadex LH-20 with an eluent system was 100% methanol. After crystallization, compound (1) was obtained. It was elucidated by UV, IR, MS and NMR spectroscopies.

Antioxidant activity

The ability of extract (n-hexane, ethyl acetate, methanol), Fraction 8 (Fr.8) and compound (1) to scavenge DPPH free radicals was assessed by using the method described by Takao *et al*⁶ with some modifications. A concentration series (200, 100, 50, 10 µg/mL) of each sample was prepared. The reaction mixture (2 mL) contained 500 µL of daily prepared DPPH solution (1mM) and various concentrations of sample and standard (quercetin) dissolved in methanol. After 30 min in the dark at room temperature, the absorbance was recorded at 515 nm. A low absorbance value indicates effective free radical scavenging. Each sample was analyzed in triplo and the average values were plotted to obtain the IC₅₀ against DPPH by linear regression. The radical scavenging activity was evaluated as the percentage of inhibition according to the following equation⁷ :

$$\% \text{ Inhibition} = [(\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control}] \times 100$$

The IC₅₀ value is the effective concentration at which 50% of DPPH radicals were scavenged. It was obtained from the graph of scavenging activity (%) versus concentration of samples. Low IC₅₀ value indicates strong ability of the extract to act DPPH scavenger⁷.

Result and Discussion

Compound (1) was obtained as yellow powders, mp. 271-273°C, C₁₃H₈O₆ (m/z 261.12 [M+H]⁺). The UV spectrum showed a maximum at 237.5, 261.0, 312.5, 375.0 nm and the IR exhibited absorption bands at 3228 cm⁻¹(OH), 1687 cm⁻¹(C=O), 1637cm⁻¹ and 1595 cm⁻¹(C=C aromatic). The ¹H NMR spectrum showed the presence of a hydrogen-bonded of hydroxyl group with carbonyl group at δH 13.85 (1H, s), two

meta-coupled aromatic protons at δ_H 6.15 and 6.29 (each 1H, *d*, $J=2$ Hz)], two *para*-coupled aromatic proton at δ_H 6.83 and 7.44 (each 1H, *s*).

In its ^{13}C NMR spectrum, the presence of conjugated carbonyl carbon, 12 aromatic carbon was observed. From these data, this compound was regarded as a tetraoxygenated xanthone derivative. Based on the long-range correlation between H-4 (δ_H 6.29) and C-3 (δ_C 159.5), H-5 and C-10a (δ_C 145.5); C-8a (δ_C 115); C-6 (δ_C 153.3); H-8 and C-10a (δ_C 145); C-6 (δ_C 153.3) in its HMBC spectrum (Figure 2, Table 1). Therefore compound (1) determined as 1,3,6,7-tetrahydroxyxanthone (Figure 3).

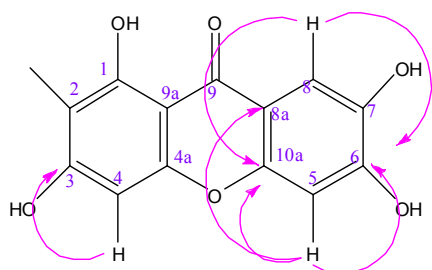


Fig. 2. Key long-range correlations of compound (1)

Table 1. ^{13}C NMR (125 MHz), ^1H NMR (500MHz) and HMBC spectrum data of 1,3,6,7-tetrahydroxyxanthone (1) in CD_3OD .

| No | ^{13}C NMR δ (ppm) | ^1H NMR δ (ppm) | HMBC δ (ppm) | | |
|-----|------------------------------------|---------------------------------|---------------------|-----|-------|
| 1 | 163.5 (C) | 13.85 (<i>s</i>) | | | |
| 2 | 98.8 (CH) | 6.15 (<i>d</i> , 2 Hz) | | | |
| 3 | 159.5 (C) | - | | | |
| 4 | 94.8 (CH) | 6.29 (<i>d</i> , 2 Hz) | 159.5 | | |
| 4a | 158 (C) | - | | | |
| 5 | 103.6 (CH) | 6.83 (<i>s</i>) | 145 | 115 | 153.3 |
| 6 | 153.3 (C) | | | | |
| 7 | 163.2 (C) | | | | |
| 8 | 109.2 (CH) | 7.44 (<i>s</i>) | 153.3 | 145 | |
| 8a | 115 (C) | - | | | |
| 9 | 181.3 (C) | - | | | |
| 9a | 125.1 (C) | - | | | |
| 10a | 145 (C) | - | | | |

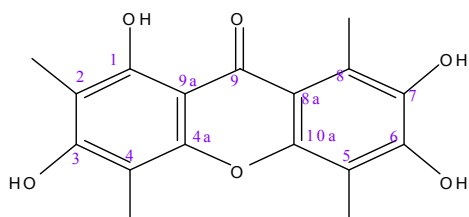


Fig.3. Structure of compound (1) ; 1,3,6,7-tetrahydroxyxanthone

The antioxidant activity assay of different extracts of *Garcinia benthami* Pierre, Fraction 8 (Fr.8) and compound (1) were determined by the use of the methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent with several variations of sample concentration and quercetin as the standard. DPPH method is used in the study of antioxidant effects of complex biological compounds and of their ability to reduce the free radicals activity⁸.

The reduction of DPPH (purple) to the corresponding of hydrazine (yellow) is fast and simple method for evaluating radical scavenging activity. The reaction can be monitored spectrophotometrically by following the decrease in absorbance at $\lambda = 515 \text{ nm}^8$. The antioxidant activity of methanol extract, Fraction 8 (Fr.8) and compound (1) is expressed in the form of IC_{50} values ($\mu\text{g/mL}$). The result are shown in Table 2.

Table 2. Antioxidant (DPPH Scavenging) activity of methanol extract, Fraction 8 (Fr.8), compound (1) and quercetin presented as IC_{50} values ($\mu\text{g/mL}$)

| Sample | IC_{50} value($\mu\text{g/mL}$) |
|------------------|-------------------------------------|
| Methanol extract | 29.92 |
| Fr.8 | 26.82 |
| Compound 1 | 8.01 |
| Quercetin | 2.97 |

Methanol extract showed the greatest activity against DPPH compared to the other extract. Methanol extract, Fraction 8 (Fr.8) and compound (1) showed the capability to scavenge the DPPH radical with IC_{50} value : $29.92 \mu\text{g/mL}$, $26.82 \mu\text{g/mL}$ and $8.01 \mu\text{g/mL}$ while the IC_{50} of quercetin as standard is $2.97 \mu\text{g/mL}$.

The high antioxidant activity of the methanol extract presumably relates with chemical compounds of phenols, flavonoids and tannins . Phenolic compounds have antioxidant activity because it is able to donate hydrogen radicals to neutralize free radicals and the phenolic radicals form will be stabilized by resonance . The high antioxidant activity of phenolic substances is attributed to their OH-moieties. The planarity of the molecule can also permits conjugation and electron delocalization. Tannins can act as an antioxidant because of its ability to stabilize the lipid fraction and its activity in the inhibition of lipoxygenase ⁹.

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