Exploration of Phenolic Compound from The Stem Bark of *Garcinia latissima* Miq.

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Abstract*—Genus Garcinia, which well known as mangosteen family. It is belonging Cluciaceae family, consists of over 100 species. It is widely distributed in Southeast Asia. There are 77 species of this genus grows throughout Indonesia. Genus Garcinia have been identified to be a rich source of phenolic compounds, including xanthones, biflavonoids, benzophenones, depsidones, and triterpenoids. Some of those have been reported to have several biological activities, such as antioxidant, antidiabetic, and anticancer. Garcinia latissima Miq. is an endemic plant growing in Indonesia, especially in Papua Island, and its neighbouring country, Papua New Guinea. It is called Dolomagata by local people in Maluku. The plant usually grows with the straight and cylindrical stem. It has short buttresses and it has 50-80 cm diameter. Previous reports have revealed antimicrobial and antioxidant activites of the leaves, fruits, and stem bark extracts of the plant. Phytochemical investigation of the stem bark of G. latissima Miq. led to the isolation of new pyranoxanthones, latisxanthones-A-D. In this work, the isolation and structural elucidation of secondary metabolites from the stem bark of G. latissima Miq. will be conducted. Three known compounds such as kaempferol (1,2), 1,3,6,7-Tetrahydroxy-2-(3-methyl-but-2-enyl)-xanthen-9-one (3), 1,3,7 trihydroxy xanthone (4) were isolated from the ethyl acetat fraction.

***Keywords*—*Garcinia latissima* Miq., Phenolic Compounds, Stem Bark, Ethyl Acetat Extract.**

I. INTRODUCTION

INDONESIA is one of the country with biodiversity, consisting of 25% of flowering plant species that reach 20,000 populations in the world. Around 40% of species are endemic to Indonesia. The plants with the most species are the family Orchidaceae (orchids) with 4,000 species, woody plants of the Dipterocarpaceae family of 386 species, the family Myrtaceae (Eugenia) and Moraceae (Ficus) as many as 500 species [1] and the Clusiaceae family consisting of 40 genus [2]. Local communities in several regions in Indonesia have long used a variety of plants as traditional medicines for the prevention and treatment of several diseases such as metabolic disorders including diabetes mellitus and obesity, cancer therapy and infectious diseases caused by bacteria and parasites. Traditional medicine uses medicinal plants as its main ingredient and is consumed as an herbal herb. The ability of plants to prevent and treat diseases because there are active substances contained in them called secondary metabolites [3].

Plant metabolism produces primary metabolites and secondary metabolites. Primary metabolite compounds, namely polysaccharides, proteins are used for growth. While secondary metabolites such as terpenoids, alkaloids, phenolics are used to adapt to the environment, weather, temperature (protectant), predators and as self-defense. This compound is also used to attract insects in the process of pollination and also as a repellant. Some plants produce secondary metabolites in limited abundance. The metabolic process in plants produce different compounds depending on the ecological interactions of plants and their environment [4].

The Clusiaceae family has one genus which spreads in the tropical forests of Southeast Asia, West Africa and North America, namely *Garcinia*. This plant has been widely used as a treatment for microbial infections, cancer and prevention of metabolic disorders such as diabetes and obesity [5][6]. The previous studies reported the compounds of genus *Garcinia* could be used as antioxidants, antifungals and antibacterials. *Garcinia mangostana* is one of the species which has some activities. They included 1,3,6,7-tetrahydroxy-2,8- (3-methyl-2-butenyl) xanthones, 1,3,6-trihydroxy-7-methoxy-2, 8- (3-methyl-2-butenyl) xanthone, epicatechin, α-mangostin, β-mangostin, γ-mangostin, 8-hydroxycubrasanton, gartanin, smeathsanton as anti-oxidants. Some compounds from *G. mangostana* which are active as anti-bacterial namely α-mangostin, γ-mangostin, garcinone D, mangostanine. Demethylcalcabalone compounds. α-mangostin, γ-mangostin is also active as an anti-fungal [7]. Some isolated compounds from the species *G. brasiliensis* yaitumorelloflavone (fukugentin) and morelloflavone-7'-O-β-D-glucosyl showed antioxidant activity [8].

One of the *Garcinia* species in Indonesia is *Garcinia latissima* Miq. It grows with the distribution area covering Seram Island, Maluku, Papua (cultivated in the Bogor Botanical Gardens). In the research conducted by Neneng Ambarwati et al., leaf extract, fruit and stem bark of *Garcinia latissima* Miq. have antimicrobial activity, antibacterial [9][10]. Whereas the research related to isolate compound was carried out by Ito at 1997 [11]. Based on the result, the biosynthesis pathaway can be made (Figure 1). The main objective of this result is to explore the chemical constituents and to complete the biosynthesis pathaway.

II. METHOD

A. General Experiment Procedures

NMR spectra were recorded on Bruker 400 AVANCE spetrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) in acetone-d_6. Column chromatography (CC) and radical chromatography (chromatotron model 7924T, Harrison Research) were carried out on silica gel 60 GF_{254} (Merck) and silica gel 60 (63-200 μm; Merck). Size-exclusion chromatography was perfomed with Sephadex LH-20 (25-100 μm; GE Healthcare). TLC analysis was used with precoated silica gel 60 GF_{254} (0.25 mm; Merck).

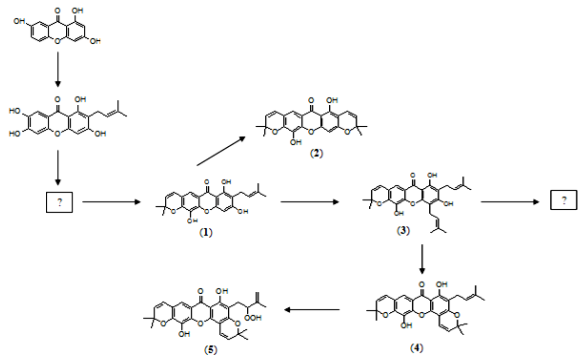

Figure 1. The Biosynthesis Pathaway.

Figure 2. The EtOAC Extract.

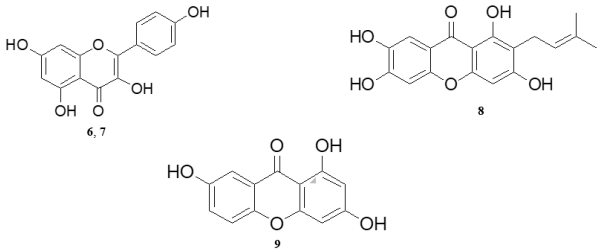

Figure 3. Isolated Compounds 1-4.

B. Plant Material

The stem bark of *Garcinia latissima* Miq. were collected from North Halmahera Islands, Indonesia. The plant was identified by Mr. Sudarsono and Mr. Ridwan from Bogor Botanical Garden Indonesia. The specimen voucher (No. IV.C.338) was deposited at the Bogor Botanical Garden, Indonesia.

C. Extraction and Isolation

The dried stems of *G. latissima* Miq. (2.0 kg) were grind into powder and extracted by maceration at room temperation with EtOAc (3 x 15 L) for three days. The solvent was evaporated under reduced pressure to obtain residue (240.0 g). The EtOAc fraction (Figure 2) was subjected to VLC on silica gel (90.0 g) using n-hexane, CH_2Cl_2, EtOAc, MeOH to obtain six fractions (a-f). Fraction d (20 g) was subjected to VLC using n-hexane: CH_2Cl_2 with gradient 10%, 20%, 30%, 50%, 100% to yield subfractions d1-d7. d6 was chromatographed on Sephadex LH-20 column eluted with CH_2Cl_2: MeOH (1:1, *v/v*) to obtain $d6_a$-$d6_b$. $d6_a$ was fractionated to CC on silica gel using gradient CH_2Cl_2: EtOAc (100:0, 0:100) to yield subfractions G1-G5. G4 was separated using Sephadex LH-20 with CH_2Cl_2: MeOH (1:1, *v/v*) to obtain $G4_1$-$G4_7$. Compound **6** (1.6 mg), **7** (1.5 mg), **8** (5.3 mg), and **9** (1.0 mg) was yielded from subfraction $G4_1$ (4.2 mg) and $G4_7$ (7.3 mg) by separation using radial chromatography (chromatotron) with n-hexane: EtOAc (75:25, *v/v*).

Table 1.
^{1}H NMR (400 Mhz) Spectroscopy Data of Compounds **6** and **7** in Acetone-D_6

Kaempferol	Compound **6** and **7**
6.27 (1H, d, *J* = 2.2 Hz, H-6)	6.27 (1H, s)
6.54 (1H, d, *J* = 2.2 Hz, H-8)	6.54 (1H, s)
8.14 (2H, d, *J* = 8.0 Hz, H-2', 6')	8.16 (2H, d, *J* = 8.8 Hz)
7.02 (2H, d, *J* = 8.0 Hz, H-3', 5')	7.02 (2H, d, *J* = 8.8 Hz)

Table 2.
^{1}H NMR (400 MHZ) Spectroscopy Data of Compounds **8** in Acetone-D_6

1,3,6,7-Tetrahydroxy-2-(3-methyl-but-2-enyl)-xanthen-9-one	Compound **8**
12.85 (1H, s, 5-OH)	13.4 (1H, s)
7.77 (1H, dd, *J* = 8 Hz, H-8)	7.48 (1H, s)
7.32 (1H, dd, *J* = 7.6 Hz, H-7)	6.84 (1H, s)
6.32 (1H, s, H-2)	6.38 (1H, s)
5.3 (1H, t, *J* = 6 Hz)	5.21 (1H, t)
1,88 (3H, s)	1,71 (3H, s)
3.57 (2H, d, *J* = 6.4 Hz)	3.29 (2H, d, *J* = 7.2 Hz)

Table 3.
^{1}H NMR (400 MHZ) Spectroscopy Data of Compounds **9** IN Acetone-D_6

1,3,7-Trihidroxyxanthone	Compound **9**
13.17 (1H, s, 5-OH)	13.0 (1H, s)
7.62 (1H, d, *J* = 8.8 Hz, H-8)	7.5 (1H,)
6.98 (1H, d, *J* = 8.8 Hz, H-7)	7.3 (1H,)
6.43 (1H, d, *J* = 2 Hz, H-2)	6.43 (1H, d, *J* = 1.6 Hz)
6.23 (1H, d, *J* = 2 Hz, H-2)	6.27 (1H, d, *J* = 1.6 Hz)

1. Kaempferol (**6**)(**7**)

Yellow powder. ^{1}H (acetone-d_6) NMR spectroscopic data, see Table 1.

2. 1,3,6,7-Tetrahydroxy-2-(3-methyl-but-2-enyl)-xanthen -9-one (**8**)

Yellow armophorus powder. ^{1}H (acetone-d_6) NMR spectroscopic data, see Table 2.

3. 1,3,7-Trihidroxyxanthone (**9**)

Yellow armophorus powder. 1H (acetone- d_6) NMR spectroscopic data, see Table 3.

III. RESULTS AND DISCUSSION

The EtOAC fraction from the stem of *G. latissima* Miq. was subjected to various chromatographic methods to yield flavonoid and xanthone. The know compounds were identified as kaempferol (**6** and **7**), 1,3,6,7-Tetrahydroxy-2-(3-methyl-but-2-enyl)-xanthen-9-one(**8**) and 1,3,7-Trihidroxyxanthone (**9**). The compounds were elucidated using ^{1}H NMR and they were compared with the literature.

Compound **6** was isolated as yellow powder. The ^{1}H NMR showed hydrogen bonded at δ_H 12.20 (1H, s, 1-OH). The aromatic group in ring A were observed at δ_H 6.27 (1H, s), 6.54 (1H, s). The proton at δ_H 8.16 (2H, d, *J* = 8.8 Hz) and 7.02 (2H, d, *J* = 8.8 Hz) showed the symmetric proton in ring B. The compound was known as kaempferol.

Compound **7** was isolated as yellow powder. The hydrogen bonded were showed at δ_H 12.20 (1H, s, 1-OH). Proton aromatic group were at δ_H 6.29 (1H, s) and 6.55 (1H, s). Compound **6** as kaempferol. It was same with compound **7.**

Compound **8** was isolated as yellow armophorus

powder. The hydrogen bonded at δ_H 13.41(1H, s, 1-OH). This compound was know as 1, 3 1,3,6,7-Tetrahydroxy-2-(3-methyl-but-2-enyl)-xanthen-9-one.

Compound **9** was isolated as yellow powder. The hydrogen bonded were showed at δ_H 13.0 (1H, s, 1-OH). This compound was known as 1,3,7-Trihidroxyxanthone.

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