1.1 INTRODUCTION

1.1.1 Introduction

Garcinia belongs to in the Guttiferae family. This genus consists of many species, which are widely used as a source of edible fruits, timber, resin and various other natural products. The family Guttiferae contains about 40 genera and over 1000 species. Only 6 genera and 60 species are found in Thailand; i.e., *Calophyllum, Cratoxylum, Garcinia, Mesua, Kayea* and *Orchrocarpus* (Panthong, *et* al., 1999). *Garcinia* species are mainly trees or shrubs, often with yellow resin. The leaves are simple, opposite, or nearly opposite, or in whorls of three, leathery, often with glandular and resinous cells. The flowers are usually male or female, occurring on separate trees, or occasionally bisexual. They are borne in panicles, in tufts or singly, in the axils of the leaves, terminally, or on the old wood, and usually have 4 sepals and petals. The male flower has an indefinite number of stamens. The female or bisexual flowers have staminodes and contain the ovary two-to many-chambered. The fruit is a fleshy berry with a leathery skin, and contains 1-4 seeds (Palmer, *et al*., 1972).

Garcinia bancana **Miq**. has various local names, such as, “Cha muang paa” (ชะมวงป่า) in the southern part of Thailand (เต็ม, 2544). *G. bancana* is a large tree 20-35 m high. The trees are found scattered in undulating lowland areas and peat swamp forests. The leaves are opposite, 15-25 cm long and 6-10 cm wide. Male flowers are creamy white and are located in clusters on small woody bosses, mainly below the leaves. The flower has 4 sepals and 4 petals. The fruit is a subglobose berry, 5 cm in diameter and turns dull orange-yellow when ripe. The seeds are embedded in the edible orange pulp.

The fruit is eaten fresh. The wood from this tree is straight and hard, and is used for furniture and for poles (Phengklai, *et al*., 1991).

Figure 1 *Garcinia bancana*

1.1.2 Review of Literatures

The genus *Garcinia* (family Guttiferae, sub-family Clusiaceae), is encountered mainly in lowland rainforests and swamp forest of the tropical world. Plants in this genus are well known to be rich in a variety of compounds, e.g., xanthones (Na Pattalung, *et al*., 1994; Likhitwitayawuid, *et al*., 1997; Likhitwitayawuid, *et al*., 1998; Cao, *et al*., 1998; Ito, *et al*., 1998; Nilar, *et al*., 2002; Ito, *et al*., 2003a; Nguyen, *et al*., 2003), benzophenones (Krishnamurthy, *et al*., 1981; Gustafson, *et al*., 1992; Sang, *et al*., 2001), biflavanoids (Kapadia, *et al*., 1994), steroides (Vieira, *et al*., 2004). Some of these exhibit various biological and pharmacological activities, e.g., cytotoxic (Cao, *et al*., 1998; Kosela, *et al*., 2000), antibacterial (Ito, *et al*., 1997), antioxidant (DPPH) (Kosela, *et al*., 2000; Ito, *et al*., 2003b; Sang, *et al*., 2001, Terashima, *et al*., 2002), anti HIV (Gustafson, *et al*., 1992; Kirk, *et al*., 1992; Kosela, *et al*., 2000; Ito, *et al*., 2003a; Ito, *et al*., 2003b), antimicrobial (Kapadia, *et al*., 1994; Na Pattalung, *et al*., 1994; Iinuma, *et al*., 1998; Likhitwitayawuid, *et al*., 1998), antitumor (Ito, *et al*., 2003a; Ito, *et al*., 2003b; Sang, *et al*., 2001), anti-inflammatory (Iinuma, *et al*., 1994; Peres, *et al*., 2000), antihepatotoxic (Kapadia, *et al*., 1994), antifungal (Gopalakrishnan, *et al*.,

1997), inhibitory effect on Epstein-Barr virus early antigen (EBV-EA) activation (Ito, *et al*., 1998; Ito, *et al*., 2003a; Ito, *et al*., 2003b).

Chemical constituents isolated from 62 species of *Garcinia* genus were reported according to information from NAPRALERT database developed by University of Illinois at Chicago, Chemical Abstracts and Dictionary of Natural Products. Several types of compounds have been isolated from some plants belonging to *Garcinia* genus, e.g. *Garcinia assigu*, *G. cowa*, *G. fusca*, *G. ovalifolia* and *G. scortechinii* were demonstrated in **Table 1**.

Table 1 Compounds isolated from the plants of *Garcinia* genus

Scientific name	**Investigated part**	**Compound**	**Bibliography**
G. assigu	Stem bark	1,5-dihydroxyxanthone, **1a** 1,3,5-trihydroxyxanthone, **1b** pancixanthone-A, **1c** assiguxanthone-A, **1d** toxyloxanthone-B, **1e** maclurin, **2a**	Ito, *et al*., 1998
	Stem bark	isogarcinol 13-*O*-methyl ether, **2b** garcinol 13-*O*-methyl ether, **2c** isogarcinol or cambogin, **2d** garcinol or camboginol, **2e** clusianone, **2f** macurin, **2a**	Ito, *et al*., 2003b

Table 1 (Continued)

Scientific name	Investigated part	Compound	Bibliography
G. cowa	Stem	1,3,6-trihydroxy-7-methoxy-8-(3,7-dimethyl-2,6-octadienyl)xanthone or rubraxanthone, **1f**	Lee, *et al*., 1977
	Latex	cowanin, **1g** cowanol, **1h** cowaxanthone, **1i** 1,3,6-trihydroxy-7-methoxy-2,5-*bis* (3-methyl-2-butenyl)xanthone, **1j** norcowanin, **1k**	Na Pattalung, *et al*., 1994
	Stem bark	7-*O*-methylgarcinone E, **1l**	Likhitwitayawuid, *et al*., 1997
	Stem bark	7-*O*-methylgarcinone E, **1l** cowanin, **1g** cowanol, **1h** cowaxanthone, **1i** *β*-mangostin, **1m**	Likhitwitayawuid, *et al*., 1998
	Leave, fruits, rinds	(-)-hydroxycitric acid, **3a** (-)-hydroxycitric acid lactone, **3b** oxalic acid, **3c** citric acid, **3d**	Jena, *et al*., 2002

Table 1 (Continued)

Scientific name	Investigated part	Compound	Bibliography
	Stem bark	(2E,6E,10E)-(+)-4*β*-hydroxy-3-methyl-5*β*-(3,7,11,15-tetramethyl-hexadeca-2,6,10,14-tetraenyl)-cyclohex-2-en-1-one, **4a** 4-(1,1-dimethyl-2-propenyl)-1,5,6-trihydroxy-3-methoxy-2-(3-methyl-2-butenyl)xanthone or nigrolineaxanthone E, **1n** rubraxanthone, **1f**	Wahyuni, *et al*., 2004.
G. fusca	Stem bark	fuscaxanthone A, **1o** fuscaxanthone B, **1p** fuscaxanthone C, **1q** fuscaxanthone D, **1r** fuscaxanthone E, **1s** fuscaxanthone F, **1t** fuscaxanthone G, **1u** fuscaxanthone H, **1v** cowaxanthone, **1i** *β*-mangostin, **1m** cowanin, **1g** rubraxanthone, **1f** *α*-mangostin, **1w**	Ito, *et al*., 2003

Table 1 (Continued)

Scientific name	Investigated part	Compound	Bibliography
		cowanol, **1h** norcowanin, **1k** 7-*O*-methylgarcinone E, **1l**	
G. ovalifolia	Leaves	guttiferone A, **2g** guttiferone E, **2h** isoxanthochymol, **2i**	Gustafson, *et al*., 1992
G. scortechinii	Twigs	scortechinone A, **1x** scortechinone B, **1y** scortechinone C, **1z**	Rukachaisirikul, *et al*., 2000
	Latex	scortechinone D, **1aa** scortechinone E, **1ab** scortechinone F, **1ac** scortechinone G, **1ad** scortechinone H, **1ae** scortechinone I, **1af** scortechinone J, **1ag** scortechinone K, **1ah** scortechinone A, **1x** scortechinone B, **1y**	Rukachaisirikul, *et al*., 2003

Structures of compounds isolated from the plants of *Garcinia* genus

1. Xanthones

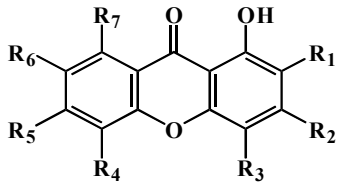


Table 2 The substituents (R_1-R_7) of xanthone

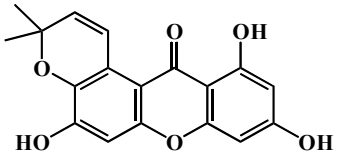
No	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Compound
1a	H	H	H	OH	H	H	H	1,5-dihydroxyxanthone
1b	H	OH	H	OH	H	H	H	1,3,5-trihydroxyxanthone
1c	H	OH	W	OH	H	H	H	pancixanthone-A
1d	H	OH	W	OH	OH	H	H	assiguxanthone-A
1g	X	OH	H	H	OH	OCH_3	Z	cowanin
1h	Y	OH	H	H	OH	OCH_3	Z	cowanol
1i	Z	OH	H	H	OH	OCH_3	H	cowaxanthone
1j	X	OH	H	X	OH	OCH_3	H	1,3,6-trihydroxy-7-methoxy-2,5-*bis* (3-methyl-2-butenyl)xanthone
1k	X	OH	H	H	OH	OH	Z	norcowanin
1l	X	OH	H	X	OH	OCH_3	X	7-*O*-methylgarcinone E
1m	X	OCH_3	H	H	OH	OCH_3	X	*β*-mangostin

W= 1,1-dimethylallyl

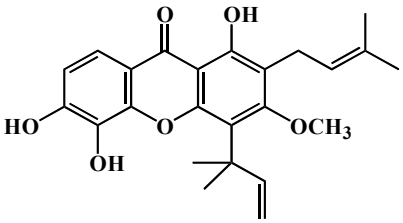
X= 3-methyl-2-butenyl or prenyl

Y= 3-methyl-4-hydroxy-2-butenyl

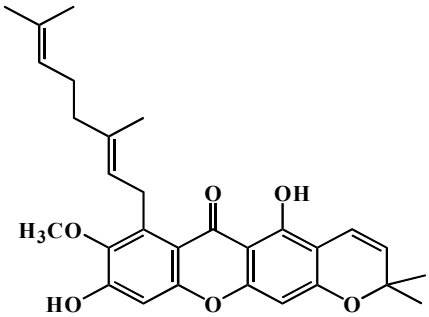
Z= 3,7-dimethyl-2,6-octadienyl or geranyl



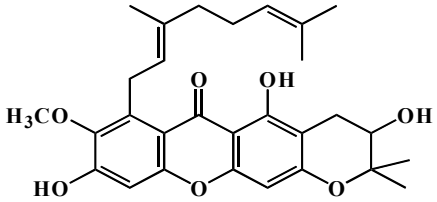
1e: toxyloxanthone-B



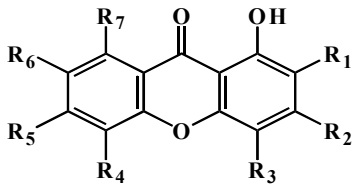
1n: nigrolineaxanthone E



1o: fuscaxanthone A

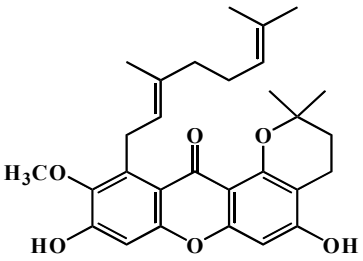


1p: fuscaxanthone B

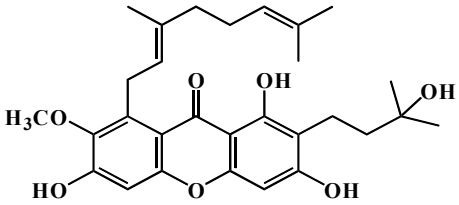


	R_1	R_2	R_3	R_4	R_5	R_6	R_7	
1f:	H	OH	H	H	OH	OCH_3	Geranyl	:rubraxanthone
1q:	Prenyl	OCH_3	H	H	OCH_3	OCH_3	Prenyl	:fuscaxanthone C
1r:	Y	OCH_3	H	H	OH	OCH_3	Prenyl	:fuscaxanthone D
1s:	H	OH	Geranyl	H	H	OH	H	:fuscaxanthone E
1t:	H	OH	H	H	H	OH	Geranyl	:fuscaxanthone F
1w:	Prenyl	OH	H	H	OH	OCH_3	Prenyl	:α-mangostin

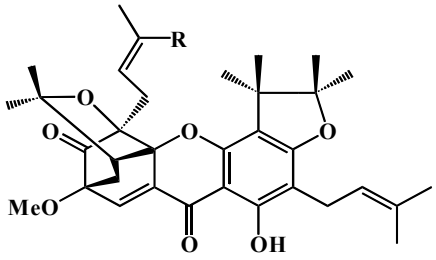
Y= 3-methyl-4-hydroxy-2-butenyl



1u: fuscaxanthone G



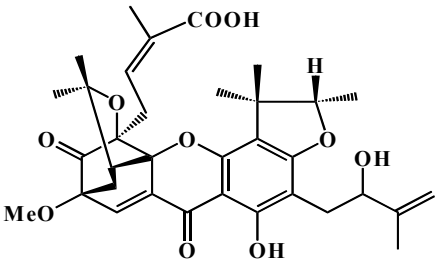
1v: fuscaxanthone H



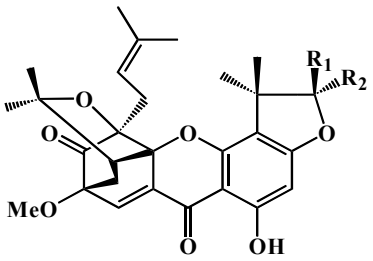
R

1x: CH_3 :scortechinone A

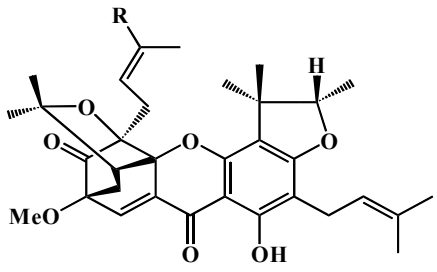
1y: CO_2H :scortechinone B



1z: scortechinone C

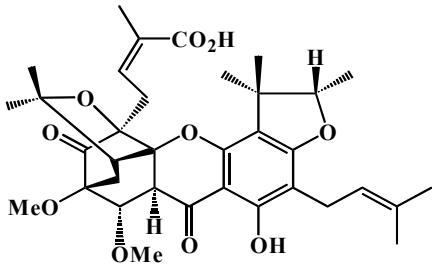


	R_1	R_2	
1aa:	Me	H	:scortechinone D
1ab:	Me	H	:scortechinone E

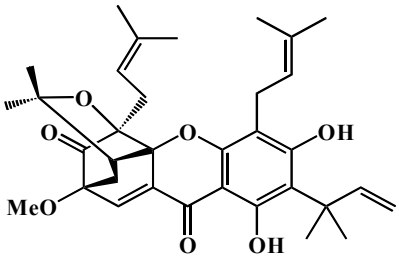


R

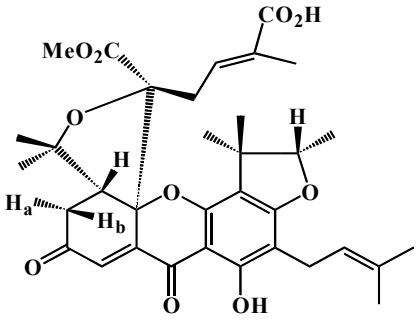
1ac:	CO_2H	:scortechinone F
1ad:	CO_2Me	:scortechinone G
1ae:	CHO	:scortechinone H



1af: scortechinone I

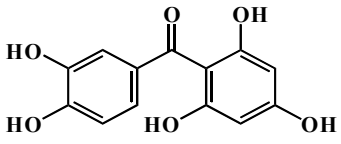


1ag: scortechinone J

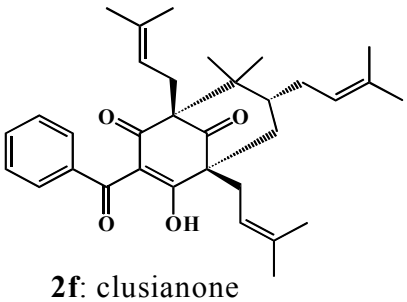


1ah: scortechinone K

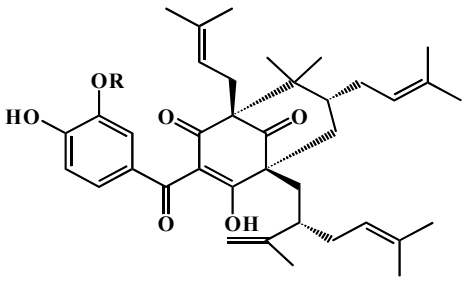
2. Benzophenones



2a: maclurin



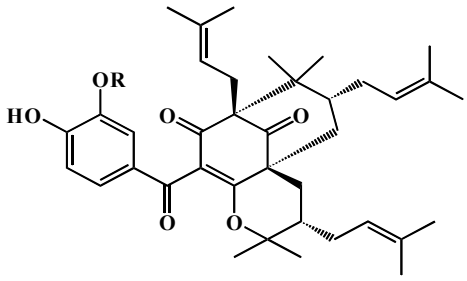
2f: clusianone



R

2c: CH_3 :garcinol 13-*O*-methyl ether

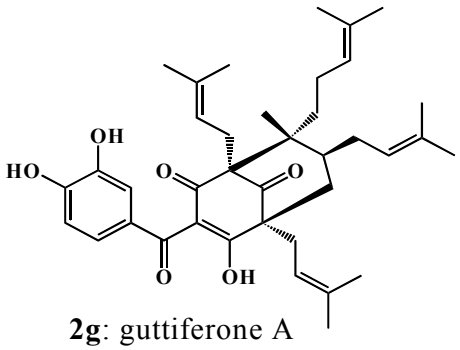
2e: H :garcinol or camboginol



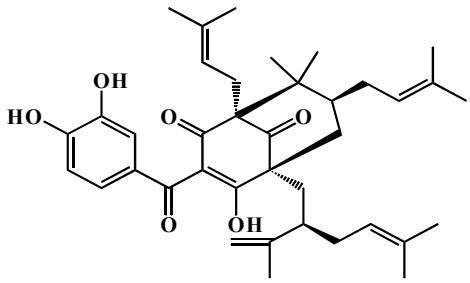
R

2b: CH_3 :isogarcinol 13-*O*-methyl ether

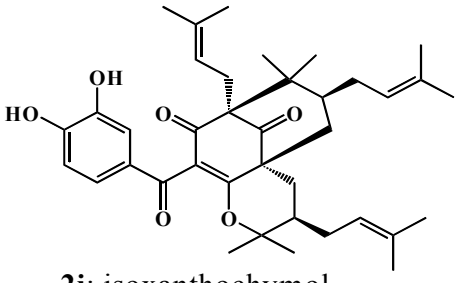
2d: H :isogarcinol or cambogin



2g: guttiferone A

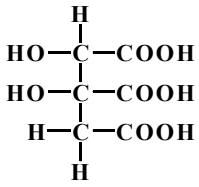


2h: guttiferone E

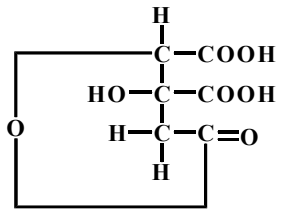


2i: isoxanthochymol

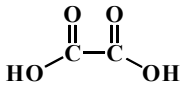
3. Acids



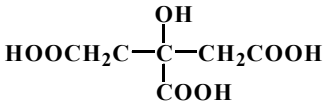
3a: (-)-hydroxycitric acid



3b: (-)-hydroxycitric acid lactone

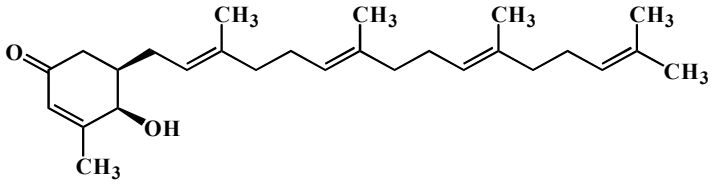


3c: oxalic acid



3d: citric acid

4. Tetraprenyltoluquinone



4a: (2E,6E,10E)-(+)-4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl)cyclohex-2-en-1-one

1.3 The Objectives

Based on NAPRALERT database, phytochemical examination on the fruits of *G. bancana* has not yet been reported. These prompted us to investigate its chemical constituents in order to provide additional information of this plant. This research involved isolation, purification and structural determination of the chemical constituents isolated from the fruits of *G. bancana*.

1.2 EXPERIMENTAL

1.2.1 Chemicals and instruments

Melting points were recorded in °C and were measured on an electrothermal melting point apparatus (Electrothermal 9100). Infrared spectra (IR) were recorded using FTS165 FT-IR spectrometer (Perkin Elmer 783). Major bands (ν) were recorded in wave number (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using UV-160A spectrophotometer (SHIMADZU). Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol solution. Nuclear magnetic resonance spectra were recorded on either 300 MHz Bruker FTNMR Ultra Shield™ spectrometer or 500 MHz Varian UNITY INOVA spectrometer. Spectra were recorded in deuterochloroform, or deuteromethanol solution and were recorded as chemical shift parameter (δ) value in ppm down field from TMS (internal standard δ 0.00). Mass spectra were recorded using MAT 95 XL Mass Spectrometer (Thermofinnigan). Spectra were recorded on high-resolution electron ionization. Optical rotation was measured in methanol solution with sodium D line (590 nm) on either an AUTOPOL® II automatic polarimeter or a JASCO DIP-370 digital polarimeter by using a 50 mm microcell (1 ml). The solvents for extraction and chromatography were distilled at their boiling point range prior to use except for petroleum ether (bp. 40-60 °C) and ethanol which were analytical grade reagent. Quick column chromatography, thin-layer chromatography (TLC), preparative thin-layer chromatography and precoated thin-layer chromatography were performed on silica gel 60 GF_{254} (Merck) or reversed-phase C-18. Column chromatography was performed on silica gel (Merck) type 100 (70–230 Mesh ASTM) or reversed-phase C-18.

1.2.2 Plant material

The fruits of Garcinia *bancana* were collected from the Pru-Toadang swamp forest, Narathiwat Province in the Southern part of Thailand. A voucher specimen has been deposited at the herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand.

1.2.3 Isolation and chemical investigation of the crude methanol extract of the fruits

The fruits of *G. bancana* were sliced into small pieces and then were extracted with methanol at room temperature, followed by filtration. The filtrates were combined and evaporated to dryness under reduced pressure to give a dark brown gum in 42.90 g. The crude methanol extract was tested for its solubility in various solvents at room temperature. The results were demonstrated in **Table 3**.

Table 3 Solubility of the crude methanol extract in various solvents at room temperature

Solvent	Solubility	Physical appearance
Petroleum ether	+++	Yellow-dark green solution
$CHCl_3$	+++	Dark yellow solution
EtOAc	++	Light yellow solution
MeOH	++++	Dark brown solution
H_2O	+	Yellow solution
10% HCl	+	Light brown solution

Table 3 (Continued)

Solvent	Solubility	Physical appearance
10% NaOH	+++	Brown-black solution
10% $NaHCO_3$	++	Brown-red solution

Symbol meaning + partially soluble ++ moderately soluble +++ well soluble ++++ very well – soluble

The solubility results indicated that the methanol extract contained non-polar and polar chemical constituents. Some constituents might be acidic.

The crude methanol extract (42.90 g) was separated by quick column chromatography over silica gel using 100% hexane, chloroform-hexane gradient, 100% chloroform, methanol-chloroform gradient and finally with 100% methanol. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford thirteen fractions. The results were shown in **Table 4**.

Table 4 Fractions obtained from the crude methanol extract by quick column chromatography over silica gel

Fraction	Eluent	Weight (g)	Physical appearance
A	100% Hexane	0.1660	Bright yellow gum mixed with yellow solid
B	2-70% $CHCl_3$/Hexane	0.7505	Yellow gum
C	100% $CHCl_3$- 5% MeOH/$CHCl_3$	8.5537	Green-yellow gum
D	5% MeOH/$CHCl_3$	4.9845	Brown-yellow gum
E	5-10% MeOH/$CHCl_3$	4.8461	Brown gum mixed with brown solid
F	10% MeOH/$CHCl_3$	1.3323	Pale brown gum
G	10% MeOH/$CHCl_3$	3.3980	Brown-black gum mixed with black solid
H	30% MeOH/$CHCl_3$	3.5719	Brown-black gum
I	30% MeOH/$CHCl_3$	3.5687	Brown gum
J	30% MeOH/$CHCl_3$	1.5407	Brown gum
K	30-50% MeOH/$CHCl_3$	1.7245	Brown-red gum
L	50% MeOH/$CHCl_3$	0.5464	Brown-red gum
M	70% MeOH/$CHCl_3$- 100% MeOH	2.9578	Brown gum

Fraction A showed no major UV-active spot on normal phase TLC with 5% acetone in hexane as a mobile phase (3 runs). It was not further investigated.

Fraction B showed four spots under UV-S on normal phase TLC with 5% acetone in hexane as a mobile phase (3 runs) with the R_f values of 0.32, 0.47, 0.57 and 0.68. Further separation by column chromatography over silica gel using 100% hexane and increasing the polarity with acetone until 100% acetone and finally increasing the polarity with methanol until 50% methanol in acetone. Fractions with the similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six fractions, as shown in **Table 5**.

Table 5 Fractions obtained from **fraction B** by column chromatography over silica gel

Fraction	Eluent	Weight (g)	Physical appearance
B1	100% Hexane	0.1207	Yellow gum
B2	100% Hexane	0.3297	Yellow-orange gum
B3	2-10% Acetone/Hexane	0.1670	Yellow gum
B4	30% Acetone/Hexane	0.0121	Yellow solid
B5	30% Acetone/Hexane	0.0073	Yellow gum
B6	30% Acetone/Hexane-50% MeOH/Acetone	0.0775	Brown-yellow gum

Fraction B1 showed no major UV-active spot on normal phase TLC with 2% acetone in hexane as a mobile phase (2 runs). It was not further investigated.

Fraction B2 the chromatogram characteristics on normal phase TLC with 5% acetone in hexane as a mobile phase (2 runs) showed four spots under UV-S with the R_f values of 0.39, 0.53, 0.59 and 0.73. Further purification was performed by column chromatography over silica gel with pure hexane and increasing the polarity with

acetone until pure acetone. Subfractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to afford four subfractions, as shown in **Table 6**.

Table 6 Subfractions obtained from **fraction B2** by column chromatography over silica gel

Subfraction	Eluent	Weight (g)	Physical appearance
B2-1	100% Hexane - 5% Acetone/Hexane	0.0010	Bright yellow viscous liquid
B2-2	5-10% Acetone/Hexane	0.2266	Yellow viscous liquid
B2-3	10-30% Acetone/Hexane	0.0464	Yellow viscous liquid
B2-4	30% Acetone/Hexane-100% Acetone	0.0149	Yellow viscous liquid

Subfraction B2-1 contained no major UV-active spot on normal phase TLC with 3% acetone in hexane as a mobile phase (2 runs). It was not further investigated.

Subfraction B2-2 its chromatogram characteristics on normal phase TLC with 3% acetone in hexane as a mobile phase (2 runs) showed four spots under UV-S with the R_f values of 0.23, 0.32, 0.41 and 0.51. It was separated by column chromatography over silica gel with 100% hexane and increasing the polarity with acetone up to 100% acetone to afford five subfractions, as shown in **Table 7**.

Table 7 Subfractions obtained from **subfraction B2-2** by column chromatography over silica gel

Subfraction	Eluent	Weight (g)	Physical appearance
B2-2-1	100% Hexane	0.0005	White solid
B2-2-2	5-10% Acetone/Hexane	0.0049	Yellow gum mixed with yellow solid
B2-2-3	10-20% Acetone/Hexane	0.1639	Yellow gum mixed with yellow solid
B2-2-4	20-40% Acetone/Hexane	0.0424	Bright yellow gum mixed with yellow solid
B2-2-5	50% Acetone/Hexane-100% Acetone	0.0100	Yellow gum

Subfraction B2-2-1 contained no major UV-active spot on normal phase TLC with 10% acetone in hexane as a mobile phase (2 runs). It was not further investigated.

Subfraction B2-2-2 the chromatogram characteristics on normal phase TLC with 10% acetone in hexane as a mobile phase (2 runs) showed three major spots under UV-S with the R_f values of 0.37, 0.56 and 0.70. Because it was obtained in low quantity, it was not further investigated.

Subfraction B2-2-3 displayed two major spots under UV-S on normal phase TLC using 2% ethyl acetate in petroleum ether as a mobile phase (3 runs) with the R_f values of 0.41 and 0.66. Further purification by preparative TLC on silica gel plates

with 2% ethyl acetate in petroleum ether as a mobile phase (6 runs) afforded three bands.

Band1 to band3 were obtained as yellow viscous liquid in 17.6, 25.6 and 15.6 mg, respectively. The chromatogram of all three bands on normal phase TLC with 2% ethyl acetate in petroleum ether as a mobile phase (2 runs) showed two major spots under UV-S with the R_f values of 0.24 and 0.49. Since all bands showed two spots on normal phase TLC, no attempted investigations were performed.

Subfraction B2-2-4 displayed one major spot under UV-S on normal phase TLC with 2% ethyl acetate in petroleum ether as a mobile phase (3 runs) with the R_f value of 0.81. Further purification by preparative TLC on silica gel plates with 2% ethyl acetate in petroleum ether as a mobile phase (6 runs) afforded two bands.

Band1 was obtained as a yellow solid in 1.5 mg. Its chromatogram characteristics on normal phase TLC with 2% ethyl acetate in petroleum ether as a mobile phase (2 runs) showed one major spot under UV-S with the R_f value of 0.63. Its ^{1}H NMR spectrum indicated that it contained some impurities. Because of low quantity of this band, it was not further separated.

Band2 was obtained as a yellow solid in 3.5 mg. The chromatogram characteristics on normal phase TLC with 2% ethyl acetate in petroleum ether as a mobile phase (2 runs) showed one major spot under UV-S with the R_f value of 0.26. Its ^{1}H NMR spectral data suggested that it consisted of some impurities. Because it was obtained in low quantity, further investigation was not performed.

Subfraction B2-2-5 showed no major UV-active spot on normal phase TLC with 10% acetone in hexane (2 runs) and 20% acetone in hexane (1 run) as mobile phases. It was not further separated.

Subfraction B2-3 the chromatogram characteristics on normal phase TLC with 2% acetone in hexane (5 runs) as a mobile phase showed one major spot under UV-S with the R_f value of 0.43. It was separated by column chromatography over silica gel with pure hexane, gradually enriched with acetone until pure acetone, followed by increasing methanol up to pure methanol. Subfractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to afford two subfractions, as shown in **Table 8**.

Table 8 Subfractions obtained from **subfraction B2-3** by column chromatography over silica gel

Subfraction	Eluent	Weight (g)	Physical appearance
B2-3-1	100% Hexane	0.0090	Yellow viscous liquid
B2-3-2	5% Acetone/Hexane-100% MeOH	0.0364	Yellow viscous liquid

Subfraction B2-3-1 the chromatogram characteristics on normal phase TLC with 10% ethyl acetate in hexane as a mobile phase (3 runs) showed one major spot under UV-S with the R_f value of 0.71. Further purification by precoated TLC on silica gel plates with 5% ethyl acetate in hexane as a mobile phase (8 runs) afforded a yellow viscous liquid in 3.4 mg. Its chromatogram characteristics on normal phase TLC with 5% ethyl acetate in hexane as a mobile phase (2 runs) showed one major spot under

UV-S with the R_f value of 0.40. Its ^1H NMR spectral data indicated that it contained some impurities. Because of low quantity, no attempted separation was carried out.

Subfraction B2-3-2 showed no distinct UV-active spot on normal phase TLC with 10% ethyl acetate in hexane (3 runs) and 20% ethyl acetate in hexane (2 runs) as mobile phases. It was not further investigated.

Subfraction B2-4 the chromatogram characteristics on normal phase TLC with 20% acetone in hexane as a mobile phase (2 runs) showed long tail and no UV-active spot. Therefore, no attempted purification was performed.

Fraction B3 contained one major spot and two minor spots under UV-S on normal phase TLC with the R_f values of 0.70, 0.27 and 0.17, using 5% acetone in hexane as a mobile phase (2 runs). It was purified by column chromatography over silica gel using 100% hexane and increasing the polarity with acetone and finally with 70% acetone in hexane. Subfractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 9**.

Table 9 Subfractions obtained from **fraction B3** by column chromatography over silica gel

Subfraction	Eluent	Weight (g)	Physical appearance
B3-1	100% Hexane	0.0048	Yellow viscous liquid
B3-2	5% Acetone/Hexane	0.0244	Yellow viscous liquid

Table 9 (Continued)

Subfraction	Eluent	Weight (g)	Physical appearance
B3-3	5% Acetone/Hexane	0.0467	Yellow viscous liquid mixed with yellow solid
B3-4	5% Acetone/Hexane	0.0572	Yellow viscous liquid mixed with yellow solid
B3-5	10% Acetone/Hexane-70% Acetone/Hexane	0.0194	Yellow viscous liquid mixed with white solid

Subfraction B3-1 the chromatogram characteristics on normal phase TLC with 3% acetone in hexane as a mobile phase (2 runs) showed no major UV-active spot. Therefore, no attempted purification was carried out.

Subfraction B3-2 contained a single spot under UV-S on normal phase TLC with 5% acetone in hexane as a mobile phase (2 runs) with the R_f value of 0.57. Purified on precoated TLC with 1% acetone in hexane as a mobile phase (9 runs) afforded two bands.

Band1 was obtained as a yellow viscous liquid in 17.9 mg. Its chromatogram characteristics on normal phase TLC with 1% acetone in hexane as a mobile phase (5 runs) showed a single spot under UV-S with the R_f value of 0.50. Its ^{1}H NMR spectrum indicated that it contained some impurities. Further attempted purification by precoated TLC on silica gel plates with 5% acetone in hexane as a mobile phase (5 runs) afforded a yellow viscous liquid in 7.7 mg. It showed one major spot under UV-S on normal phase TLC with 5% acetone in hexane as a mobile phase (2 runs) with the

R_f value of 0.31. The separation was not successful because its ^{1}H NMR spectral data contained some impurities. Thus, it was not investigated further.

Band2 was obtained as a yellow viscous liquid in 6.5 mg. The chromatogram characteristics on normal phase TLC with 1% acetone in hexane as a mobile phase (5 runs) showed no spot under UV-active. Thus, it was not further purified.

Subfraction B3-3 displayed only one UV-active spot on normal phase TLC with 5% acetone in hexane as a mobile phase (2 runs) with the R_f value of 0.59 and many inseparable spots under UV-S. Because of low quantity, it was not further investigated.

Subfraction B3-4 (**GB1**) the chromatogram characteristics on normal phase TLC with 5% acetone in hexane as a mobile phase (2 runs) showed one major spot with the R_f value of 0.59 and many UV-active spots. The solid was separated by washing with methanol to give **GB1** as a white solid (1.0 mg), melting at 138.2-139.2 ° C. The chromatogram characteristics on normal phase TLC with 5% acetone in hexane as a mobile phase (3 runs) showed one distinct spot under UV-active with the R_f value of 0.59, which appeared as a purple spot in vanilin sulfuric acid reagent and subsequently heating. The ^{1}H NMR spectra results were identical to a mixture of β-sitosterol and stigmasterol **(GB1)**.

FT-IR (KBr) ν_{cm-1}	3442 (OH stretching)
^{1}H NMR ($CDCl_3$) (δppm) (300 MHz)	5.35 (*d, J* = 5.5 Hz, 2H), 5.15 (*dd, J* = 15.0, 8.4 Hz, 1H), 5.01 (*dd, J* = 15.0, 8.4 Hz, 1H), 3.52 (*m*, 2H), 2.29-2.10 (*m*), 2.03-1.90 (*m*), 1.87-1.79 (*m*), 1.67-1.52 (*m*), 1.49-1.31 (*m*), 1.25-1.07 (*m*),

1.01 (*s*), 0.93 (*s*), 0.91(*s*), 0.87-0.76 (*m*), 0.70 (*s*), 0.68 (*s*)

Subfraction B3-5 displayed no spot under UV-S on normal phase TLC with 5% acetone in hexane as a mobile phase (3 runs). No further investigation was performed.

Fraction B4 the chromatogram characteristics on normal phase TLC with 5% acetone in hexane as a mobile phase (3 runs) appeared no definite spot under UV-active. Therefore, no attempted purification was carried out.

Fraction B5 (GB2) contained one major spot and long tail under UV-S on normal phase TLC with 5% acetone in hexane as a mobile phase (3 runs) with the R_f value of 0.25. This fraction was washed with hexane to give **GB2** as a white solid (1.0 mg). Its chromatogram on normal phase TLC with 10% acetone in hexane as a mobile phase (2 runs) demonstrated a single spot under UV-S with the R_f value of 0.10. The ^{1}H NMR spectra were identical to 1-hydroxy-3,6,7-trimethoxyxanthone (**GB2)**, melting at 214.6-215.0 °C.

UV(MeOH) λ_{max} nm (log ε)	245 (4.20), 261 (4.18), 314 (4.06), 358 (3.64)
FT-IR (KBr) ν_{cm-1}	3372 (OH stretching), 1646 (C=O stretching), 1598 (C=C aromatic ring)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	13.12 (*s*, 1H), 7.57 (*s*, 1H), 6.88 (*s*, 1H), 6.41 (*d*, *J* = 2.1 Hz, 1H), 6.36 (*d*, *J* = 2.1 Hz, 1H), 4.02 (*s*, 3H), 3.97 (*s*, 3H), 3.89 (*s*, 3H)

Fraction B6 displayed many inseparable UV-active spots on normal phase TLC with 5% acetone in hexane (3 runs) and 10% acetone in hexane (3 runs) as mobile phases. Thus, no further investigation was performed.

Fraction C contained one yellow major spot and two minor spots under UV-S on normal phase TLC with 20% acetone in hexane as a mobile phase (4 runs) with the R_f values of 0.24, 0.13 and 0.09. It was further separated by column chromatography over silica gel using pure hexane, gradually enriched with ethyl acetate until pure ethyl acetate followed by increasing methanol up to pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give five fractions, as shown in **Table 10**.

Table 10 Fractions obtained from **fraction C** by column chromatography over silica gel

Fraction	Eluent	Weight (g)	Physical appearance
C1	100% Hexane-50% EtOAc/Hexane	0.1248	Yellow viscous liquid
C2	50% EtOAc/Hexane	0.0782	Green viscous liquid
C3	60% EtOAc/Hexane	3.6081	Green viscous liquid
C4	60-90 % EtOAc/Hexane-100% EtOAc	2.2644	Green viscous liquid
C5	2% MeOH/EtOAc-100% MeOH	0.7630	Green-brown viscous liquid

Fraction C1 showed no interesting spot on normal phase TLC with 30% ethyl acetate in hexane (3 runs). Therefore, no attempted investigation was performed.

Fraction C2 displayed a single spot and three minor spots on normal phase TLC with 30% ethyl acetate in hexane (3 runs) as a mobile phase with the R_f values of 0.61 for a major spot and 0.30, 0.22 and 0.09 for minor spot. Fraction C2 was separated by column chromatography over silica gel using 100% hexane containing increasing portion of ethyl acetate until 100% ethyl acetate followed by increasing methanol up to pure methanol. Subfractions with the similar chromatogram were combined and evaporated to dryness under reduced pressure to give three subfractions, as shown in **Table 11**.

Table 11 Subfractions obtained from **fraction C2** by column chromatography over silica gel

Subfraction	Eluent	Weight (g)	Physical appearance
C2-1	100% Hexane - 40% EtOAc/Hexane	0.0269	Yellow viscous liquid
C2-2	40-60% EtOAc/Hexane	0.0239	Green viscous liquid
C2-3	80% EtOAc/Hexane - 100% MeOH	0.0171	Yellow -brown viscous liquid

Subfraction C2-1 showed many inseparable spots under UV-active on normal phase TLC with 50% dichloromethane in hexane as a mobile phase (3 runs). No further separation was conducted.

Subfraction C2-2 displayed one major spot under UV-S on normal phase TLC using 50% dichloromethane in hexane as a mobile phase (3 runs) with the R_f value of 0.38. Thus, purification by preparative TLC on silica gel plates with 100% dichloromethane as a mobile phase (4 runs) gave three bands, as shown in **Table 12.**

Table 12 Bands obtained from **subfraction C2-2** by preparative TLC on silica gel plates

Band	Weight (g)	Physical appearance
1	0.0017	Yellow-orange gum
2	0.0010	Yellow gum mixed with yellow solid
3	0.0086	Yellow gum

Band1 showed one spot and long tail on normal phase TLC using 50% dichloromethane in hexane as a mobile phase (2 runs) under UV-S with the R_f value of 0.76. Because it was obtained in low quantity, no attempted investigation was conducted.

Band2 displayed a single UV-active spot on normal phase TLC with 50% dichloromethane in hexane as a mobile phase (2 runs) with the R_f value of 0.13. Based on the ^{1}H NMR spectral data, this band contained some impurities. It was not further investigated.

Band3 contained one UV-active spot on normal phase TLC using 50% dichloromethane in hexane as a mobile phase (2 runs) with the R_f value of 0.09. Its ^1H NMR spectral data indicated that it contained some impurities. No attempted purification was carried out.

Subfraction C2-3 demonstrated no major spot and long tail under UV-S on normal phase TLC with 70% dichloromethane in hexane as a mobile phase (2 runs). It was not further investigated.

Fraction C3 displayed a single yellow spot on normal phase TLC using 30% ethyl acetate in hexane (3 runs) as a mobile phase with the R_f value of 0.28. It was further purified by column chromatography over silica gel using 100% hexane containing increasing portion of ethyl acetate up to 100% ethyl acetate and finally increasing the polarity with methanol up to 100% methanol. Subfractions with the similar chromatogram were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 13**.

Table 13 Subfractions obtained from **fraction C3** by column chromatography over silica gel

Subfraction	**Eluent**	**Weight (g)**	**Physical appearance**
C3-1	2-20% EtOAc/Hexane	0.0096	Yellow viscous liquid
C3-2	30-50% EtOAc/Hexane	0.0187	Yellow viscous liquid

Table 13 (Continued)

Subfraction	Eluent	Weight (g)	Physical appearance
C3-3	60-80 % EtOAc/Hexane	0.5873	Green viscous liquid
C3-4	50% EtOAc/Hexane-100% MeOH	2.9779	Brown viscous liquid

Subfraction C3-1 the chromatogram characteristics on normal phase TLC with 10% acetone in hexane as a mobile phase (3 runs) showed one major spot under UV-S with the R_f value of 0.71. Further purification by preparative TLC on silica gel plates with 10% acetone in hexane as a mobile phase (2 runs) gave a white solid (3.2 mg). Its chromatogram showed one major spot under UV-S on normal phase TLC using 10% acetone in hexane as a mobile phase (2 runs) with the R_f value of 0.60. Based on the ^1H NMR spectral data, this band contained some impurities. It was not investigated.

Subfraction C3-2 showed one UV-active spot on normal phase TLC using 10% acetone in hexane as a mobile phase (3 runs) with the R_f value of 0.78. Further separation by preparative TLC on silica gel plates with 10% acetone in hexane as a mobile phase (2 runs) afforded two bands, as shown in **Table 14**.

Table 14 Bands obtained from **subfraction C3-2** by preparative TLC on silica gel plates

Band	Weight (g)	Physical appearance
1	0.0046	Yellow viscous liquid
2	0.0137	Yellow viscous liquid

Band1 showed many inseparable spots under UV-S on normal phase TLC with 10% acetone in hexane as a mobile phase (2 runs). Because it was obtained in low quantity, no attempted purification was carried out.

Band2 contained one major UV-active spot on normal phase TLC with 10% acetone in hexane as a mobile phase (2 runs) with the R_f value of 0.13. Its ^1H NMR spectrum indicated that it contained some impurities. Therefore, no attempted investigation was performed.

Subfraction C3-3 showed one yellow spot on normal phase TLC under UV-S using 100% chloroform as a mobile phase (2 runs) with the R_f value of 0.39. Further attempted separation was performed by column chromatography over silica gel using pure hexane and increasing the polarity by chloroform until pure chloroform and finally increasing the polarity by methanol until 80% methanol in chloroform. Subfractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to afford three subfractions, as shown in **Table 15**.

Table 15 Subfractions obtained from **subfraction C3-3** by column chromatography over silica gel

Subfraction	Eluent	Weight (g)	Physical appearance
C3-3-1	100% Hexane-80% $CHCl_3$/Hexane	0.0054	White solid
C3-3-2	100% $CHCl_3$-5% MeOH/ $CHCl_3$	0.5398	Yellow-green gum
C3-3-3	10-80% MeOH/$CHCl_3$	0.0028	Brown gum

Subfraction C3-3-1 showed no major spot under UV-active on normal phase TLC with 20% acetone in hexane as a mobile phase (3 runs). Therefore, no attempted investigation was performed.

Subfraction C3-3-2 contained a single spot under UV-S on normal phase TLC using 20% dichloromethane in hexane as a mobile phase (3 runs) with the R_f value of 0.20. This fraction was purified by column chromatography over silica gel eluted with 100% hexane and increasing the polarity by dichloromethane until 100% dichloromethane and finally increasing the polarity by methanol until 100% methanol. Subfractions with the similar chromatogram were combined and evaporated to dryness under reduced pressure to give three subfractions, as shown in **Table 16**.

Table 16 Subfractions obtained from **subfraction C3-3-2** by column chromatography over silica gel

Subfraction	Eluent	Weight (g)	Physical appearance
C3-3-2-1	100% Hexane-60% CH_2Cl_2/Hexane	0.0369	Green-brown gum
C3-3-2-2	50% CH_2Cl_2/Hexane-2% MeOH/ CH_2Cl_2	0.3360	Brown-yellow gum
C3-3-2-3	5% MeOH/ CH_2Cl_2 100% MeOH	0.0470	Brown gum

Subfraction C-3-3-2-1 showed many UV-active spots on normal phase TLC with 30% dichloromethane in hexane as a mobile phase (4 runs). No attempted purification was performed.

Subfraction C3-3-2-2 contained one major spot under UV-S on normal phase TLC using 30% dichloromethane in hexane as a mobile phase (4 runs) with the R_f value of 0.09 but its chromatogram showed many UV-active spots on reverse phase TLC using 40% water in methanol (6 runs) and 20% water in methanol (6 runs) as mobile phases. Further purification by column chromatography over reverse phase silica gel was performed. Elution was conducted with 50% water in methanol followed by increasing amount of methanol and finally with pure methanol. Subfractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to afford three subfractions, as shown in **Table 17**.

Table 17 Subfractions obtained from **subfraction C3-3-2-2** by column chromatography over reverse phase silica gel

Subfraction	Eluent	Weight (g)	Physical appearance
C3-3-2-2R1	50-80% MeOH/H_2O	0.0420	Dark brown solid mixed with brown solid
C3-3-2-2R2	80-90% MeOH/H_2O	0.3364	Dark brown liquid
C3-3-2-2R3	100% MeOH	0.0045	Green-yellow gum

Subfraction C3-3-2-2R1 showed one pale yellow spot under UV-active on normal phase TLC with 30% dichloromethane in hexane as a mobile phase (4 runs)

with the R_f value of 0.04. The ^1H NMR spectral data indicated that they contained some impurity. This subfraction was not further separated because of limitation of time.

Subfraction C3-3-2-2R2 showed many UV-active spots on reverse phase TLC with 50% water in methanol (7 runs) as a mobile phase. Because of limitation of time, it was not conducted further.

Subfraction C3-3-2-2R3 showed many inseparable spots under UV- S on reverse phase TLC using 50% water in methanol (7 runs) as a mobile phase. Because of low quantity, no attempted purification was carried out.

Subfraction C3-3-2-3 showed long tail under UV-active on normal phase TLC with 30% dichloromethane in hexane as a mobile phase (4 runs). No attempted investigation was performed.

Subfraction C3-3-3 contained no UV-active spot on normal phase TLC with 30% acetone in hexane as a mobile phase (3 runs). Therefore, it was not further separated.

Subfraction C3-4 showed no definite UV-active spot on normal phase TLC using 10% acetone in hexane as a mobile phase (3 runs). No attempted purification was conducted.

Fraction C4 showed four UV-active spots on normal phase TLC using 30% ethyl acetate in hexane as a mobile phase (3 runs) with the R_f values of 0.04, 0.09, 0.19 and 0.24. Further separation by column chromatography over silica gel was performed.

Elution was conducted initially with hexane, followed by increasing the polarity with ethyl acetate until 100% ethyl acetate and then increasing amount of methanol up to 100% methanol. Subfractions with the similar chromatogram were combined and evaporated to dryness under reduced pressure to afforded four subfractions, as shown in **Table 18**.

Table 18 Subfractions obtained from **fraction C4** by column chromatography over silica gel

Subfraction	Eluent	Weight (g)	Physical appearance
C4-1	100% Hexane - 30% EtOAc/Hexane	0.0996	Yellow gum mixed with yellow solid
C4-2	30% EtOAc/Hexane	0.7318	Yellow-brown gum
C4-3	40% EtOAc/Hexane - 100%EtOAc	1.2118	Green-yellow gum mixed with yellow solid
C4-4	2% MeOH/EtOAc - 100% MeOH	0.4258	Dark brown gum

Subfractions C4-1 showed no definite spot under UV-active on normal phase TLC using 20% ethyl acetate in hexane (11 runs) as a mobile phase. No further purification was attempted.

Subfraction C4-2 showed many inseparable spots under UV-active on normal phase TLC using 20% ethyl acetate in hexane (11 runs) as a mobile phase. It was not further investigated.

Subfraction C4-3 was recrystallized using acetone in hexane, upon standing at 10 °C, afforded a yellow solid (**GB3**, 0.0190 g**)**, melting at 232.5-233.2 °C. Its chromatogram showed only one spot on normal phase TLC under UV-S with the R_f value of 0.36 using 20% ethyl acetate in hexane as a mobile phase.

$[\alpha]_D^{29}$	-231.58° (c = 0.19, ethanol)
UV(MeOH) λ_{max} nm (log ε)	233 (4.15), 276 (4.27), 317 (3.87)
FT-IR (KBr) ν_{cm-1}	3468 and 3369 (OH stretching), 1719, 1681 and 1606 (C=O stretching)
^{1}H NMR ($CDCl_3/CD_3OD$) (δ ppm) (300 MHz)	7.34 (*d*, *J* = 2.1 Hz, 1H), 6.98 (*dd*, *J* = 8.1, 2.1 Hz, 1H), 6.73 (*d*, *J* = 8.1 Hz, 1H), 5.17 (*m*, 1H), 4.88 (*m*, 1H), 4.86 (*m*, 1H), 3.03 (*dd*, *J* = 14.1, 3.6 Hz, 1H), 2.67-2.59 (*m*, 1H), 2.65-2.56 (*m*, 1H), 2.41 (*dd*, *J* = 13.2, 5.1 Hz, 1H), 2.25 (*d*, *J* = 14.4 Hz, 1H), 2.21-2.15 (*m*, 1H), 2.12-1.97 (*m*, 2H), 1.96 (*dd*, *J* = 14.4, 7.5 Hz, 1H), 1.43 (*m*, 1H), 1.39 (*m*, 1H), 1.75 (*s*, 3H), 1.66 (*s*, 3H), 1.65 (*s*, 3H), 1.58 (*s*, 3H), 1.57 (*s*, 3H), 1.55 (*s*, 3H), 1.22 (*s*, 3H), 1.14 (*s*, 3H), 0.96 (*s*, 3H), 0.98-0.90 (*m*, 1H), 0.89 (*s*, 3H)
^{13}C NMR ($CDCl_3/CD_3OD$) (δ ppm) (75 MHz)	207.23, 194.35, 193.22, 171.48, 150.36, 144.35, 134.62, 133.64, 133.01, 130.01, 125.19, 124.92, 123.96, 121.38, 119.64, 114.27, 114.15, 86.59, 68.21, 51.22, 46.26, 46.15, 42.80, 39.42, 29.61, 29.25, 28.63, 28.33, 26.72, 26.02, 25.84, 25.71, 25.51, 22.42, 21.21, 18.03, 17.95, 17.89

DEPT (135°) ($CDCl_3$) CH : 124.92, 123.96, 121.38, 119.64, 114.27, 114.15, 46.26, 42.80

CH_2 : 39.42, 29.61, 29.25, 28.33, 25.51

CH_3 : 28.63, 26.72, 26.02, 25.84, 25.71, 22.42, 21.21, 18.03, 17.95, 17.89

Subfraction C4-4 contained many UV-active spots on normal phase TLC with 30% ethyl acetate in hexane (5 runs) as a mobile phase. It was not conducted further.

Fraction C5 showed no distinct spot on normal phase TLC using 50% ethyl acetate in hexane (2 runs) and 70% ethyl acetate in hexane (1 runs) as mobile phases. Therefore, further separation was not performed.

Fractions D to M displayed no definite spot on normal phase TLC using 20% acetone in hexane (2 runs) as a mobile phase. They were not further investigations because of limitation of time.

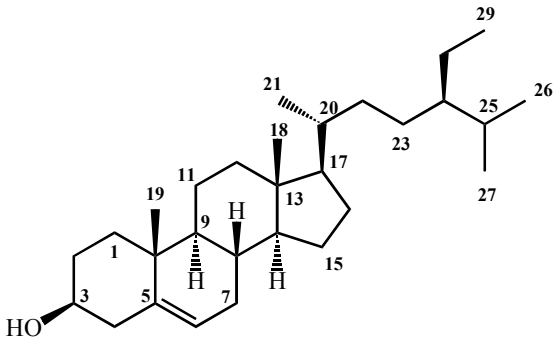
1.3 RESULTS AND DISCUSSION

The crude methanol extract from the fruits of *G. bancana* was purified by chromatographic methods to yield a mixture of of β-sitosterol and stigmasterol (**GB1**) and two pure compounds; one xanthone (**GB2**) and one benzophenone (**GB3**). Their structures were determined by 1D and 2D NMR spectroscopic data and/or comparison of ^{1}H and/or ^{13}C spectral data with those reported in the literature. The ^{13}C NMR signals were assigned from DEPT, HMQC and HMBC spectra.

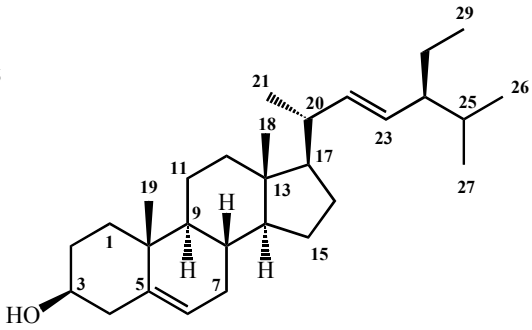
1.3.1 Structure determination of compounds isolated from the fruits of *G. bancana*

1.3.1.1 Compound GB1: β-sitosterol (1) and stigmasterol (2)

Compound **GB1** was obtained as a white solid, melting at 138.2-139.0 °C. The IR spectrum (**Figure 3**) exhibited an absorption band at 3442 cm^{-1} for a hydroxyl group. The ^{1}H NMR spectrum (**Figure 4**) showed characteristic signals of olefinic protons of stigmasterol and β-sitosterol [δ_H 5.01 (1H, *dd, J* = 15.0, 8.4 Hz), 5.15 (1H, *dd*, *J* = 15.0, 8.4 Hz) and 5.35 (2H, *d*, *J* = 5.5 Hz)]. The ratio of integral of olefinic protons at δ_H 5.01, 5.15 for stigmasterol and 5.35 for both compounds, 1:1:2, suggested that **GB1** was a mixture of β-sitosterol (**1**) and stigmasterol (**2**) in a ratio of 1:1.



(**1**)



(**2**)

1.3.1.2 Compound GB2: 1-hydroxy-3,6,7-trimethoxyxanthone

Compound **GB2** was isolated as a white solid, melting at 214.6-215.0 °C. Its UV spectrum (**Figure 5**) (λ_{max} 245, 261, 314 and 358 nm) indicated the presence of a xanthone functionality. The IR spectrum (**Figure 6**) showed absorption bands at 3372 cm^{-1} (OH) and 1646 cm^{-1} (α,β-unsaturated C=O). The ^{1}H NMR spectrum (**Figure 7**) (**Table 19**) showed the presence of a chelated hydroxy proton [δ_H 13.12 (1H, *s*)], two singlet aromatic protons [δ_H 7.57 (1H, *s*) and 6.88 (1H, *s*)], two sets of doublet signal of two *meta* aromatic protons [δ_H 6.41 (1H, *d, J* = 2.1 Hz) and 6.36 (1H, *d, J* = 2.1 Hz)] and three methoxyl groups [δ_H 4.02 (3H, *s*), 3.97 (3H, *s*) and 3.89 (3H, *s*)]. The appearance of a chelated hydroxy proton at δ_H 13.12 suggested that the hydroxyl group was located at a *peri* position (C-1) to a carbonyl group. The HMBC correlations (**Figure 9**) (**Table 19**) between the *meta* aromatic proton at δ_H 6.41 with C-2, C-3, C-4a and C-9a and the other *meta* aromatic proton at δ_H 6.36 with C-1, C-4 and C-9a established the location of two *meta* aromatic protons at C-4 and C-2, respectively. The methoxy protons (δ_H 3.89) showed a correlation with C-3, indicating the

attachment of the methoxyl group at C-3. Irradiation of the methoxy protons, in the NOE spectrum (**Figure 10**), enhanced signals of H-4 and H-2, supporting the location of the methoxyl group at C-3. A singlet signal of the lowest-field aromatic proton at δ_H 7.57 was assigned to H-8, *peri* to the carbonyl functionality, based on the HMBC correlation, with C-6, C-9 and C-10a. Irradiation of H-8 caused an NOE enhancement (**Figure 11**) of the methoxy protons (δ_H 3.97), suggesting that this methoxyl group was located at C-7. The correlation of the methoxy protons with an oxyquaternary carbon (C-7), in the HMBC spectrum (**Figure 9**) (**Table 19**), comfirmed the location of the methoxyl group. The other singlet signal at δ_H 6.88 was attributed to H-5, due to its cross peaks in HMBC spectral data (**Figure 9**) (**Table 19**) with quaternary carbons at C-6, C-7, C-8a and C-10a. The linkage of the methoxyl group at C-6 was established by a ^3J correlation between the methoxy protons (δ_H 4.02) and C-6, in HMBC data (**Figure 9)** (**Table 19**). In addition, the enhancement of the methoxy protons (δ_H 4.02, 6-OCH_3), upon irradiation at H-5 in the NOE spectrum (**Figure 12**), indicated the presence of the methoxyl group at C-6. Therefore, **GB2** was identified as 1-hydroxy-3,6,7-trimethoxyxanthone which was previously isolated from *Polygala tenuifolia* (Ikeya, *et al*., 1991).

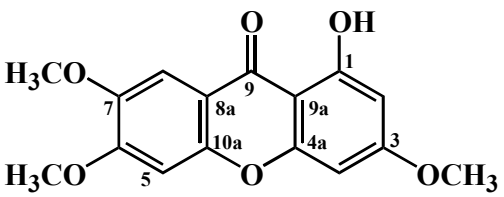


Table 19 The NMR data of compound **GB2** and 1-hydroxy-3,6,7-trimethoxy-xanthone in $CDCl_3$

Position	GB2		HMBC Correlation	1-hydroxy-3,6,7-trimethoxyxanthone[b]	
	δ_H (mult., J_{Hz})	δ_C (C-Type)		δ_H (mult., J_{Hz})	δ_C (C-Type)
1		~164 (C)[a]			163.2 (C)
1-OH	13.12 (1H, *s*)			12.98 (1H, *s*)	
2	6.36 (2H, *d*, 2.1)	96.91 (CH)	C-1, C-4, C-9a	6.33 (2H, *d*, 2.4)	107.9 (C)
3		~166 (C)[a]			166.0 (C)
3-OCH_3	3.89 (3H, *s*)	55.77 (OCH_3)	C-3	3.88 (3H, *s*)	55.8 (OCH_3)
4	6.41 (2H, *d*, 2.1)	92.59 (CH)	C-2, C-3, C-4a, C-9a	6.37 (2H, *d*, 2.4)	92.5 (CH)
4a		~157 (C)[a]			157.2 (C)
5	6.88 (1H, *s*)	99.51 (CH)	C-6, C-7, C-8a, C-10a	6.83 (1H, *s*)	99.5 (CH)
6		~156 (C)[a]			155.6 (C)
6-OCH_3	4.02 (3H, *s*)	56.51 (OCH_3)	C-6	4.00 (3H, *s*)	-
7		~147 (C)[a]			146.7 (C)
7-OCH_3	3.97 (3H, *s*)	56.38 (OCH_3)	C-7	3.98 (3H, *s*)	56.5 (OCH_3)
8	7.57 (1H, *s*)	104.62 (CH)	C-6, C-9, C-10a	7.52 (1H, *s*)	104.6 (C)
8a		113.28 (C)			113.3 (C)
9		~180 (C=O)[a]			179.8 (C=O)
9a		~104 (C)[a]			103.5 (C)
10a		~157 (C)[a]			152.4 (C)

[a] The chemical shift values of carbons were obtained from HMBC data

[b] ^1H NMR 500 MHz and ^{13}C NMR 125 MHz of 1-hydroxy-3,6,7-trimethoxyxanthone in $CDCl_3$ (Ikeya, *et al*., 1991)

1.3.1.3 Compound GB3: cambogin

Compound **GB3** was isolated as a yellow amorphous solid, melting at 232.5-233.2 °C (ref. 240 °C); $[\alpha]_D^{29}$ -231.58°, c = 0.19, ethanol (ref. $[\alpha]_D^{25}$ -211°, ethanol) (Sahu, *et al*., 1989). Its UV spectrum (**Figure 13**) (λ_{max} 233, 276 and 317 nm) revealed a chromophore with extended conjugation. Its IR spectrum (**Figure 14**) showed the presence of hydroxyl groups (3468-3369 cm^{-1}) and three carbonyl groups (1719, 1681 and 1606 cm^{-1}). The ^1H NMR spectrum (**Figure 15**) (**Table 20**) showed the presence of three aromatic protons at δ_H 7.34 (1H, *d, J* = 2.1 Hz), 6.98 (1H, *dd, J* = 8.1, 2.1 Hz) and 6.73 (1H, *d, J* = 8.1 Hz), three prenyl groups {[δ_H 5.17 (1H, *m*), 2.12-1.97 (2H, *m*), 1.75 (3H, *s*), 1.57 (3H, *s*)], [δ_H 4.88 (1H, *m*), 2.65-2.56 (1H, *m*), 2.21-2.15 (1H, *m*), 1.66 (3H, *s*), 1.65 (3H, *s*)] and [δ_H 4.86 (1H, *m*), 2.67-2.59 (1H, *m*), 2.41 (1H, *dd*, *J* = 13.2, 5.1 Hz), 1.55 (3H, *s*), 1.58 (3H, *s*)]}, four methyl groups on saturated carbons at δ_H 1.22 (3H, *s*), 1.14 (3H, *s*), 0.96 (3H, *s*) and 0.89 (3H, *s*), in addition to other protons [δ_H 3.03 (1H, *dd*, *J* = 14.1, 3.6 Hz), 2.25 (1H, *d*, *J* = 14.4 Hz), 1.96 (1H, *d*, *J* = 14.4, 7.5 Hz), 1.43 (1H, *m*), 1.39 (1H, *m*) and 0.98-0.90 (1H, *m*)]. The ^{13}C NMR spectrum (**Figure 16**) (**Table 20**) showed 38 resonances for 38 carbon atoms: fifteen quaternary carbons (δ_C 207.23, 194.35, 193.22, 171.48, 150.36, 144.35, 134.62, 133.64, 133.01, 130.01, 125.19, 86.59, 68.21, 51.22 and 46.15), eight methine carbons (δ_C 124.92, 123.96, 121.38, 119.64, 114.27, 114.15, 46.26 and 42.80), five methylene carbons (δ_C 39.42, 29.61, 29.25, 28.33 and 25.51) and ten methyl carbons (δ_C 28.63, 26.72, 26.02, 25.84, 25.71, 22.42, 21.21, 18.03, 17.95 and 17.89). These data indicated that compound **GB3** was a polyprenylated benzophenone derivative. The lowest-field aromatic proton (δ_H 7.34) showed strong cross peaks with C-10 (δ_C 193.32), C-13 (δ_C 144.35), C-14 (δ_C 150.36) and C-16 (δ_C 123.96), in the HMBC spectrum (**Figure 19**) (**Table 20**), suggesting that this aromatic proton was located at C-12 (δ_C 114.27).

These data also indicated that hydroxyl groups attached the C-13 and C-14 positions. Consequently, two other aromatic protons at δ_H 6.98 and 6.73 were attributed to H-16 and H-15, respectively, due to the HMBC correlation: H-16 with C-10 and C-15 (δ_C 114.15) and H-15 with C-11 (δ_C 130.01), C-13, C-14 and C-16. In the HMBC data, the methylene protons (δ_H 2.67-2.59 and 2.41, H-17) of the first prenyl group showed cross peaks with C-4 (δ_C 68.21), C-9 (δ_C 207.23), C-18 (δ_C 119.64) and C-19 (δ_C 134.62), indicating the location of this prenyl group at C-4. The *gem*-dimethyl protons (δ_H 1.14 and 0.96) at C-5 (δ_C 46.15) were indicated by the HMBC correlations with C-4 and C-5. The HMBC correlation between the methylene protons (δ_H 2.65-2.56, H-24) of the second prenyl group with C-6 (δ_C 46.26) and C-25 (δ_C 124.92) suggested that the second prenyl group was located at C-6. The HMQC spectrum (**Figure 18**) revealed that the carbon at δ_C 39.42 carried the methylene protons (δ_H 2.25 and 1.96), which were then attributed to H-7 due to the HMBC correlations with C-1 (δ_C 171.48), C-6 and C-8 (δ_C 51.22). The other methylene protons (δ_H 3.03 and 0.98-0.90) were assigned to H-29, in the HMBC spectrum, showed cross peaks with C-8, C-9 (δ_C 207.23), C-30 (δ_C 42.80), C-31 (δ_C 46.26) and C-34 (δ_C 29.61). The methylene carbon (C-34) also correlated to methylene protons (δ_H 2.12-1.97) of the third prenyl group, in the HMQC spectrum. The ^3J correlation between H-29 with C-34, in the HMBC data, supporting the location of the third prenyl group at C-30. The remaining *gem*-dimethyl protons (δ_H 1.22 and 0.89) at C-31 were established by the HMBC correlations with C-30 and C-31. Direct comparison of its melting point, specific rotation (Sahu, *et al*., 1989) and ^{13}C NMR data with those of cambogin previously isolated from *G. indica* (Sang, *et al*., 2002) indicated that compound **GB3** was cambogin.

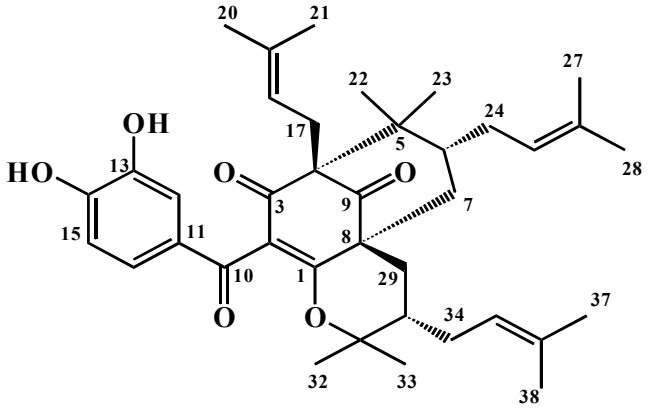


Table 20 The NMR data of compound **GB3** and cambogin in $CDCl_3/CD_3OD$

Position	GB3		HMBC Correlation	cambogin[b]
	δ_H (mult., J_{Hz})	δ_C (C-Type)		δ_C (C-Type)
1		171.48 (C)		171.9 (C)
2		125.19 (C)		124.8 (C)
3		194.35 (C)		193.3 (C)
4		68.21 (C)		68.3 (C)
5		46.15 (C)		46.2 (C)
6	1.43 (1H, *m*)	46.26 (CH)	C-4	46.1 (CH)
7	2.25 (1H, *d*, 14.4)	39.42 (CH_2)	C-1, C-6, C-8, C-9, C-24	40.8 (CH_2)
	1.96 (1H, *dd*, 14.4, 7.5)		C-1, C-6, C-8	
8		51.22 (C)		51.2 (C)
9		207.23 (C=O)		207.3(C=O)
10		193.32 (C=O)		194.7 (C=O)
11		130.01 (C)		129.9 (C)
12	7.34 (1H, *d*, 2.1)	114.27 (CH)	C-10, C-13, C-14, C-16	115.7 (CH)
13		144.35 (C)		144.9 (C)

Table 20 (Continued)

Position	GB3		HMBC Correlation	cambogin[a]
	δ_H (mult., J_{Hz})	δ_C (C-Type)		δ_C (C-Type)
14		150.36 (C)		150.8 (C)
15	6.73 (1H, *d*, 8.1)	114.15 (CH)	C-11, C-13, C-14, C-16	114.6 (CH)
16	6.98 (1H, *dd*, 8.1, 2.1)	123.96 (CH)	C-10, C-15	121.4 (CH)
17	2.67-2.59 (1H, *m*), 2.41 (1H, *dd*, 13.2, 5.1)	25.51 (CH_2)	C-4, C-9, C-18, C-19 C-4, C-18, C-19	25.8 (CH_2)
18	4.86 (1H, *m*)	119.64 (CH)		119.7 (CH)
19		134.62 (C)		134.6 (C)
20	1.58 (3H, *s*)	26.02 (CH_3)	C-21	25.7 (CH_3)
21	1.55 (3H, *s*)	17.89 (CH_3)	C-18, C-19	17.8 (CH_3)
22	1.14 (3H, *s*)	22.42 (CH_3)	C-4, C-5, C-23	22.2 (CH_3)
23	0.96 (3H, *s*)	26.72 (CH_3)	C-4, C-5, C-22	26.3 (CH_3)
24	2.65-2.56 (1H, *m*), 2.21-2.15 (1H, *m*)	29.25 (CH_2)	C-6, C-25 -	29.5 (CH_2)
25	4.88 (1H, *m*)	124.92 (CH)		125.3 (CH)
26		133.01 (C)		133.7 (C)
27	1.66 (3H, *s*)	25.84 (CH_3)	C-28	25.5 (CH_3)
28	1.65 (3H, *s*)	18.03 (CH_3)	C-25, C-26	17.9 (CH_3)
29	3.03 (1H, *dd*, 14.1, 3.6), 0.98-0.90 (1H, *m*)	28.33 (CH_2)	C-1, C-8, C-9, C-30, C-31, C-34 C-7, C-8, C-9, C-30 C-31, C-34	28.5 (CH_2)

Table 20 (Continued)

Position	GB3		HMBC Correlation	cambogin[a]
	δ_H (mult., J_{Hz})	δ_C (C-Type)		δ_C (C-Type)
30	1.39 (1H, *m*)	42.80 (CH)	C-31	41.1 (CH)
31		86.59 (C)		86.8 (C)
32	0.89 (3H, *s*)	28.63 (CH_3)	C-30, C-31, C-33	28.2 (CH_3)
33	1.22 (3H, *s*)	21.21 (CH_3)	C-30, C-31, C-32	21.1 (CH_3)
34	2.12-1.97 (2H, *m*)	29.61 (CH_2)		29.3 (CH_2)
35	5.17 (1H, *m*)	121.38 (CH)		124.9 (CH)
36		133.64 (C)		133.1 (C)
37	1.75 (3H, *s*)	25.71 (CH_3)	C-36	25.4 (CH_3)
38	1.57 (3H, *s*)	17.95 (CH_3)	C-35, C-36	17.7 (CH_3)

[a] ^{13}C NMR 150 MHz of cambogin in $CDCl_3/CD_3OD$ (Sang, *et al*., 2002)