PHYTOCHEMICAL STUDY ON THE FRUIT LATEX OF *G. MANGOSTANA* L. AND DEVELOPMENT OF THE HPLC-ESI-TOF-MS METHOD FOR QUANTITATIVE ANALYSIS OF THE XANTHONES FROM THE FRUIT HULL EXTRACT

A DISSERTATION

BY

AMORNMART JARATRUNGTAWEE

Presented in Partial Fulfillment of the Requirement for the

Doctor of Philosophy Degree in Applied Chemistry

At Srinakharinwirot University

May 2011

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AN ABSTRACT

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Amornmart Jaratrungtawee. (2011). *Phytochemical study on the fruit latex of G. mangostana L. and development of the HPLC-ESI-TOF-MS method for quantitative analysis of the xanthones from the fruit hull extract*. Dissertation, Ph.D. (Applied Chemistry). Bangkok: Graduate School, Srinakharinwirot University. Advisor Committee: Assoc. Prof. Dr. Sunit Suksamrarn, Assist. Prof. Dr. Apinya Chaivisuthangkura, Dr. Piyada Jittangprasert

Mangosteen or *Garcinia mangostana* contains many prenylated xanthones, of which exhibits biological activities and have been used as ingredients in various dietary supplements or mangosteen juice products. This study focuses on isolation of lead compound from the fruit latex and development of a method for quantitative analysis of xanthone compounds in the fruit hull extract of *G. magnostana*. Investigation of the chemical constituent of the fruit latex led to isolation of a new xanthone, mangostenone H (**105**) or 1,3,6-trihydroxy-7-methoxy-3-(1-methylethenyl)-8-(3-methylbut-2-enyl)-furano[2,3-b]xanthone, along with twelve known xanthones. including α-mangostin (**1**), β-mangostin (**2**), γ-mangostin (**3**), a mixture of gartanin (**4**) and 8-desoxygartanin (**5**), 9-hydroxycalabaxanthone (**10**), garcinone E (**26**), 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone (**12**), garcinone B (**14**), cowaxanthone D (**102**), mangostanin (**63**), garcinone D (**16**) and cratoxylone (**103**). The HPLC-ESI-TOF-MS method was employed successfully to simultaneous evaluate of eleven xanthones in the fruit hull extract of *G. mangostana,* especially the structural isomers garcinone D and cratoxylone. The separation of eleven xanthones was achieved within 16 min by using a gradient of acetonitrile and 2.00% (v/v) acetic acid. The calibration curve shows good linearity with correlation coefficient value higher than 0.99. The HPLC-ESI-TOF-MS method offered improvements on the sensitivity, compared with previously applied LC methods, with limits of detection down to 0.4 – 50 ng/mL. The % RSD value of intra-day and inter-day are in the range of 0.6 – 7.1, and 0.6 – 8.4, respectively.

การศึกษาองค์ประกอบทางเคมีจากยางผลมังคุด และพัฒนาวิธีการวิเคราะห์ปริมาณสารแซนโทนในสารสกัดเปลือกผลมังคุดโดยเทคนิค HPLC-ESI-TOF-MS

บทคัดย่อ

ของ

อมรมาศ จรัสรุ่งทวี

เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา
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สารจากธรรมชาติที่พบในมังคุดคือ สารแซนโทน ซึ่งมีฤทธิ์ทางชีวภาพที่น่าสนใจ ได้มีการนำมังคุดไปเป็นส่วนผสมในผลิตภัณฑ์อาหารเสริมต่าง ๆ และผลิตภัณฑ์น้ำมังคุดกันอย่างแพร่หลาย ในงานวิจัยนี้มุ่งเน้นการแยกสารบริสุทธิ์จากยางผลมังคุด และพัฒนาวิธีการวิเคราะห์ปริมาณสารแซนโทนในสารสกัดเปลือกผลมังคุด จากการศึกษาองค์ประกอบทางเคมีของยางผลมังคุด พบสารแซนโทนชนิดใหม่คือ mangostenone H (**105**) หรือ 1,3,6-trihydroxy-7-methoxy-3-(1-methylethenyl)-8-(3-mthylbut-2-enyl)-furano[2,3-b]xanthone รวมกับสารประกอบแซนโทนที่เคยพบแล้ว 12 ชนิดคือ α-mangostin (**1**), β-mangostin (**2**), γ-mangostin (**3**), ของผสมระหว่าง gartanin (**4**) และ 8-desoxygartanin (**5**), 9-hydroxy calabaxanthone (**10**), garcinone E (**26**), 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone (**12**), garcinnone B (**14**), cowaxanthone D (**102**), mangostanin (**63**), garcinone D (**16**) และ cratoxylone (**103**) เทคนิค HPLC-ESI-TOF-MS ที่พัฒนาขึ้นมานี้ได้นำไปใช้ในการแยกสารแซนโทน 11 ชนิดในสารสกัดเปลือกผลมังคุด โดยเฉพาะสารไอโซเมอร์เชิงโครงสร้าง garcinone D และ cratoxylone ในการแยกสารแซนโทน 11 ชนิดใช้เวลาในการแยกสาร 16 นาที โดยใช้ระบบที่เพิ่มความเป็นขั้วด้วย acetronitrile และ acetic acid เข้มข้นร้อยละ 2.00 โดยปริมาตร กราฟมาตรฐานที่ได้มีความเป็นเส้นตรง (R^2 > 0.99) ที่สามารถนำมาใช้หาปริมาณสารแซนโทนได้ เทคนิค HPLC-ESI-TOF-MS มีความไวในการตรวจวิเคราะห์มากกว่า เมื่อเทียบกับเทคนิค LC ที่เคยมีการรายงานก่อนหน้านี้ โดยมีขีดความสามารถต่ำสุดที่ตรวจวัดได้ (LOD) อยู่ในช่วง 0.4 – 50 ng/mL สำหรับค่าเบี่ยงเบนมาตรฐาน (% RSD) ของการวิเคราะห์ภายในวันเดียว และระหว่างวันมีค่าอยู่ในช่วง 0.6 - 7.1 และ 0.6 - 8.4 ตามลำดับ

The dissertation titled

"Phytochemical study on the fruit latex of *G. mangostana* L. and development of the HPLC-ESI-TOF-MS method for quantitative analysis of the xanthones from the fruit hull extract."

By

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The usefulness of this thesis, I dedicate to my mother and all the teachers who have taught me since my childhood.

Amornmart Jaratrungtawee

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CHAPTER 1

INTRODUCTION

Background

The mangosteen fruit originates in Southeast Asia, such as India, Sri Lanka, Burma, and Thailand (Peres; et al. 2000: 683-710). Mangosteen requires a year round, warm, very humid and equatorial climate. It is one of the most popular fruits named 'the queen of fruits' (Chin; et al. 2008: 754-8). It is considered as a member of the plant family Clusiaceae although it is often in earlier works referred to as a member of the Guttiferae family. The latin name for the mangosteen is *Garcinia mangostana* L. and the genus *Garcinia* is an honor bestowed upon Laurent Garcin by Linnaeus for his work as a botanist and naturalist in the 18th century.

Botanical Description

G. mangostana is an erect slow-growing tree that has a pyramidal crown as shown in figure 1. It attains 6–25 m in height and has dark-brown or nearly black bark color . The inner bark contains yellow, gummy, bitter latex.

Leaves are opposite, short-stalked. Shape is ovate oblong or elliptic. The leaves have a cuneate base, acute apex, entire margin; contain numerous veins that are joined together by a vein running parallel to a midrib. The leaves are thick, dark green and glossy above and yellowish-green beneath, 9–25 cm long and 4.5–11 cm wide. The petiole is 1.2–2.5 cm long.

The flowers are 4–5 cm in wide and may be male or hermaphrodite on the same tree. They form clusters of three to nine at the branch tips. There are four sepals and four ovate, thick, fleshy petals. The color of petals is green with red spots on the outside, yellowish-green inside. The flowers carry a lot of stamens although they bear no pollen as they have no anthers. Hermaphrodite flowers are situated singly or in pairs at the tips of young branch lets. Their petals are usually yellowish-green edged with red or mostly red and are quickly shed.

The fruit is capped by the prominent calyx at the stem end and with 4–8 triangular remnants of the stigma in a shape of a rosette at the apex. The fruit is round, smooth externally, 3.4–7.5 cm in diameter. The color is usually from dark-purple to red-purple. The rind is 6–10 mm thick, red in cross-section, purplish-white on the inside. The rind contains bitter yellow latex and purple, staining juice. The fruit may be seedless or have 1–5 fully developed seeds with ovoid-oblong shape. The seeds are usually flattened, 2.5 cm long and 1.6 cm wide. The arils of the seeds are represented as 4–8 triangular segments of white, juicy and soft flesh. The flesh is slightly or distinctly acid in flavor and is claimed as very luscious and delicious (Obolskiy; et al. 2009: 1048)

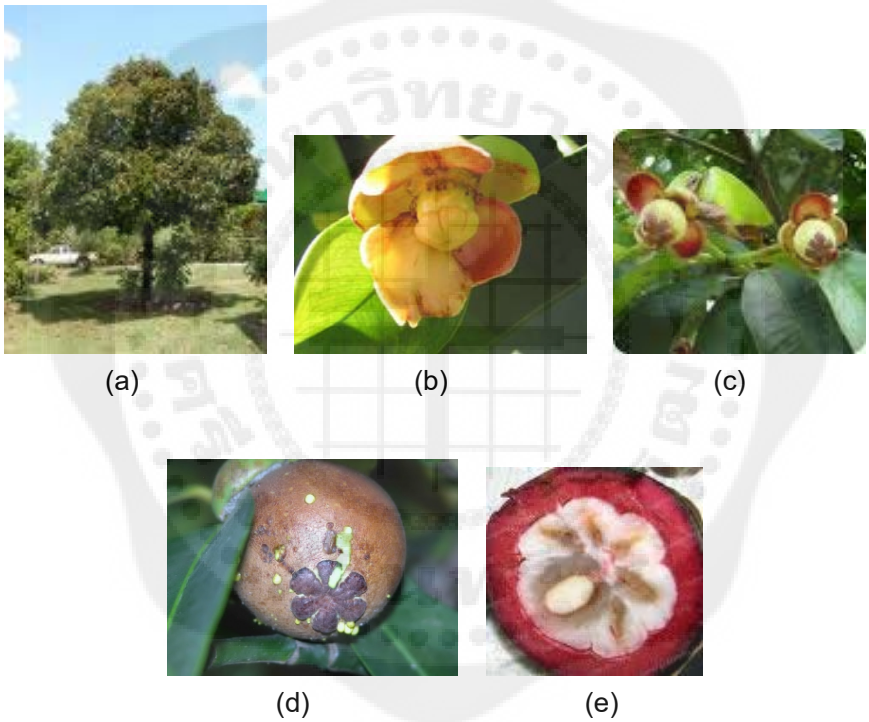


Figure 1 Photographs of mangosteen tree and state of fruit maturity
(a) mangosteen tree (b) young fruit after anthesis (c) unripe fruit
(d) ripe fruit (e) cross section of ripe fruit show aril and seed part

Ethanopharmacological Uses

The sliced and dried rind is powdered and administered to overcome dysentery. Made into an ointment, it is applied on eczema and other skin disorders. The rind decoction is taken to relieve diarrhea and cystitis, gonorrhea and gleet. It can be applied externally as

an astringent lotion. The infusion of a portion of the rind steeped in water overnight is given as a remedy for chronic diarrhea in adults and children (www.health-report.co.uk /mangosteen.htm).

Mangosteen extracts and purified constituents have been subjected to a wide array of biological tests germane particularly to infectious diseases (Voravuthikunchai; et al. 2005: 510-2), cancer chemotherapy (Akao; et al. 2008: 355-70), cancer chemoprevention (Chin; et al. 2008: 754-8) and diabetes (Muruganandan; et al. 2005: 497-501). More than eighty xanthones have been isolated from different plant parts, including 8-desoxygartanin, gartanin, garcinone C, D, E and 9-hydroxycalabaxanthone. The two most beneficial xanthones found in the mangosteen have been named as α-mangostin and γ-mangostin. Both xanthones have significant effects on cardiovascular health (Pinto; et al. 2005: 2517-38 and Sampath; et al. 2007: 336-9), antibiotic (Suksamrarn; et al. 2003: 857-9), antiviral (Chen; et al. 1996: 381-2.), and antiinflammatory (Tewtrakul; et al. 2009: 379-82.). Moreover, both xanthones from this plant are amongst the most powerful antioxidants found in nature (http://www.health-report.co.uk/mangosteen.htm).

Dharmaratne et al. studied the green fruit latex. The result showed that the latex consists of more than 75% xanthones which showed strong antibacterial (anti-MRSA and -VRE), antiinflammatory, antifungal and a number of other biological activities (Dharmaratne; et al. 2005: 239-43). It revealed that the fruit latex was another source of xanthone as same as from the fruit part.

Mangosteen is becoming a major botanical dietary supplement and has greatly increased in sales in the U.S. and around the world because the concentration levels of xanthones in mangosteen juice are rather high and their purported health benefits (http://www.mangosteenthesecret.com/mangosteen.php). The consequences of frequent intake of mangosteen botanical dietary supplements might involve significant aromatase inhibition and mangosteen-based products containing these xanthones could be specially marketed for hormone-dependent breast cancer prevention and treatment in post-menopausal women (Balunas; et al. 2008: 1161-6). Furthermore, the health-promoting benefits of mangosteen are dependent on delivery of the xanthones to target. Bioaccessibility, biotransformation and transport have been studied and the result showed

that during digestion process, α-mangostin was transfered to the aqueous fraction and used bile salt for micellarization. Cell uptake α-mangostin from micelles was dose dependent and intracellular concentrations were maximum by one hour. This result suggested that adsoption of α-mangostin was enhanced by dietary fat (Bumrungpert; et al. 2009: 54-61).

Accordingly from a number of health benefits and only two reports on fruit latex, it is interesting to study on the chemical constituents of fruit latex from *G. mangostana* and developing strategies to determine the amount of xanthones in mangosteen extract which can be applied for quality control of available mangosteen products. Thus, the fruit latex is selected for this study to search for new xanthones and of which will be used as standards in the mangosteen extracts analysis.

Objectives of the study

1. To isolate and purify components from the fruit latex of *G. mangostana*.
2. To determine the chemical structures of the isolated compounds.
3. To develop a method for determination of xanthone compounds by HPLC-ESI-TOF-MS.
4. To apply the developed method for quantification of xanthone compounds in the extract of *G. mangostana*

CHAPTER 2

REVIEW OF LITERATURES

Xanthones are secondary metabolites commonly occurring in a few higher plant families, fungi and lichens. The symmetrical nature of the xanthone nucleus coupled with its mixed biogenetic origin in higher plants necessitates that the carbons be numbered according to a biosynthetic convention. Carbons 1-4 are assigned to the acetate-derived ring A, and carbons 5-8 to the shikimate derived ring B which according to figure 2. The numbering system is based on xanthene-9-one as the basic skeleton. In cases where only ring B is oxygenated, the lowest numbers are used, except for biosynthetic discussions (Peres; et al. 2000: 683-710). Natural xanthones can be subdivided, based on the nature of substituents, into simple oxygenated xanthones, glycosylated xanthones, prenylated xanthones and their derivatives, xanthone dimers, xanthonolignoids and miscellaneous xanthones (Obolskiy; et al. 2009: 1047-65).

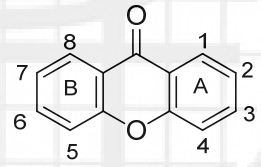


Figure 2 Structure of a xanthone

Chemical constituents of *Garcinia mangostana*

According to the previous report on *G. magostana* (Jaratrungtawee A. 2007). Xanthone derivatives are the major type of compound. However, other types of compounds including, triterpene and anthocyanidin, were also found. The compounds isolated from the different parts and various solvent extracts of mangosteen were summarized in Table 1 and Figure 3.

TABLE 1 Secondary metabolites of *G. mangostana*

References	Plant parts/ solvent extract	Compounds
Jefferson et al: 1970	Fruit hull/benzene	γ-mangostin (**3**)
Govindachari et al: 1971	Fruit hull/hexane	α-mangostin (**1**); β-mangostin (**2**); γ-mangostin (**3**); gartanin (**4**); 8-desoxy gartanin (**5**)
Du et al: 1977	Fruit hull	cyanidin-3-sophoroside (**8**); cyanidin-3-glucoside (**9**)
Sen et al: 1980	Fruit hull/ petroleum ether	1,6-dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)-6′,6′-dimethylpyrano(2′,3′:3,2) xanthone or 9-hydroxycalabaxanthone (**10**)
Sen et al: 1981	Fruit hull/ petroleum ether	α-mangostin (**1**); 1,5-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone (**11**); 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxy- xanthone (**12**)
Sen et al: 1982	Fruit hull/ dichloromethane	α-mangostin (**1**); γ-mangostin (**3**); garcinone A (**13**); garcinone B (**14**); garcinone C (**15**)
Macleod et al: 1982	Fresh fruit hull/water	Hexyl acetate; *cis*-hex-3-enyl acetate; *cis*-hex-3-en-1-ol
Sen et al:1986	Fresh fruit hull/ dichloromethane	garcinone D (**16**)
Dutta et al: 1987	Fruit hull/benzene	garcinone E (**26**)
Mahabusarakam et al: 1987	Fruit hull/benzene	α-mangostin (**1**); β-mangostin (**2**); γ-mangostin (**3**); gartanin (**4**); 1-isomangostin (**17**); 3-isomangostin (**18**); 3-isomangostin hydrate (**19**); 1-isomangostin hydrate (**20**)

TABLE 1 (continued)

References	**Plant parts/ solvent extract**	**Compounds**
Mahabusarakam et al: 1987	Aril/acetone	α-mangostin (**1**); 1,7-dihydroxy-2-(3-methyl- but-2-enyl)-3-methoxyxanthone (**12**); demethylcalabaxanthone (**21**); 1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl)xanthone (**22**); calabaxanthone (**23**); 1,7-dihydroxy-2,8-di-(3-methylbut-2-enyl)-3-methoxy xanthone (**24**)
Balasubramanian et al: 1988	Fruit hull/benzene, petroleum ether	BR-xanthone-A (**27**)
Balasubramanian et al: 1988	Fruit hull/acetone	α-mangostin (**1**); gartanin (**4**); BR-xanthone-B (**28**)
Asai et al: 1995	Fruit hull/benzene	α-mangostin (**1**); β-mangostin (**2**); γ-mangostin (**3**); gartanin (**4**); compound **11**; compound **12**; garcinone E (**26**); mangostinone (**38**)
Chairungsrilerd et al: 1996	Fresh fruit hull/ methanol	α-mangostin (**1**); γ-mangostin (**3**); gartanin (**4**); 8-desoxygar tanin (**5**); 9-hydroxycalaba xanthone (**10**); compound **23,** garcinone E (**26**); epicatechin (**40**); mangostanol (**41**)

TABLE 1 (continued)

References	Plant parts/ solvent extract	Compounds
Gopalakrishnan et al: 2000	Fruit hull/methanol	2,7-di-(3-methylbut-2-enyl)-1,3,8-trihydroxy-4-methylxanthone (**42**); 2,8-di-(3-methylbut-2-enyl)-7-carboxy -1,3-dihydroxyxanthone (**43**)
Huang et al: 2001	Fruit hull/ethanol	α-mangostin (**1**); β-mangostin (**2**); γ-mangostin (**3**); gartanin (**4**); 8-desoxygartanin (**5**); 9-hydroxycalabaxanthone (**10**); compound **11**; **12**; garcinone B (**14**); garcinone D (**16**); 1-isomangostin (**17**); 3-isomangostin (**18**); (-)-epicatechin (**40**); mangostanol (**41**); garcimagosone A (**44**); garcimangosone B (**45**); garcimangosone C (**46**); garcimangosone D (**47**); tovophyllin A (**48**); tovophyllin B (**49**); 1,3,6,7-tetrahydroxy-8-(3-methyl-2-butenyl)-*9H*-xanthone-9-one (**50**); proanthocyanidin A_1 (**51**); proanthocyanidin A_2 (**52**); procyanidin B_2 (**53**); procyanidin B_5 (**54**);

TABLE 1 (continued)

References	Plant parts/ solvent extract	Compounds
Suksamrarn et al: 2002	Young fruit/methanol	α-mangostin (**1**); β-mangostin (**2**); garcinone B (**14**); mangostinone (**38**); mangostanol (**41**); (-)-epicatechin (**40**); tovophyllin B (**49**); mangostenol (**67**); mangostenone A (**68**); mangostenone B (**69**); trapezifolixanthone (**70**)
Suksamrarn et al: 2003	Green fruit/methanol	γ-mangostin (**3**); 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone (**12**); garcinone D (**16**); mangostanin (**63**)
Suksamrarn et al: 2003	Fresh aril and seed/ methanol	α-mangostin (**1**); 9-hydroxycalabaxanthone (**10**); demethylcalabaxanthone (**21**)
Jung et al: 2006	Fruit hull/methanol	α-mangostin (**1**); γ-mangostin (**3**); gartanin (**4**); 8-desoxygartanin (**5**); garcinone D (**16**); 1-isomangostin (**17**); garcinone E (**26**); mangostinone (**38**); garcimangosone B (**45**); tovophyllin A (**48**); 8-hydroxy cudraxanthone G (**76**); mango stinone[7-methoxy-2-(3-methyl

TABLE 1 (continued)

References	Plant parts/ solvent extract	Compounds
		-2-butenyl)-8-(3-methyl-2-oxo-3-butenyl)]-1,3,6-trihydroxy xanthone (**77**); cudraxanthone G (**78**); smeathxanthone A (**79**)
Suksamrarn et al: 2006	Young fruit/ethyl acetate	α-mangostin (**1**); β-mangostin (**2**); γ-mangostin (**3**); gartanin (**4**); 8-desoxygartanin (**5**); 9-hydroxycalabaxanthone (**10**); garcinone B (**14**); garcinone C (**15**); garcinone D (**16**); demethylcalabaxanthone (**21**); garcinone E (**26**); mangostinone (**38**); mangostanol (**41**); mangostanin (**63**); mangostenone C (**80**); D (**81**); E (**82**); thwaitesixanthone (**83**); 11-hydroxy-1-isomangostin (**84**)
Chin et al: 2008	Fruit/dichloromethane	α-mangostin (**1**); γ-mangostin (**3**); mangostanin (**63**); 1,2-dihydro-1,8,10-trihydroxy-2-(2-hydroxy propan-2-yl)-9-(3-methylbut-2-enyl)furo[3,2-a]xanthen-11-one (**86**); 6-deoxy-7-demethylmangostanin (**87**)

TABLE 1 (continued)

References	Plant parts/ solvent extract	Compounds
Zhang et al: 2010	Fruit hull/ethyl acetate	garcimangosxanthonen A–C (**91-93**); α-mangostinn (**1**); γ-mangostin (**3**); garcinone C (**15**); garcinone D (**16**); trapezifolixanthone (**70**); 8-desoxygartanin (**5**); gartanin (**4**); tovophyllin A (**48**); 2-(γ,γ-dimethylallyl)-1,7-dihydroxy-3-methoxyxanthone (**12**); 1,5-dihydroxy-3-methoxy-2-prenylxanthone (**29**); garcinone B (**14**); 9-hydroxycalaba xanthone (**10**); dulxanthone D (**73**); 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (**94**)
Ryu et al: 2010	Fruit hull/ethyl acetate	mangostenone F (**95**) and mangostenone G (**96**), α-mangostin (**1**), β-mangostin (**2**), γ-mangostin (**3**), 8-desoxy gartanin (**5**), gartanin (**4**), 9-hydroxycalabaxanthone (**10**), garcinone D (**16**), mangostanol (**67**), cudraxanthone (**78**) and smeathxanthone A (**79**).

TABLE 1 (continued)

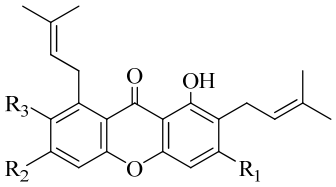
References	Plant parts/ solvent extract	Compounds
Yates et al: 1968	Latex/petroleum	α-mangostin (**1**); β-mangostin (**2**)
Dharmaratne et al: 2005	Latex/methanol	α-mangostin (**1**); β-mangostin (**2**); γ-mangostin (**3**); methoxy-β-mangostin (**95**)
Parveen et al: 1987	Leave/ethyl acetate	1,5,8-trihydroxy-2-(3-methylbut-2-enyl)-3-methoxy-xanthone (**25**)
Parveen et al: 1988	Leave/benzene	gartanin (**4**); 1,6-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxy xanthone (**29**); 1,5,8-trihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone (**25**)
Parveen et al: 1990	Leave/ethyl acetate	cycloartenol (**30**); friedelin (**31**); β-sitosterol (**32**); 9,19-cyclolanost-25-en-3β,24-diol (**33**); betulin (**34**); angiferadiol (**35**); mangiferolic acid (**36**)
Parveen et al: 1991	Leave/ethanol	3β-hydroxy-26-*nor*-9,19-cyclolanost-23-en-25-one (**37**)
Krajewski et al: 1996	Leave/methanol	2-ethyl-3-methylma-leimide-2,3,4,6-tetraacetyl-*N*-β-D-glucopyranoside (**39**)
Yates et al: 1958	Stem bark/benzene	α-mangostin (**1**)
Dharmaratne et al: 2005	Stem bark/hexane, dichloromethane	α-mangostin (**1**); β-mangostin (**2**); γ-mangostin (**3**)
Sakagami et al: 2005	Stem bark/hexane, dichloromethane	α-mangostin (**1**); β-mangostin (**2**)

TABLE 1 (continued)

References	Plant parts/ solvent extract	Compounds
Ee et al: 2006	Stem	α-mangostin (**1**); β-mangostin (**2**); garcinone D (**16**); mangostanol (**41**); mangosharin (**85**); 1,6-dihydroxy-3,7-dimethoxy-2-(3-methylbut-2-enyl)-xanthone(**97**); 9-hydroxycalabaxanthone (**10**)
Han et al: 2009	Stem bark/ dichloromethane	11-hydroxy-α-mangostin (**1**); β-mangostin (**2**); gartanin (**4**); 8-desoxygartanin (**5**); 9-hydroxycalabaxanthone (**10**); garcinone D (**16**); 3-isomangostin (**18**); mangostanin (**63**); 1-isomangostin (**84**); 11-hydroxy-3-*O*-methyl-1-isomangostin (**89**); cratoxyxanthone (**90**)
Ee et al: 2008	Stem	2,8-dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)-xanthone (**88**)
Holloway et al: 1975	Heart wood/chloroform	1,3,6,7-tetrahydroxyxanthone (**6**); maclurin (**7**)
Nilar et al: 2002	Heart wood/hot hexane	α-mangostin (**1**); β-mangostin (**2**); γ-mangostin (**3**); garciniafuran (**55**); compounds **56-63**; 6-*O*-methylmangosta nin (**64**); compounds **65-66**
Nilar et al: 2005	Heart wood/hot acetone	mangoxanthone (**71**); 3′,6-dihydroxy-2,4,4′-trimethoxy

TABLE 1 (continued)

References	**Plant parts/ solvent extract**	**Compounds**
		benzophenone (**72**); dulxanthone D (**73**); 1,3,7-tri hydroxy-2-methoxyxanthone (**74**); 1,3,5-trihydroxy-13,13-dimethyl-2*H*-pyran[7,6-*b*]xan then-9-one (**75**)
Ee et al: 2008	Root	α-mangostin (**1**); β-mangostin (**2**); γ-mangostin (**3**); gartanin (**4**); garcinone D (**16**); mangostanol (**41**)



α-mangostin (**1**): R_1=R_2=OH, R_3=OMe

β-mangostin (**2**): R_1=R_3=OMe, R_2=OH

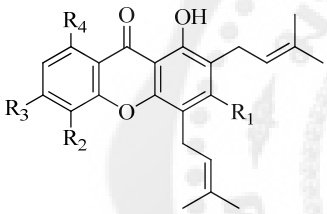
γ-mangostin (**3**): R_1=R_2=R_3=OH

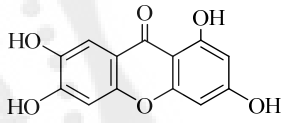
1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl)xanthone (**22**): R_1=R_3=OH, R_2=H

1,7-dihydroxy-2,8-di-(3-methylbut-2-enyl)-3-methoxy xanthone (**24**): R_1=OMe, R_2=H, R_3=OH

2,8-di-(3-methylbut-2-enyl)-7-carboxy -1,3-dihydroxyxanthone (**43**): R_1=OH, R_2=H, R_3=COOH

methoxy-β-mangostin (**95**): R_1=R_2=R_3=OMe





1,3,6,7-tetrahydroxyxanthone (**6**)

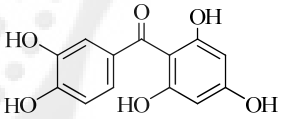
gartanin (**4**): R_3=H, R_1=R_2=R_4=OH

8-desoxygartanin (**5**): R_3=R_4=H, R_1=R_2=OH

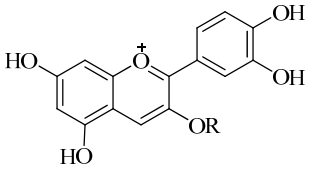
garcinone A (**13**): R_1=R_3=OH, R_2=R_4=H

8-hydroxycudraxanthone G (**76**): R_3=H, R_2=R_4=OH, R_1=OMe

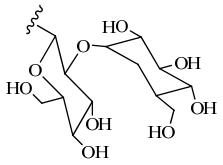
cudraxanthone G (**78**): R_3=R_4=H, R_2=OH, R_1=OMe



maclurin (**7**)



sopgorosyl =



cyanidin-3-sophoroside (**8**): R = sophorosyl

cyanidin-3-glucoside (**9**): R = glucosyl

glucosyl =

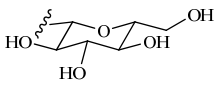
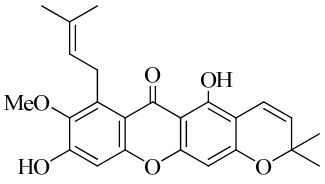
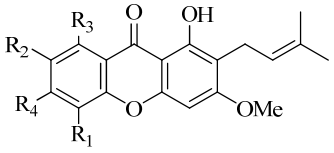


Figure 3 Structure of isolated compounds from *G. mangostana*



9-hydroxycalabaxanthone (**10**)

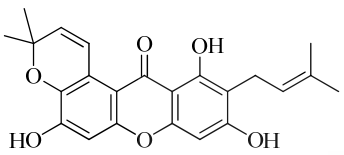


1,5-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone (**11**): R_1=OH, R_2=R_3=R_4=H

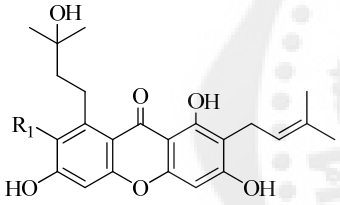
1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone (**12**): R_1=R_3=R_4=H, R_2=OH

1,5,8-trihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone (**25**): R_1=R_3=OH, R_2=R_3=R_4=H

1,6-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone (**29**): R_1=R_2=R_3=H, R_4=OH

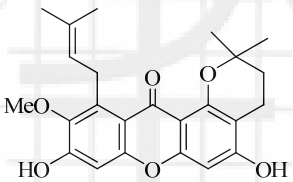


garcinone B (**14**)

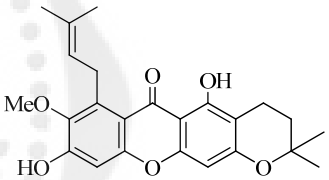


garcinone C (**15**): R=OH

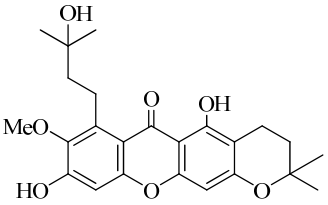
garcinone D (**16**): R=OMe



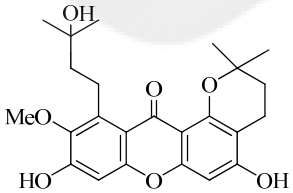
1-isomangostin (**17**)



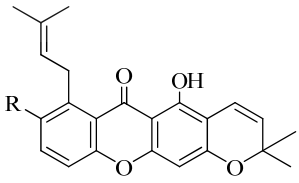
3-isomangostin (**18**)



3-isomangostin hydrate (**19**)



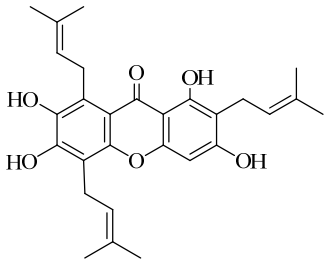
1-isomangostin hydrate (**20**)



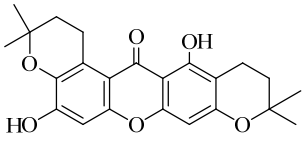
demethylcalabaxanthone (**21**): R=OH

calabaxanthone (**23**): R=OMe

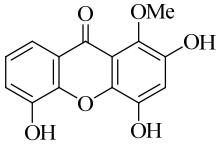
Figure 3 (continued)



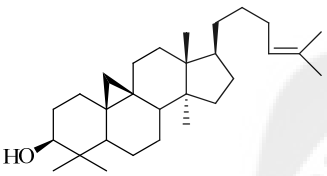
garcinone E (**26**)



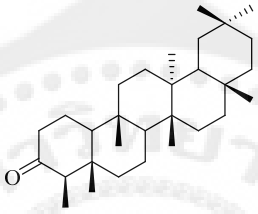
BR-xanthone A (**27**)



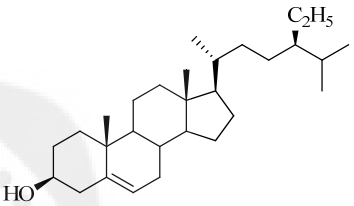
BR-xanthone B (**28**)



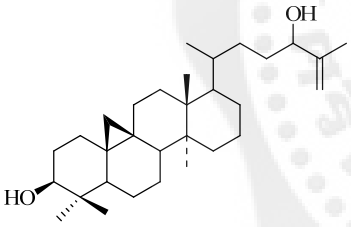
cycloartenol (**30**)



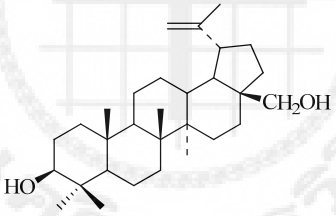
friedelin (**31**)



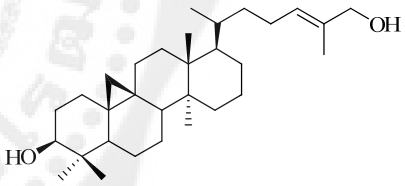
β-sitosterol (**32**)



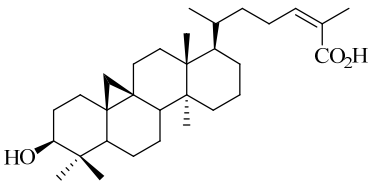
9,19-cyclolanost-25-en-3β,24-diol (**33**)



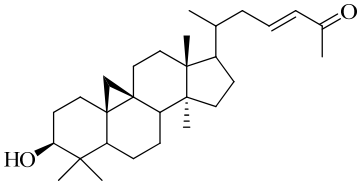
betulin (**34**)



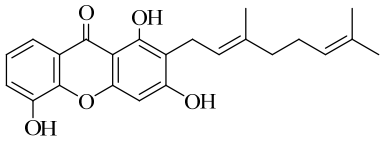
angiferadiol (**35**)



mangiferolic acid (**36**)

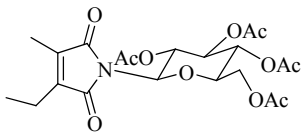


3β-hydroxy-26-nor-9,19-cyclolanost-23-en-25-one (**37**)

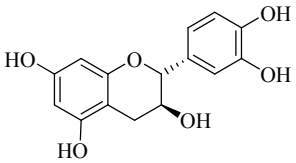


mangostinone (**38**)

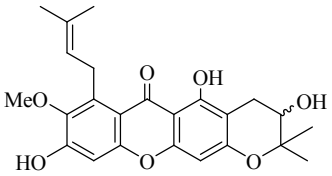
Figure 3 (continued)



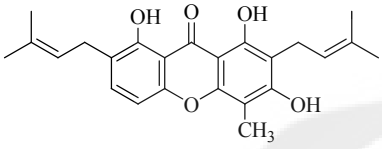
2-ethyl-3-methylma-leimide-2,3,4,6-tetraacetyl-N-β-D-glucopyranoside (**39**)



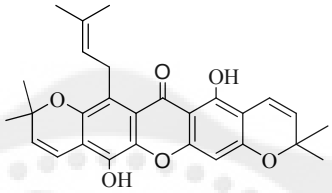
epicatechin (**40**)



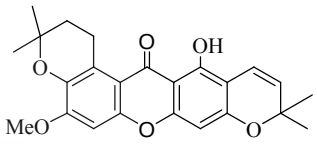
mangostanol (**41**)



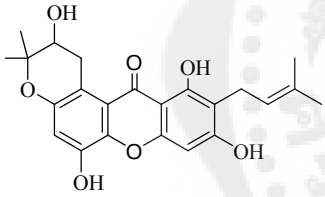
2,7-di-(3-methylbut-2-enyl)-1,3,8-trihydroxy-4-methylxanthone (**42**)



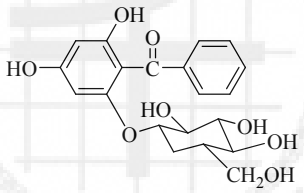
garcimangosone A (**44**)



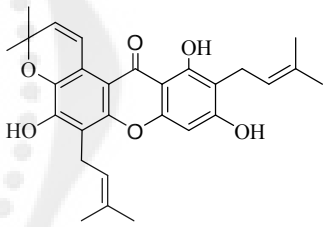
garcimangosone B (**45**)



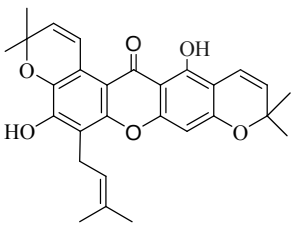
garcimangosone C (**46**)



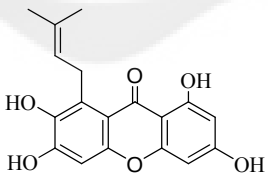
garcimangosone D (**47**)



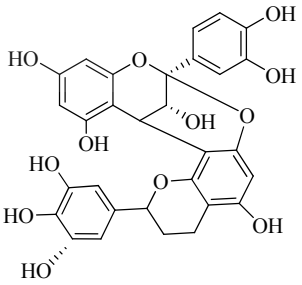
tovophyllin A (**48**)



tovophyllin B (**49**)

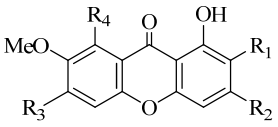


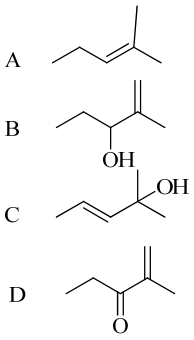
1,3,6,7-tetrahydroxy-8-(3-methyl-2-butenyl)-9*H*-xanthone-9-one (**50**)



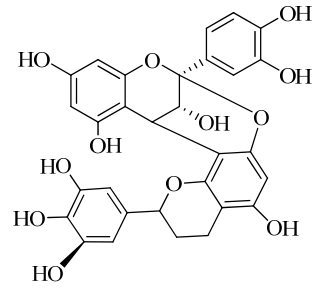
proanthocyanidin A_1 (**51**)

Figure 3 (continued)

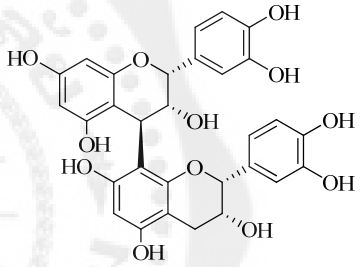




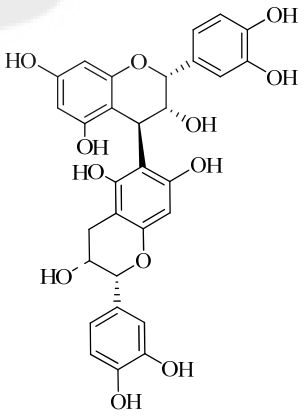
- 1-hydroxy-8-(2-hydroxy-3-methylbut-3-enyl) -3,6,7-trimethoxy-2-(3-methylbut-2-enyl)xanthone (**56**): R_1 = A, R_2 = R_3 = OMe, R_4 = B
- 1,6-dihydroxy-8-(2-hydroxy-3-methylbut-3-enyl)-3,7-dimethoxy-2-(3-methylbut-2-enyl)xanthone (**57**): R_1 = A, R_2 = OMe, R_3 = OH, R_4 = B
- 1,6-dihydroxy-2-(2-hydroxy-3-methylbut-3-enyl)-3,7-dimethoxy-8-(3-methylbut-2-enyl)xanthone (**58**): R_1 = B, R_2 = OMe, R_3 = OH, R_4 = A
- 1-hydroxy-2-(2-hydroxy-3-methylbut-3-enyl) -3,6,7-trimethoxy-8-(3-methylbut-2-enyl)xanthone (**59**): R_1 = B, R_2 = R_3 = OMe, R_4 = A
- 1,3-dihydroxy-2-(2-hydroxy-3-methylbut-3-enyl) -6,7-dimethoxy-8-(3-methylbut-2-enyl)xanthone (**60**): R_1 = B, R_2 = OH, R_3 =OMe, R_4 = A
- (16E)-1,6-dihydroxy-8-(3-hydroxy-3-methylbut-1 -enyl)-3,7-dimethoxy-2-(3-methylbut-2-enyl)xanthone (**61**): R_1 = A, R_2 = OMe, R_3 = OH, R_4 = C
- (16E)-1-hydroxy-8-(3-hydroxy-3-methylbut-1 -enyl)-3,6,7-trimethoxy-2-(3-methylbut-2-enyl)xanthone (**62**): R_1 = A, R_2 = R_3 = OMe, R_4 = C
- 1,6-dihydroxy-3,7-dimethoxy-2-(3-methylbut-2-enyl) xanthone (**65**): R_1 = A, R_2 = OMe, R_3 = R_4 = OH
- 1,6-dihydroxy-3,7-dimethoxy-2-(3-methylbut-2-enyl) -8-(2-oxo-3-methylbut-3-enyl)xanthone (**66**): R_1 = A, R_2 = OMe, R_3 = OH, R_4 = D



proanthocyanidin A_2 (**52**)

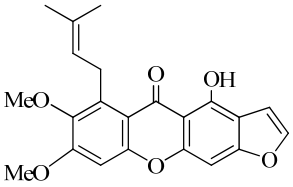


procyanidin B_2 (**53**)

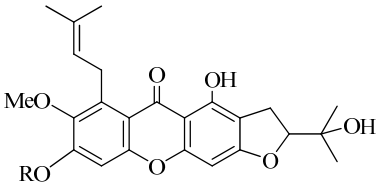


procyanidin B_5 (**54**)

Figure 3 (continued)

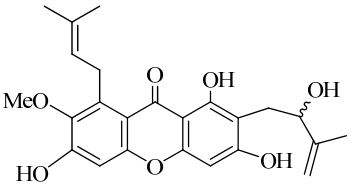


garciniafuran (**55**)

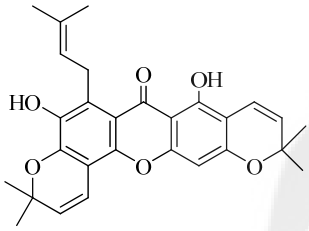


mangostanin (**63**) R = H

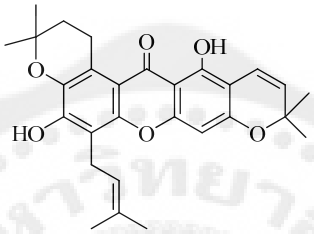
6-*O*-methylmangostanin (**64**) R = Me



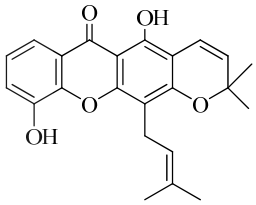
mangostenol (**67**)



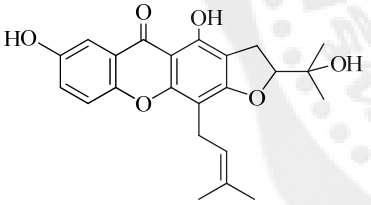
mangostenone A (**68**)



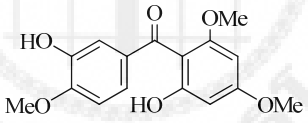
mangostenone B (**69**)



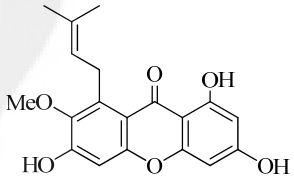
trapezifolixanthone (**70**)



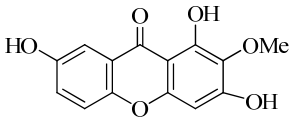
mangoxanthone (**71**)



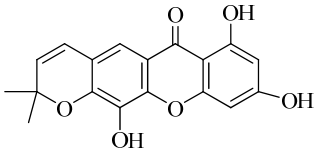
3 ,6-dihydroxy-2,4,4 -trimethoxy benzophenone (**72**)



dulxanthone D (**73**)

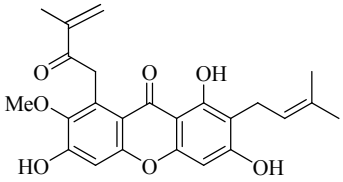


1,3,7-trihydroxy-2-methoxyxanthone (**74**)

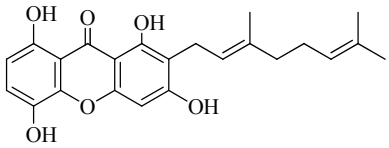


1,3,5-trihydroxy-13,13-dimethyl-2*H*-pyran[7,6-*b*]xan then-9-one (**75**)

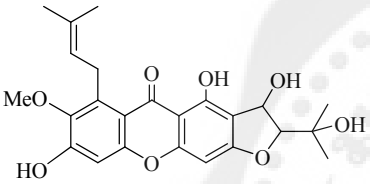
Figure 3 (continued)



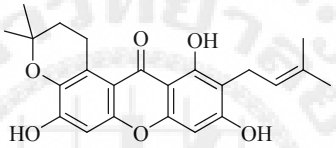
mangostinone[7-methoxy-2-(3-methyl -2-butenyl) -8-(3-methyl-2-oxo-3-butenyl)]-1,3,6-trihydroxy xanthone (**77**)



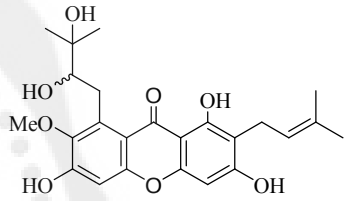
smeathxanthone A (**79**)



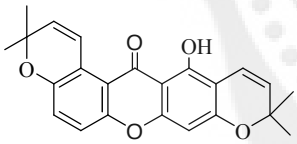
mangostenone C (**80**)



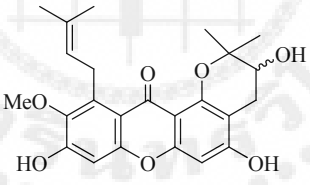
mangostenone D (**81**)



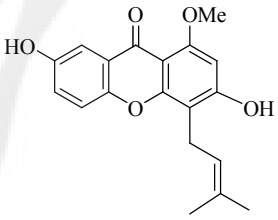
mangostenone E (**82**)



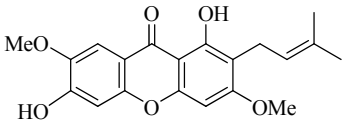
thwaitesixanthone (**83**)



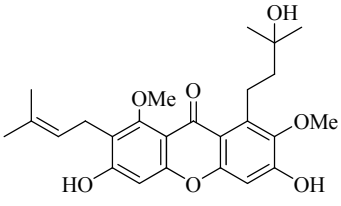
11-hydroxy-1-isomangostin (**84**)



mangosharin (**85**)

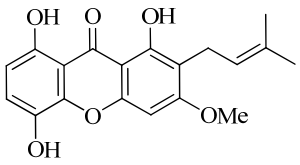


1,6-dihydroxy-3,7-dimethoxy-2-(3-methylbut-2-enyl)-xanthone (**97**)

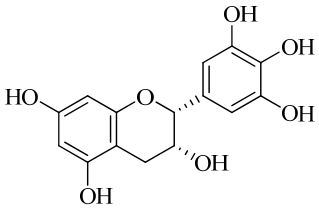


3,6-dihydroxy-1-(3-hydroxy-3-methylbutyl)-2,8-dimethoxy-7-(3-methylbut-2-enyl)-9Hxanthen-9-one (**98**)

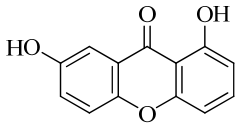
Figure 3 (continued)



1,5,8-trihydroxy-3-methoxy-2 -(3-methylbut-2-enyl) xanthone (**99**)



epigallocatechin (**100**)

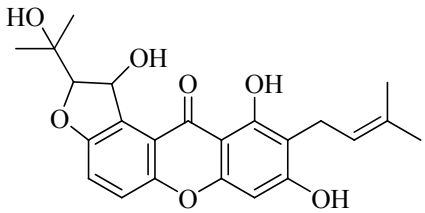


euxanthone (**101**)

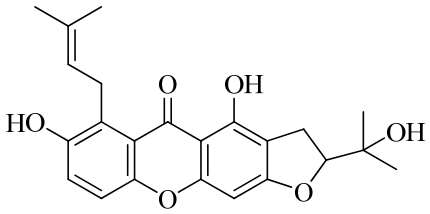
Figure 3 (continued)

In continuation of the literature search for chemical constituents, reports on *G. mangostana* have been collected as follows:

The study on dichloromethane extract of *G. mangostana* fruit, collected in China and/or southeast Asia, led to the isolation and identification of five compounds, including two new xanthones, 1,2-dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methylbut-2-enyl)furo [3,2-a]xanthen-11-one (**86**) and 6-deoxy-7-demethylmangostanin (**87**), along with three known compounds, γ-mangostin (**3**), mangostanin (**63**), and α-mangostin (**1**). The structures of compounds **86** and **87** were determined by analysis of their spectroscopic data. (Chin; et al. 2008: 754-758)

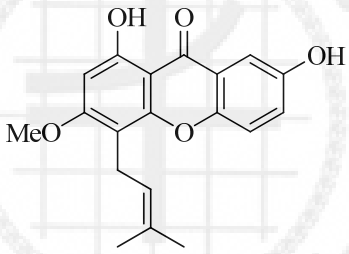


1,2-dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methylbut-2-enyl)furo[3,2-a]xanthen-11-one (**86**)



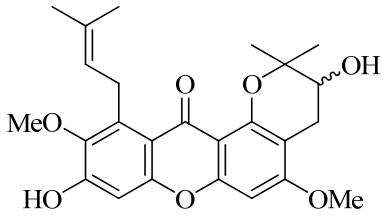
6-deoxy-7-demethylmangostanin (**87**)

The biological active compounds from the stem and root of *G. mangostana* were investigated. The stem furnished 2,8-dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)xanthone (**88**), which was a new xanthone. Meanwhile, the root bark of the same plant furnished six xanthones, namely α-mangostin (**1**), β-mangostin (**2**), γ-mangostin (**3**), garcinone D (**16**), mangostanol (**41**), and gartanin (**4**). (Ee; et al. 2008: 475-479)

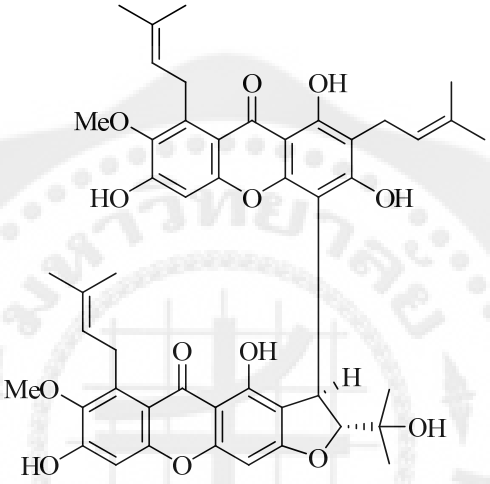


2,8-dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)xanthone (**88**)

Han et al. found a new xanthone, 11-hydroxy-3-*O*-methyl-1-isomangostin (**89**), along with 10 known compounds, including, 11-hydroxy-1-isomangostin (**84**), mangostanin (**63**), 3-isomangostin (**18**), α-mangostin (**1**), β-mangostin (**2**), garcinone D (**16**), 1,6-dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)-6′,6′-dimethylpyrano(2′,3′:3,2)xanthone (**10**), 8-desoxygartanin (**5**), gartanin (**4**), and a bixanthone, cratoxyxanthone (**90**), from the chloroform soluble extract of stem bark which was collected in Indonesia. (Han; et al. 2009: 2028-2031)

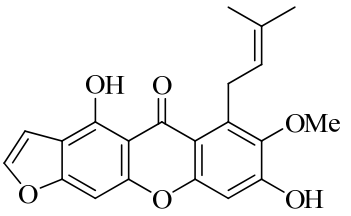


11-hydroxy-3-*O*-methyl-1-isomangostin (**89**)

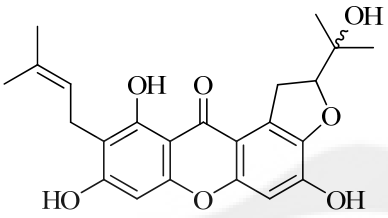


cratoxyxanthone (**90**)

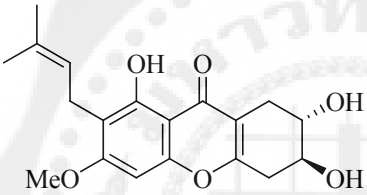
In 2010, Zhang et al. investigated on ethyl acetate extract from fruit hull of *G. mangostana* which were collected in China. It furnished two new prenylated xanthones and a new prenylated tetrahydroxanthone, garcimangosxanthonen A–C (**91-93**), along with fourteen known xanthones α-mangostinn (**1**), γ-mangostin (**3**), garcinone C (**15**), garcinone D (**16**), trapezifolixanthone (**70**), 8-desoxygartanin (**5**), gartanin (**4**), tovophyllin A (**48**), 2-(γ,γ-dimethylallyl)-1,7-dihydroxy-3-methoxyxanthone (**12**), 1,5-dihydroxy-3-methoxy-2-prenylxanthone (**29**), garcinone B (**14**), 9-hydroxycalabaxanthone (**10**), dulxanthone D (**73**) and 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)xanthone (**94**). (Zhang; et al. 2010: 595-599)



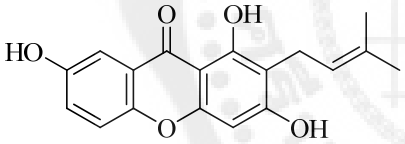
garcimangosxanthone A (**91**)



garcimangosxanthone B (**92**)

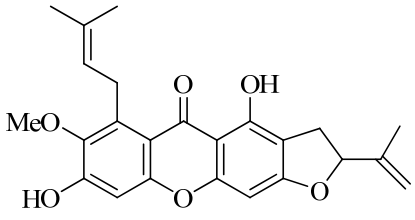


garcimangosxanthone C (**93**)

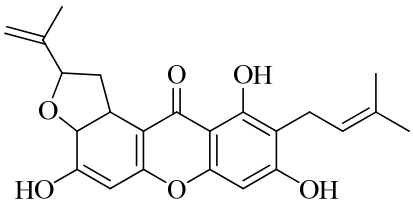


1,3,7-trihydroxy-2-(3-methylbut-2-enyl)xanthone (**94**)

Ryu studied on chloroform extract from fruit hull of *G. mangostana*, which were collected form Vietnam. The two new compounds were isolated, mangostenone F (**95**) and mangostenone G (**96**), along with ten known compounds α-mangostin (**1**), β-mangostin (**2**), γ-mangostin (**3**), 8-desoxygartanin (**5**), gartanin (**4**), 9-hydroxycalabaxanthone (**10**), garcinone D (**16**), mangostanol (**67**), cudraxanthone (**78**) and smeathxanthone A (**79**). (Ryu; et al. 2010: 6258-6264)



mangostenone F (**95**)



mangostenone G (**96**)

Analytical methods for determination of xanthone compounds from *G. mangostana*

There are various mangosteen products available in the market, such as mangosteen juice, mangosteen dietary supplement, bath & shower gel, cosmetic, which contain mangosteen fruit or the rind, or a concentrate of the mangosteen fruit. Hence, it is important to develop a method for determination of major and minor xanthone contents in this plant species for quality control of the products. Several analytical techniques have been used for the determination of xanthones which are summarized as follows:

In 1971, Jefferson et al. employed the gas chromatographic technique for quantitative analysis of trimethylsilyl ether of mangostin. This method has been achieved using a column containing 2% silicone rubber on silanised Chromosorb W (80–100 mesh). (Jefferson; et al. 1971: 2539-2543)

Comparisons of the separation selectivity and efficiency for analysis of xanthones by using various CE methods, including capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), microemulsion electrokinetic capillary chromatography (MEEKC), and capillary electrochromatography (CEC). These techniques indicated that different CE modes greatly varied in the separation selectivity and efficiency for analysis of xanthones. Moreover, compared with traditional chromatographic methods, e.g., high performance liquid chromatography (HPLC), CE shows higher separation ability and analytical speed for these xanthones. (Bo; et al. 2003: 993-1003)

The determination of six xanthones, including α-mangostin (**1**), 8-desoxygartannin (**5**), gartanin (**4**), β-mangostin (**2**), 3-isomangostin (**18**), and 9-hydroxycalabaxanthone (**10**) was achieved by HPLC-PDA technique. Separation was performed on a Phenomenex Luna C18(2) (150mm × 3.00 mm, 5μm) column. The xanthones were identified by retention time, ultraviolet (UV) spectra and quantified by LC–PDA at 320 nm. The precision of the method presented as relative standard deviation (R.S.D.), which was less than 4.6%. The recovery was in the range

from 96.58% to 113.45%. The limits of detection (LOD) for six xanthone compounds were less than 0.248 μg/mL. The identity of the peaks was further confirmed by high performance liquid chromatography with time-of-flight mass spectrometry (HPLC–TOF-MS) system coupled with electrospray ionization (ESI) interface. This method was successful in the qualitative and quantitative evaluation of four *G. mangostana* products. (Ji; et al. 2007: 1270-1276)

Walker quantified six xanthones, including α-mangostin (**1**), 8-desoxygartannin (**5**), gartanin (**4**), β-mangostin (**2**), 3-isomangostin (**18**), and 9-hydroxycalabaxanthone (**10**) was performed by HPLC-PDA technique which used Waters Nova-Pak C-18 (3.9 mm x 150 mm) with 30-min gradient of 65–90% methanol in 0.1% formic acid at the flow rate of 1.0 mL/min. Detection wavelength was 254 nm. This analytical method was applied to the analysis of these xanthones in the rind of the mangosteen fruit. (Walkerl. 2007: 1229-1234)

The evaluation of total mangostins was studied, using α-mangostin **(1)** as reference standard, in the dried powder and the ethanolic extract of the fruit rinds of *G. mangostana* collected from 13 locations from the East and the South of Thailand. The UV-spectrophotometric method (detected at 320 nm) was validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ). The linearity was found over the range of 2-20 μg/mL with regression coefficient (r^2) of 0.9999. Intra- and inter-day precisions showed relative standard deviation (%RSD) less than 2%. Accuracy of the method determined by a recovery study conducted at 3 different levels was 100.68%. The LOD and LOQ were 0.1622 and 0.4915 μg/mL, respectively. The total mangostin contents in all dried powder samples were in the range of 8.51 ± 0.05 to 11.50 ± 0.02 %w/w while in the crude ethanolic extracts were 30.19 ± 0.16 to 45.61 ± 0.09 %w/w. The average content of total mangostins (10.39 ± 1.04 % of the dried powder) from the South was higher than from the East. (Pothitirat W, et al. 2008; 161-166)

In the same year, Pothitirat et al also developed another method for determination of α-mangostin (**1**) content in the dried unripe and ripe fruit rinds of *G. mangostana* by using Thin-layer chromatography densitometric method. The method was validated for linearity, precision, accuracy, limit of detection and limit of quantitation. The linearity was found over the range of 100-500 ng/spot with regression coefficient of 0.999. Intra-day and inter-day precision studies showed the relative standard deviation was less than 2%. Accuracy of the method was determined by a recovery study conducted at 3 different levels, and the average recovery was

99.49%. The LOD and LOQ were 40 and 100 ng, respectively. The α-mangostin (**1**) content in their extract and the dried ripe fruit (16.65 ± 0.38 (4.38 ± 0.10) (%w/w) were more than the unripe fruit and their extract (10.48 ± 0.83 (3.14 ± 025) %w/w). (Pothitirat; et al. 2008: 1145-1148)

Chaivisuthangkura et al. developed an HPLC-PDA method for establish a chemical fingerprint of the fourteen xanthone compounds, including 11-hydroxy-1-isomangostin (**84**), garcinone C (**15**), garcinone D (**16**), γ-mangostin (**3**), 8-desoxygartanin (**5**), gartanin (**4**), α-mangostin (**1**), garcinone E (**26**), demethylcalabaxanthone (**21**), 9-hydroxycalabaxanthone (**10**) (10), β-mangostin (**2**), mangostenone A (**68**), calabaxanthone (**23**), and tovophyllin B (**49**), in mangosteen fruit. Separation was performed on a C18 column (150 mm x 4.6 mm, 4μm) and mobile phase consisting of acetonitrile (component A), 2%v/v acetic acid solution (component B), and n-butanol (component C). The gradient program (A:B:C) was from 30:70:0 to 45:45:10 (%v/v/v) in 2 min, from 45:45:10 to 80:15:5 in 23 min, from 80:15:5 to 80:20:0 in 2 min, from 80:20:0 to 95:5:0 in 8 min, and hold at 95:5:0 for 25 min. The flow rate was 0.5 mL/min. The total separation time of this method was 60 min. It was also applied for quantitative analysis of the two major xanthones (α- and γ-mangostin) in crude extracts of mangosteen fruit hull. (Chaivisuthangkura; et al. 2009: 315-318)

Yodhnu et al. set up a validated HPLC method for quality control and quantify of α-mangostin **(1)** from mangosteen peel extract. The assay shown good linearity (r^2 > 0.999), sensitivity (LOD = 0.02 μg/mL and LOQ = 0.08 μg/mL), precision (intra-day variation ≤ 1.8%, inter-day variation ≤ 4.3%), specificity, and with good recovery. Total analysis was 8 min. (Yodhnu; et al. 2009: 185-189)

In 2009, Zarena et al. extracted the fruit hull of *G. mangostana* by with and without alcohol as entrainer at 30MPa and 50 ˚C in supercritical carbon dioxide (SC-CO_2). It was found that entrainer increases the rate of extraction and yield substantially. SC-CO_2 and 4% ethanol at 20MPa, 40 ˚C was investigated by HPLC–ESI-MS for characterization of the xanthones. The column was RP C-18 (150×4.6 mm, 5μm) and mobile phase consisting of (A) water with 0.5% acetic acid, (B) acetonitrile with 0.5% acetic acid and (C) isopropanol, which were applied in the following gradient elution(A:B:C): 40:40:20 (% v/v/v) in 10 min to 5:70:25 in 35 min; finally in the next 10 min to 100% B at flow rate of 1 mL/min. Electrospary ionization (ESI), in negative mode was used to detect and confirmed the structure of twelve xanthone compounds,

including epigallocatechin (**99**), garcinone C (**15**), 3-isomangostin (**18**), 3,6-dihydroxy-1-(3-hydroxy-3-methylbutyl)-2,8-dimethoxy-7-(3-methylbut-2-enyl)-9H-xanthen-9-one(**97**), mangostanol (**41**), 8-desoxygartanin (**5**), gartanin (**4**), α-mangostin (**1**), garcinone E (**26**), 9-hydroxycalabaxanthone (**10**), β-mangostin (**2**) and 1,5,8-trihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone (**98**), based on the fragmentation characteristics and information available in the literature. (Zarena; et al. 2009: 330-337)

CHAPTER 3

EXPERIMENTAL

Source of Plant Material

The yellow dried fruit latex of *G. mangostana* was collected from the local market area in Bangkok, in July 2008. Dried fruit hull of *G. mangostana* was purchased from Eastern, Chanthaburi province in June 2005, and Southern, Chumphon province, areas of Thailand in February 2006.

General Techniques

1. Thin-Layer chromatography (TLC)

Technique : One dimension, ascending

Adsorbent : Silica gel 60 F_{254} precoated on aluminium plate (Merck 1.05554)

Layer Thinkness : 1.25 mm

Plate size : 1 x 5 cm and 2 x 5 cm

Detecion : 1. Spots on TLC were visualized under ultraviolet light at wavelengths of 254 and 365 nm.

2. Developing agent. Anisaldehyde-sulphuric reagent (2.5% in absoluted ethanol containing 3.4% sulphuric acid and 1.0% glacial acetic acid). After heating of TLC plate at 100-110 ^{o}C for 1-2 minutes, the spots of organic compounds will give specific colors with this reagent

2. Column chromatography (CC)

Absorbent : 1. Silica gel 60, particle size < 0.063 mm (Merck 1.07729)

2. Silica gel 60, particle size 0.040-0.063 mm (Merck 1.09385)

Packing method : Slurry packing method

Sample loading : The sample were dissolved in a small volume of suitable organic solvent. The solution were mixed with silica gel particle size < 0.063 mm or 0.040-0.063 mm, depending on the type of silica gel in column. The sample were evaporated under reduced pressure and added onto the top of column.

Elution : After loading of sample onto the column, and appropriate solvent system were used as a mobile phase in the isocratic or gradient systems.

3. Centrifugal Thin-Layer chromatography

Absorbent : Silica gel 60 PF_{254} containing $CaSO_4$ (Merck 1.07749)

Packing method : Sorbent layers on rotors are produced by casting sorbent-binder mixtures followed by scraping down to 1mm, 2mm or 4mm thickness with a roting scraping tool. Before use rotor coated, it should be dried in oven at 70°C 1 hour for activation of chromatotron rotor.

Sample loading : Dissolved the sample in a small volume (0.5-1 mL) of ethyl acetate or methanol. Turn on the rotor and introduce the sample solution with a dropper or syringe into solvent inlet. Allow a few minutes for solvent to drain from the rotor.

Elution : After loading of sample into the chromatotron, an appropriate solvent system were used as a mobile phase in the isocratic or gradient systems.

Physical Property

Melting points

Melting points were measured on Griffin melting point apparatus in degree Celsius of temperature.

Instruments

1. Infrared (IR) Absorption Spectra

IR spectra were recorded on Perkin Elmer FT-IR spectrum BX spectrophotometer by using potassium bromide (KBr) disc or film.

2. Ultraviolet (UV) Absorption Spectra

UV spectra were obtained on a Shimadzu UV-2401 PC spectrophotometer.

3. Mass Spectra

Electrospray ionization (ESI) mass spectra were measured on Finnigan LC-Q mass spectrometer.

Electrospray ionization-time-of-flight (ESI-TOF-MS) mass spectra (Bruker Daltonics GmbH, Bremen, Germany) were measured on micrOTOF-Q II, an orthogonal acceleration quadrupole time-of-flight (Q-TOF) mass sepectrometer equipped with electrospray interface. (Archemica International Co, Ltd.)

4. Nuclear Magnetic Resonance (NMR) Spectra

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker Avance 300 FT-NMR spectrometer.

5. High Performance Liquid Chromatography

HPLC chromatogram were recorded on Ultimate 3000 HPLC system (DIONEX, Germany) equipped with an online degasser, dual gradient pump, an auto-plate sample and a thermostatically controller column compartment.

Part I Extraction and Isolation of the dried fruit latex of *G. mangostana*

1. Extraction of the dried fruit latex of *G. mangostana*

The dried fruit latex (40 g), contaminated with fruit hull material, was dissolved in 300 mL of ethyl acetate 3 times for each 30 minutes and the mixture filtered. Evaporation of this filtrate under reduced pressure (about 40 °C) to give the ethyl acetate extract (35 g). The extraction procedure is shown in Scheme 1.

2. Isolation of xanthone compound from the dried fruit latex of *G. mangostana*

A portion of the ethyl acetate extract of the dried fruit latex (35 g) was separated into 6 broad fractions (Fr.1-6, GM-E1 – GM-E13) by chromatography on silica gel column (0.040-0.063 mm, 150 g), eluting sequentially with *n*-hexane, *n*-hexane-acetone, acetone and methanol with increasing amounts of the more polar solvent (96:4 = 1.5 L; 94:6 = 4 L; 92:8 = 5 L; 88:12 = 5 L; 85:15 = 4 L; 80:20 = 2.75 L; 70:30 = 5 L; 50:50 = 2L; methanol = 1L, collected each 1 L) .

2.1 Isolation of compounds GM-E2, GM-E4, GM-E5, GM-E6 and GM-E7

Fraction 2 (3.24 g, eluted with 6% acetone-*n*-hexane) was subjected to silica gel column chromatography (0.040-0.063 mm, 85 g) employing a gradient of *n*-hexane to acetone (1% increment of acetone, each 200 mL) resulting in 10 subtractions. Subfraction 5 (248.3 mg, eluted with 4% acetone-*n*-hexane) yield compound GM-E5 (9-hydroxycalabaxanthone (**10**), 248.3 mg). Compound GM-E2 (β-mangostin (**2**), 7.1 mg) were isolated from subfraction 7 (114.0 mg, eluted with 5% acetone-*n*-hexane) by precipitation in *n*-hexane and washed the yellow solid with cool *n*-hexane (10 mL).

Subfraction 9 (164.7 mg, eluted with 6% acetone-*n*-hexane) was further purified by silica gel column chromatography (0.040-0.063 mm, 10 g) employing a gradient of *n*-hexane to acetone (100:0 = 100 mL; 99.75:0.25 = 300 mL; 99.5:0.5 = 400 mL; 99:1 = 500 mL; 98:2 = 200 mL; 97:3 = 200 mL) to obtain compound GM-E4 (a mixture of gartanin (**4**) and 8-

desoxygartanin (**5**), 7.8 mg) and GM-E7 (1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl) xanthone (**12**), 9.0 mg).

Subfraction 10 (213.0 mg, eluted with 7% acetone-*n*-hexane) was rechromatographed over silica gel (0.040-0.063 mm, 10 g) with *n*-hexane-acetone (0.25% increment of acetone, each 100 mL), to afford compound GM-E6 (garcinone E (**26**), 17.3 mg).

These compounds were identified as 9-hydroxycalabaxanthone (**10**), β-mangostin (**2**), a mixture of gartanin (**4**) and 8-desoxygartanin (**5**), 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone (**12**) and garcinone E (**26**), respectively (see Scheme 2).

2.2 Isolation of compounds GM-E1 and GM-E8

The TLC pattern of fraction 3 showed a big green spot with R_f value of 0.45 (30% acetone-*n*-hexane), corresponded to R_f value of α-mangostin. So, fraction 3 contained α-mangostin as the major compound. GM-E1 (α-mangostin (**1**), 8.7 g) was isolated by precipitation of fraction 3 in 8% acetone-*n*-hexane (15 bottles, each 50 mL) for 24 hours. The crystallized compound was washed with cool *n*-hexane (500 mL) and dried in vacauo to give yellow solid. The residual portion of fraction 3 (8.36 g, eluted with 8% acetone-*n*-hexane) was subjected to a silica gel column, with *n*-hexane-acetone as solvent system, to provide 13 subfractions. GM-E8 (mangostenone H or 1,3,6-trihydroxy-7-methoxy-3-(1-methylethenyl)-8-(3-methylbut-2-enyl)-furano[2,3-b]xanthone, 5.0 mg) was obtained after repeated column chromatography (less than 0.063 mm, 19 g) of subfraction 3 (845 mg, eluted with 20% acetone-*n*-hexane) with *n*-hexane-acetone (1% increment of acetone, each 200 mL) as eluting solvent.

Compounds GM-E1 and GM-E8 were subsequently identified as α-mangostin (**1**) and mangostenone H or 1,3,6-trihydroxy-7-methoxy-3-(1-methylethenyl)-8-(3-methylbut-2-enyl)-furano[2,3-b]xanthone, respectively (see Scheme 2).

2.3 Isolation of compounds GM-E3, GM-E10, GM-E9 and GM-E11

Fraction 4 (6.57 g, eluted with 12-20% acetone-*n*-hexane) yield GM-E3 (γ-mangostin (**3**), 1.6 g) by precipitation in 12% acetone-*n*-hexane (7 bottles, each 50 mL). This crystallized compound was washed by using cool dichloromethane (250 mL) and dried in vacauo to give yellow solid. The residual portion of fraction 4 was applied to a silica gel column (less than 0.063 mm, 65 g) using *n*-hexane and *n*-hexane-acetone with increasing

amount of the more polar solvent (2% increment of acetone, each 200 mL) to give 13 subfractions.

Subfraction 9 (166.9 mg, eluted with 7% acetone-*n*-hexane) was fractionated by centrifugal thin-layer chromatography (Silica gel 60 PF_{254} containing $CaSO_4$, plate of 2.0 mm thickness) and eluted with *n*-hexane-ethyl acetate of increasing polarity (1% increment of ethyl acetate, each 400 mL) to provide 6 subfractions (Fr. 9a-9f). Compound GM-E10 (cowaxanthone D (**102**), 2.9 mg) was obtained from subfraction 9a (2.9 mg, eluted with 5% ethyl acetate-*n*-hexane) as pale yellow solid.

Repeated chromatography of subfraction 9e (44 mg, eluted with 5% ethyl acetate-*n*-hexane) with centrifugal thin-layer chromatography (Silica gel 60 PF_{254} containing $CaSO_4$, plate of 1.0 mm thickness) eluted with *n*-hexane-dichloromethane of increasing polarity (10% increment of dichloromethane, each 100 mL, 1% methanol-dichloromethane, each 100 mL) to provide 7 subfractions (Fr. 9e-1 – 9e-7). Compound GM-E11 (mangostanin (**63**), 4.7 mg) was purified by preperative TLC (Silica gel 60 F_{254} precoated on aluminium plate, 5x7 cm, 100% dichloromethane, 3 elutions) of subfraction 9e-6 (8 mg, eluted with 1% methanol-dichloromethane).

Subfraction 11 (152.1 mg eluted with 9% acetone-*n*-hexane) was fractionated by silica gel column chromatography (less than 0.063 mm, 12 g) eluted with dichloromethane-methanol (0.5% increment of methanol, each 100 mL) as eluent to provide 15 subfractions (Fr. 11a-11o). Compound GM-E9 (garcinone B (**14**), 0.5 mg) was obtained in subfraction 11b (0.5 mg, eluted with dichloromethane) as pale yellow solid.

Compounds GM-E3, GM-E10, GM-E11 and GM-E9 were identified as γ-mangostin (**3**), cowaxanthone D (**102**), mangostanin (**63**) and garcinone B (**14**), respectively (see Scheme 2).

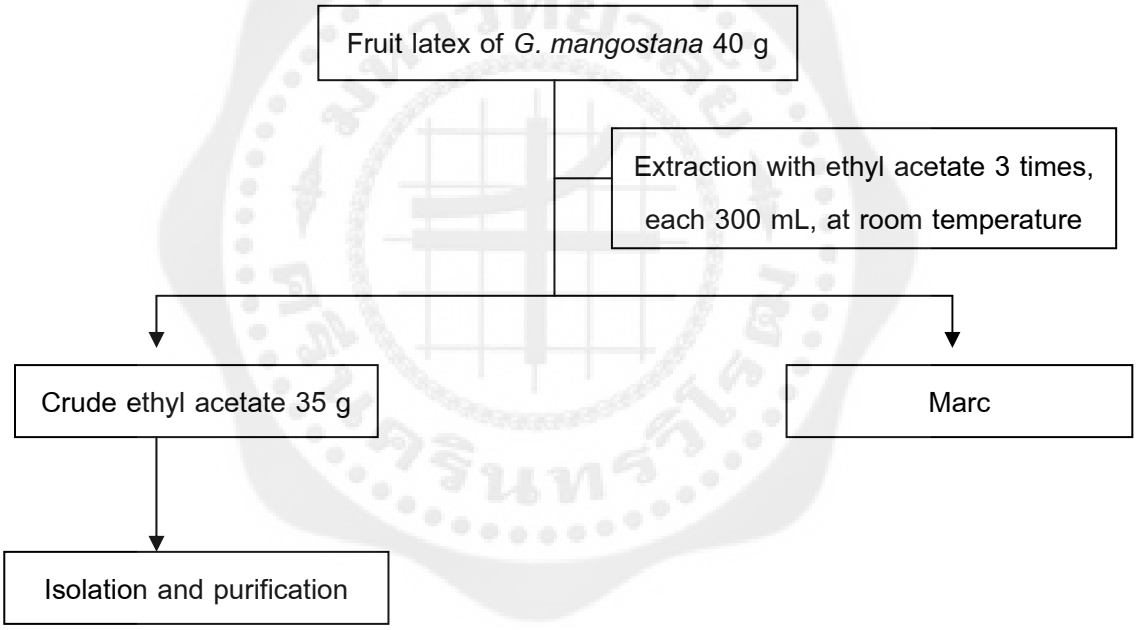
2.4 Isolation of compounds GM-E12 and GM-E13

Garcinone D and cratoxylone are structural isomer. These compounds showed the same polarity when used 40% acetone-*n*-hexane as mobile phase, 6 elutions in TLC plate. Spots on the TLC plate were monitored under UV light and using anisaldehyde-H_2SO_4 reagent. Both sport showed green color. We observed the different R_f value when TLC plate was developed in 40% acetone-*n*-hexane, 12 elutions. So, we were carefully increasing small amount of polar solvent in column chromatography. Fraction 5 (6.33 g, 30% acetone-hexane), contained the mixture of garcinone D and cratoxylone, was subjected to silica gel

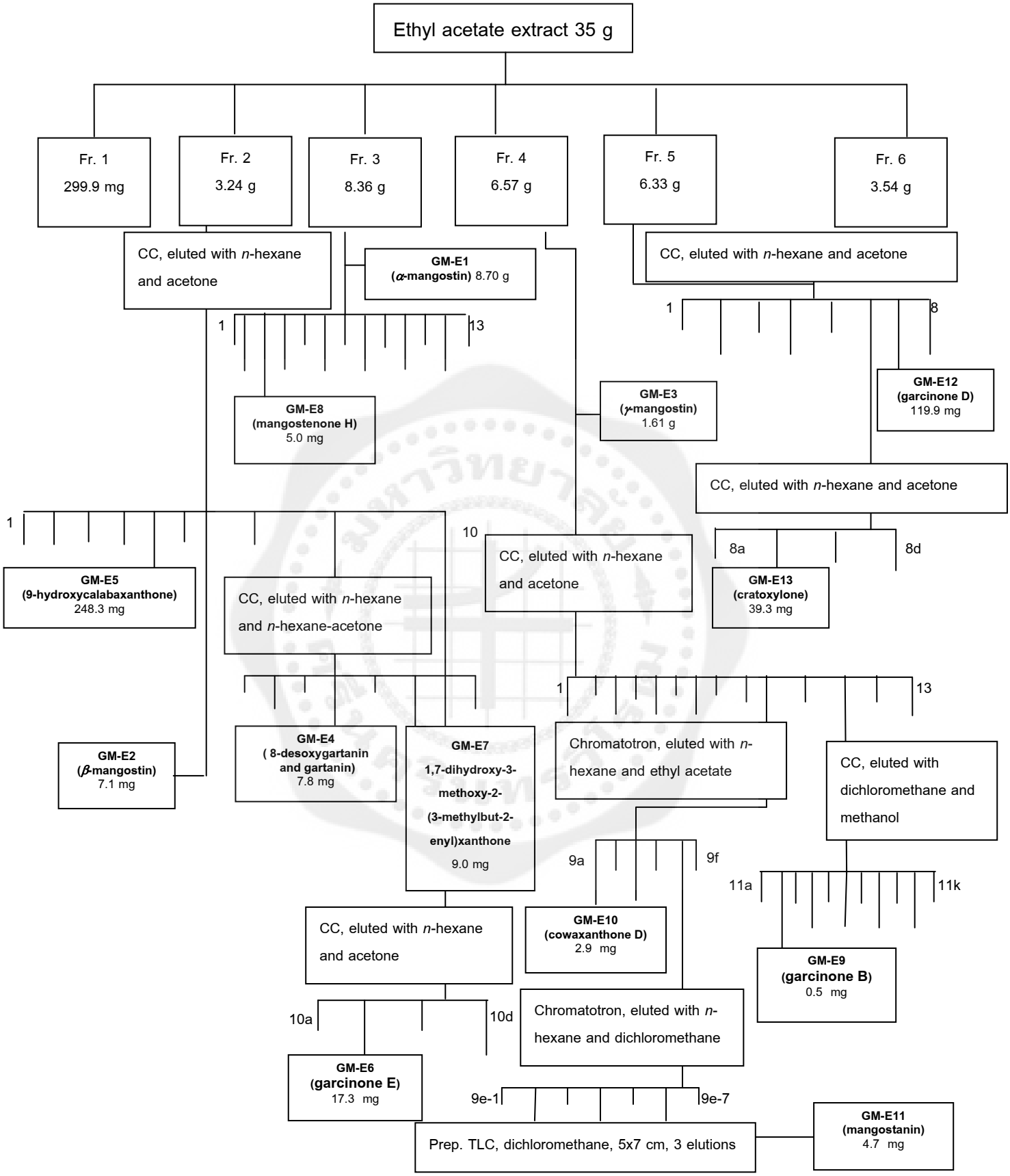
chromatography (0.040-0.063 mm, 81 g) employing solvent gradient *n*-hexane-acetone (2% increment of acetone, each 400 mL) and 8 subfractions were collected. Compound GM-E12 (garcinone D (**16**), 119.9 mg) was contained in subfraction 7 (543.6 mg, 25% acetone-*n*-hexane) as pale yellow solid.

Subfraction 6 (560.3 mg, 16% acetone-*n*-hexane) was further column chromatographed over silica gel column (less than 0.063 mm, 15 g) using *n*-hexane-acetone (90:10 = 100 mL; 85:15 = 200 mL; 82:18 = 200 mL, 80:20 = 200 mL, 75:25 = 100 mL) as eluting solvent to provide compound GM-E13 (cratoxylone (**103**), 39.3 mg).

Compounds GM-E12 and GM-E13 were identified as garcinone D (**16**) and cratoxylone.



Scheme 1 Extraction procedure for the fruit latex of *G. mangostana*



Scheme 2 Isolation of compounds from the ethyl acetate extract of *G. mangostana* fruit latex

Physical and spectral data of the isolated compounds in ethyl acetate extract

1. Compound GM-E1 (α-mangostin, sss4924)

Yellow solid 8.7 g, soluble in acetone and methanol

m.p. : 171-172 °C [lit. 180-182 °C (Yates; & Stout. 1958: 1691-1700)]

R_f : 0.45 (40% acetone-*n*-hexane), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3421, 3268, 2925, 2963, 1645, 1609, 1580, 1456, 1281, 1185, 1077, 1050, 984, 849, 629

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 1-OH δ 13.75 (s), H-4 δ 6.27 (s), H-5 δ 6.81 (s), H-11 δ 3.43 (2H, d, J = 7.1 Hz), H-12 δ 5.26 (1H, t, J = 7.1 Hz), H-14 δ 1.67 (3H, *s*), H-15 δ 1.75 (3H, s), H-16 δ 4.07 (2H, d, J=6.1 Hz), H-17 δ 5.26 (1H, t, J = 6.1 Hz), H-19 δ 1.81 (s), H-20 δ 1.82 (s) and 7-OMe δ 3.78 (3H, s).

2. Compound GM-E2 (β-mangostin, sss4447)

Yellow solid 7.1 mg, soluble in dichloromethane and acetone

m.p. : 162-164 °C [lit. 173-175 °C (Suwannapoch. 2002: 30), 175-176 °C (Yates; et al. 1968: 3770-3772), 162-163 °C (Sheikh Mohd Ghazali; et al. 2010: 134-142)]

R_f : 0.37 (20% acetone-*n*-hexane), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3398, 1645, 1602, 1461, 1432, 1282, 1201, 1168, 1150, 1114, 1049, 991, 840, 793

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 1-OH δ 13.40 (s), H-4 δ 6.32 (s), H-5 δ 6.81 (s), 7-OMe δ 3.79 (s), 3-OMe δ 3.88 (s), H-11 δ 3.33 (2H, d, J = 7.3 Hz), H-12 δ 5.22 (1H, m), H-14 δ 1.77 (s), H-15 δ 1.66 (s), H-16 δ 4.07 (2H, d, J = 5.7 Hz), H-17 δ 5.22 (1H, m), H-19 δ 1.81 (s) and H-20 δ 1.66 (s).

3. Compound GM-E3 (γ-mangostin, sss3209)

Yellow solid 1.61 g, soluble in acetone and methanol

m.p. : 194-196 °C [lit. 194-196 °C (Komutiban. 2004: 37), 207-210 °C (Mahabusarakam; Wiriyachitra; & Taylor. 1987: 474-478)]

R_f : 0.45 (40% acetone-*n*-hexane), a yellow coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3557, 3420, 3239, 2913, 1645, 1618, 1497, 1459, 1282, 1197, 1172, 1075, 1042, 847, 811

^{1}H NMR : δ ppm, 300 MHz, in CD_3COCD_3; 1-OH δ 13.93 (s), H-4 δ 6.36 (s), H-5 δ 6.80 (s), H-11 δ 3.33 (2H, d, J = 7.2 Hz), H-12 δ 5.30 (1H, t, J = 7.2 Hz), H-14 and H-15 δ 1.62 (s), H-16 δ 4.17 (2H, d, J = 6.6 Hz), H-17 δ 5.30 (1H, t, J = 6.6 Hz), H-19 δ 1.77 (s) and H-20 δ 1.82 (s).

4. Compound GM-E4 (a mixture of gartanin and 8-desoxygartanin, sss4406)

Yellow solid 7.8 mg, soluble in dichloromethane and acetone

R_f : 0.53 (30% acetone-*n*-hexane), a green coloration with anisaldehyde-H_2SO_4 reagent

5. Compound GM-E5 (9-hydroxycalabaxanthone, sss4597)

Yellow solid 248.3 mg, soluble in dichloromethane and acetone

m.p. : 153-154 °C [lit. 156-157 °C (Sen; et al. 1980: 2223-2225)]

R_f : 0.37 (20% acetone-*n*-hexane), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3521, 3427, 2978, 2927, 1653, 1601, 1573, 1464, 1436, 1317, 1286, 1175, 1118, 1041, 981, 889, 837, 772, 708

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 1-OH δ 13.67 (s), H4 δ 6.22 (s), H5 δ 6.81 (s), 7-OMe δ 3.78 (s), H11 δ 6.70 (1H, d, J=10.0 Hz), H12 δ 5.55 (1H, d, J=10.0 Hz), H14 and H15 δ 1.46 (s), H16 δ 4.06 (2H, d, J=6.4 Hz), H17 δ 5.24 (1H, t, J=6.4 Hz), H19 δ 1.81 (s) and H20 δ 1.67 (s).

6. Compound GM-E6 (garcinone E, sss4479)

Yellow solid 17.3 mg, soluble in dichloromethane and acetone

m.p. : 142-146 °C [lit. 152.5-155.5 °C (Sakai; et al. 1993 : 958-960)]

R_f : 0.38 (20% acetone-*n*-hexane), a yellow coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3422, 2971, 2920, 1645, 1617, 1447, 1277, 1190, 1042

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 1-OH δ 13.82 (s), H-4 δ 6.31 (s), H-11 δ 3.44 (2H, d, J = 7.2 Hz), H-12 δ 5.60 (1H, m), H-14 δ 1.68 (s), H-15 δ 1.75 (s), H-16 δ 4.28 (2H,

d, J = 6.3 Hz), H-17 δ 5.60 (1H, m), H-19 δ 1.85 (s), H-20 δ 1.75 (s), H-21 δ 3.58 (2H, d, J = 7.5 Hz), H-22 δ 5.60 (1H, m), H-24 δ 1.82 (s) and H-25 δ 1.85 (s).

7. Compound GM-E7 (1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone, sss4477)

Yellow solid 9.0 mg, soluble in dichloromethane and acetone

m.p. : 216-218 °C [lit. 220-222 °C (Sen; et al. 1980: 2223-2225)]

R_f : 0.52 (30% acetone-*n*-hexane), a yellow coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3427, 3246, 2971, 2920, 1649, 1609, 1584, 1483, 1443, 1309, 1234, 1172, 1093

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 1-OH δ 12.79 (s), H-4 δ 6.40 (s), H-5 δ 7.32 (1H, d, J = 8.7 Hz), H-8 δ 7.46 (1H, d, J = 3.0 Hz), H-6 ca δ 7.24 obscured signal, H-11 δ 3.35 (2H, d, J = 6.6 Hz), H-12 δ 5.21 (1H, t, J = 6.6 Hz), H-14 δ 1.71 (s), H-15 δ 1.67 (s) and 3-OMe δ 3.89 (s).

8. Compound GM-E8 (mangostenone H or 1,3,6-trihydroxy-7-methoxy-3-(1-methylethenyl)-8-(3-methylbut-2-enyl)-furano[2,3-b]xanthone, sss2917)

Yellow solid 5.0 mg, soluble in dichloromethane and acetone

m.p. : 131-133 °C

R_f : 0.47 (30% acetone-*n*-hexane), a yellow coloration with anisaldehyde-H_2SO_4 reagent

UV nm (log ε) : 205.60(4.44), 240.60(4.26), 260.00(4.32), 275.40(4.37), 286.60(4.42), 301.20(4.51), 314.20(4.41), 367.40(3.62)

IR ν_{max}^{KBr} cm^{-1} : 3443, 2920, 1650, 1643, 1632, 1615, 1463, 1433, 1374, 1294, 1231, 1170, 118, 1038, 981, 800, 699, 634, 590

HRTOFMS (APCI, -ve) *m/z* : 405.1342 $[M-H]^-$ (calcd 405.1344 for $C_{24}H_{22}O_6$-H)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 1-OH δ 14.10 (s), H-4 δ 6.88 (s), H-5 δ 6.87 (s), 7-OMe δ 3.80 (s), H-16 δ 4.10 (2H, d, J = 10.2 Hz), H-17 5.27 δ (1H, t, J = 10.2 Hz), H-19 δ 1.69 (3H, s), H-20 δ 1.83 (3H, s), H-11 δ 6.81 (1H, s), H-14a δ 5.16 (1H, br s), H-14b δ 5.72 (1H, br s) and H-15 δ 2.10 (3H, s).

9. Compound GM-E9 (garcinone B, sss3456)

Pale yellow solid 0.5 mg, soluble in dichloromethane and acetone

R_f : 0.40 (dichloromethane), a yellow coloration with anisaldehyde-H_2SO_4 reagent

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 1-OH δ 13.67 (s), H-4 δ 6.29 (s), H-5 δ 6.80 (s), H-11 δ 3.43 (2H, d, J = 7.2 Hz), H-12 δ 5.27 (1H, m), H-14 δ 1.75 (s), H-15 δ 1.82 (s), H-16 δ 8.00 (1H, d, J = 10.2 Hz), H-17 δ 5.80 (1H, d, J = 10.2 Hz), H-19 and H-20 δ 1.47 (s).

10. Compound GM-E10 (cowaxanthone D, sss3502)

Pale yellow solid 0.5 mg, soluble in dichloromethane and acetone

m.p. : 222-224 °C [lit. 210.0-210.7 °C (Panthong; et al. 2006: 999-1004)]

R_f : 0.54 (30% acetone-*n*-hexane), a yellow coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3483, 3057, 3007, 2920, 1715, 1645, 1635, 1624, 1591, 1558, 1490, 1456, 1432, 1362, 1306, 1288, 1273, 1223, 1166, 1132, 1109, 1093, 1017, 903, 842, 811, 734, 702, 645, 619, 530, 489

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 1-OH δ 13.31 (s), H-4 δ 6.29 (s), H-5 δ 6.73 (s), 3-OMe δ 3.84 (s), H-11 δ 3.29 (2H, d, J = 7.2 Hz), H-12 δ 5.15 (1H, br t, J = 7.2 Hz), H-14 δ 1.72 (s), H-15 δ 1.61 (s), H-16 δ 7.95 (1H, d, J = 10.2 Hz), H-17 δ 5.75 (1H, d, J = 10.2 Hz), H-19 and H-20 δ 1.43 (s).

11. Compound GM-E11 (mangostanin, sss3458)

Pale yellow solid 0.5 mg, soluble in dichloromethane and acetone

m.p. : 182-183 °C [lit. 215-217 °C (Nilar; et al. 2002: 541-548)]

R_f : 0.33 (dichloromethane), a green coloration with anisaldehyde-H_2SO_4 reagent

ESMS (+ve): *m/z* (% relative intensity): 427.5 $[M+H]^+$ (100)

^{1}H NMR : δ ppm, 300 MHz, in 2% CD_3OD-$CDCl_3$; 1-OH δ 13.66 (s), H-4 δ 6.22 (s), H-5 δ 6.72 (s), 7-OMe δ 3.73 (s), H-11a δ 3.12 (1H, dd, J = 9.1, 15.9 Hz), H-11b δ 3.03 (1H, dd, J = 8.5, 15.9 Hz), H-12 δ 4.72 (1H, dt, J = 8.5, 9.1 Hz), H-14 δ 1.18 (3H, s), H-15 δ 1.29 (3H, s), H-16 δ 4.04 (2H, d, J = 5.8 Hz), H-17 δ 5.21 (1H, d, J = 5.8 Hz), H-19 δ 1.64 (3H, s) and H-20 δ 1.78 (3H, s).

12. Compound GM-E12 (garcinone D, sss3301)

Pale yellow solid 119.9 mg, soluble in acetone and methanol

m.p. : 206-208 °C [lit. 210-211 °C (Komutiban. 2004: 37), 202-204 °C (Sen; et al. 1986: 1157-1158)]

R_f : 0.45 (30% acetone-*n*-hexane, 6 elutions), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3406, 3260, 2971, 2934, 1649, 1609, 1577, 1459, 1302, 1277, 1194, 1078, 905, 840, 822

^{1}H NMR : δ ppm, 300 MHz, in CD_3COCD_3; 1-OH δ 13.81 (s), H-4 δ 6.38 (s), H-5 δ 6.80 (s), 7-OMe δ 3.83 (s), H-11 δ 3.32 (2H, d, J = 6.3 Hz), H-12 δ 5.26 (1H, t, J = 6.3 Hz), H-14 δ 1.76 (s), H-15 δ 1.63 (s), H-16 δ 3.45 (2H, m), H-17 ca δ 1.72, H-19 and H-20 δ 1.29 (s).

13. Compound GM-E13 (cratoxylone, sss3300)

Pale yellow solid 28.5 mg, soluble in acetone and methanol

m.p. : 229-230 °C [lit. gum (Bennett; et al. 1993: 1245-1251)]

R_f : 0.45 (30% acetone-*n*-hexane, 6 elution)), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3589, 3408, 3159 2971, 2932, 1646, 1604, 1560, 1474, 1451, 1372, 1332, 1298, 1277, 1254, 1208, 1189, 1160, 1092, 1079, 1019, 1053, 975, 934, 903, 818, 842, 757, 628, 660, 588, 495, 450, 390

ESMS (+ve): *m/z* (% relative tintensity): 429 $[M+H]^+$ (55)

^{1}H NMR : δ ppm, 300 MHz, in CD_3COCD_3; 1-OH δ 13.78 (s), H-4 δ 6.36 (s), H-5 δ 6.81 (s), 7-OMe δ 3.78 (s), H-11 δ 2.74 (2H, dt, J = 5.7, 7.9 Hz), H-12 δ 1.69 (2H, dt, J = 5.7, 7.9 Hz), H-14 and H-15 δ 1.24 (s), H-16 δ 4.11 (2H, d, J = 6.3 Hz), H-17 δ 5.26 (2H, t, J = 6.3 Hz), H-19 δ 1.81 (s) and H-20 δ 1.63 (s).

Part II Development of the determination of xanthone compounds in *G. mangostana*

1. Reagents and materials

1.1 Reagents

HPLC-grade acetronitrile and analytical grade acetic acid, sodium hydroxide, iso-propanol, formic acid and ethyl acetate were purchased form Merck (Damstadt, Germany). Deionized-distilled water purified by Millipore Milli-Q system (Thermo Fisher Scientific, USA) was used in all experiments.

1.2 HPLC column

Acclaim® RSLC 120 C18 column (2.1 x 50.0 mm i.d., 2.2 μm) supplied by DIONEX Technologies (CA, USA).

1.3 Membrane filter

Nylon filter membranes (0.45 μm pore size, 47 mm and 13 mm i.d.) supplied by FILTREX Technologies (USA) were used to remove particulate materials from mobile phase, standard and sample solutions.

2. Preparation of standard and sample solutions

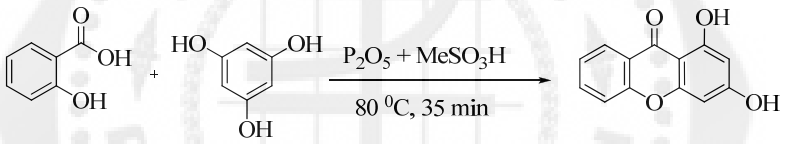
The followings are descriptions of the preparation procedures of standard and sample solutions employed in this work. The eleven xanthone standards, including garcinone C, garcinone D, γ-mangostin, 8-desoxygartanin, gartanin, α-mangostin, garcinone E, demethylcalabaxanthone, 9-hydroxycalabaxanthone and β-mangostin were isolated from the young fruit and cratoxylone was obtained from the dried fruit latex. All standards were identified by ^{1}H and ^{13}C nuclear magnetic resonance (NMR), mass spectral (MS) analysis and also compared the data with the literature data.

2.1 Internal standard solution

2.1.1 Internal standard preparation

The 1,3-dihydroxyxanthone was used as internal standard. This xanthone was prepared by using the oxidative coupling reaction (Eaton; et al. 1973: 4071-4073, Pillai; et al. 1986: 717-723) as follows:

Firstly, a solution of 10% phosphoruspentaoxide (P_2O_5) in methanesulfonic acid (MsOH) was prepared by dissolving P_2O_5 (1 g, 7.09 mmol) in MsOH (10 mL, 15.4 mmol) and the reaction mixture was stirred at 90°C for 30 minutes to give clear solution. After cooling this solution at room temperature, salicylic acid (1.34 g, 9.7 mmol) and phloroglucinol (1.262 g, 10 mmol) were dissolved in 10% P_2O_5 (5 mL) to give dark orange solution and then the solution was stirred at 80°C for 35 minutes. The reaction was monitored every 5 minutes by TLC technique. The solution was poured into ice bath and stirred 2 hours at room temperature until the orange solid was appeared. The residue was filtered and washed out the excess of MsOH with water (100 mL) and then dried in high vacuum pressure. The orange solid (2 g) were obtained. The residue was further purified via chromatography using silica gel (less than 0.063 mm, 85 g) and solvent system composed of *n*-hexane-acetone (100:0 = 100 mL, 90:10 = 900 mL). The pale yellow solid were obtained (1.59 g, 10 mmol, 71.6% yield). The purity of 1,3-dihydroxyxanthone was more than 97% by MS analysis



Physical and spectral data of 1,3-dihydroxyxanthone

Pale yellow solid 1.59 g, soluble in acetone and methanol

m.p. : 254-255°C [lit. 255–256°C (Annals of Tropical Medicine and Parasitology. 2003: 683-688)]

R_f : 0.50 (30% acetone-*n*-hexane)

IR ν_{max}^{KBr} cm^{-1} : 3482, 3397, 3331, 2925, 2847, 1654, 1610, 1569, 1508, 1485, 1457, 1352, 1312, 1284, 1223, 1193, 1165, 1077, 1030, 1009, 929, 872, 855, 828, 788, 763, 727

UV nm (log ε) : 360.0 (3.79), 347.5 (3.87), 307.5 (3.91), 260.0 (3.95), 231.0 (4.25).

ESMS (-ve): *m/z* (% relative tintensity): 227.6 $[M-H]^-$ (100)

HRTOFMS (ESI, -ve) *m/z* : 227.0356 $[M-H]^-$ (calcd 227.0338 for $C_{13}H_8O_4$-H)

^{1}H NMR : δ ppm, 300 MHz, in CD_3COCD_3; 1-OH δ 12.89 (s), H-2 δ 6.41 (1H, d, J = 2.08 Hz), H-4 δ 6.81 (1H, d, J = 2.08 Hz), H-5 δ 7.50 (1H, dd, J = 7.15, 0.97 Hz), H-6 δ 7.81 (1H, ddd, J = 8.69, 7.15, 1.6 Hz), H-7 δ 7.43 (1H, ddd, J = 8.69, 8.14, 0.97 Hz), H-8 δ 8.16 (1H, dd, J = 8.14, 1.6 Hz).

2.1.2 Internal standard solution at concentration of 50 μg/mL

1,3-Dihydroxyxanthone (0.05 mg) was dissolved in 1.00 mL of acetronitrile. The solution was stored in the refrigerator at 4°C. It was used as stock solution for further dilution.

2.2 Standard solutions

2.2.1. Xanthone standard solutions at concentration of 1000 μg/mL

Each xanthone (1.00 mg) was dissolved in 1.00 mL of acetronitrile. The solution was stored in the refrigerator at 4°C. It was used as stock solution for further dilution.

2.2.2. Xanthone standard solutions at concentration of 200 μg/mL

A 200 μL aliquot of each standard xanthone solution was transferred into a 2.00 mL of amber vial and then made up to 1.00 mL with acetronitrile. These solutions were used as the secondary stock solution for further dilution.

2.2.3. Working xanthone standard solutions

2.2.3.1 Standard solutions of garcinone C, garcinone D, cratoxylone, γ-mangostin and garcinone E

Working standard solutions at the concentration of 1, 3, 5, 7 and 15 μg/mL were freshly prepared by transferring of the stock solution 200 μg/mL at exact volume of 5, 15, 25, 35 and 75 μL, respectively, into 2.00 mL of amber vial and making to 1.00 mL with acetonitrile. All standard solutions were added with internal standard solution at final concentration of 0.5 μg/mL.

2.2.3.2 Standard solutions of 8-desoxygartanin, gartanin, demethylcalabaxanthone, 9-hydroxycalabaxanthone and β-mangostin

Working standard solutions at the concentration of 1, 3, 5 and 7 μg/mL were freshly prepared by transferring of the stock solution (200 μg/mL) at exact volume of 5, 15, 25 and 35 μL, respectively, into 2.00 mL of amber vial and the solution was then made to 1.00 mL with acetonitrile. All standard solutions were added with internal standard solution at final concentration of 0.5 μg/mL.

2.2.3.3 Standard solution of α-mangostin

Working standard solutions at the concentration of 10, 20, 50, 90 and 150 μg/mL were freshly prepared by transferring of the stock solution 1000 μg/mL at exact volume of 10, 20, 50, 90 and 150 μL, respectively, into 2.00 mL of amber vial and the solution was then made to 1.00 mL with acetonitrile. These standard solutions were added with internal standard solution at final concentration of 10.0 μg/mL.

2.3 Sample preparation

The dried fruit hull of *G. mangostana* (500 g) from the Eastern and Southern areas of Thailand was extracted with ethyl acetate (4L, 12 siphons) by Soxhlet extractor. The solvent was evaporated using rotatory evaporator reduced pressure followed by in vacauo to give a yellow brown solid. This yellow brown solid were pulverized to provide yellow powder of the extract (42 g, 8.4% yield). Each portion of the yellow powder (5.00 mg) was dissolved in 10.0 mL of acetonitrile : water (70:30 v/v) for further dilution. A 200 μL aliquot of this solution was transferred into 2.00 mL of amber vial and making to 1.00 mL with acetonitrile : water (70:30 v/v). This sample solution was added with internal standard solution at final concentration of 10.0 μg/mL for determination the amount of α-mangostin. A 199.5 μL aliquot of this solution was transferred into 2.00 mL of amber vial and added with internal standard solution at final concentration of 0.50 μg/mL for determination of others.

3. Procedures

The following sections describe the development of chromatographic technique for determination of xanthone compounds in mangosteen extracts. The optimization of chromatographic separation, analytical performance of the developed HPLC method and analysis of xanthone in samples from the Eastern and Southern areas of Thailand are given below.

3.1 Chromatographic study

This work modified the previous HPLC method (Chaivisuthangkura; et al. 2009: 315-318) for determination of prenylated xanthones in the extract of the fruit hull of

G. mangostana. The gradient elution system of acetonitrile and 2.00% (v/v) acetic acid were studied to obtain a good separation of xanthone compounds. All the operations and data analysis were controlled by Hystar, micrOTOF control, DataAnalysis 4.0 and QuantAnalysis 2.0 software (Bruker Daltonik GmbH, Bremen, Germany). The optimum condition is given below

Column :	Acclaim® RSLC 120 C18 (2.1mm x 50.0mm i.d., 2.2 μm)
Mobile phase :	45-55% acetonitrile at 0-3 min, 55-75% acetonitrile at 3-12 min, 75-95% acetonitrile at 12-15 min, 95-45% acetonitrile at 15-16 min and 45% acetonitrile at 16-20 min
Flow rate :	0.3 mL/min
Sample volume :	1 μL
MS conditions:	drying gas, N_2 (flow rate, 8.0 mL/min);
	drying gas temperature, 150 $^{\circ}$C;
	capillary voltage, 3000 V;
	nebulizer gas, N_2 (3.0 bar);
	end plate offset, 500 V;
	mode, negative mode with mass range of 50-1000 m/z.

3.2 Analytical Performance of HPLC-MS method

Validation parameters for xanthones determination using the development method in term of linearity, repeatability and reproducibility, limit of detection and quantification were investigated using the optimized conditions.

3.2.1 Linearity

The calibration curves of xanthone compounds were investigated by triplicate injections of standard garcinone C, garcinone D, cratoxylone, γ-mangostin and garcinone E in the concentration range of 1 to 15 μg/mL, 8-desoxygartanin, gartanin, demethylcalabaxanthone, 9-hydroxycalabaxanthone and β-mangostin in the concentration

range of 1 to 7 μg/mL and α-mangostin in the concentration range of 10 to 150 μg/mL using HPLC-MS system as described in section 3.1. A five-point calibration curve was established for the linear least-squares regression of peak area ratios between the analyte and internal standard versus relative concentrations of working standard and internal standard solutions.

3.2.2 Precision

The repeatability (intra-day) and reproducibility (inter-day) of the method were determined using the percentage of relative standard deviation (% RSD) of five replicate analyses of working standard solution within one day for repeatability and over a period of three consecutive days for reproducibility. The concentration at 4 μg/mL for ten xanthones and at 40 μg/mL for α-mangostin were use in this study. The percentage of relative standard deviation (% RSD) of peak area ratios between the analyte and internal standard and retention time were calculated.

3.2.3 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) were calculated from the concentration of each xanthone compound that gave a signal-to-noise ratio of 3:1 and 10:1, respectively. These parameters were evaluated by triplicate analyse.

3.3 Quantification of xanthone compounds in the mangosteen extracts

The samples were prepared as described in section 2.3 and analyzed by using the optimum condition as given in section 3.1. These sample solutions were directly determined for analysis of other xanthone compounds. The diluted sample solutions for five-folds were determined for measurement of α-mangostin. The concentration of xanthone compounds in the samples were obtained in mg/g unit.

CHAPTER 4
RESULTS AND DISCUSSION

The experimental results of this work are divided into two parts. The first part (Section 4.1) involves the identification and structure elucidation of xanthone compounds obtained from the dried fruit latex of *G. mangostana* by using the spectroscopic techniques and by comparison the data with previously reported values.

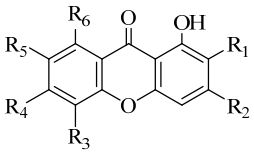
The second part (Section 4.2) involves the development of HPLC-ESI-TOF-MS method for determination of xanthones in the fruit hull extracts of *G. mangostana* obtained from the Eastern and Southern areas of Thailand. The optimized method was also used to separate eleven xanthones. The measurement of xanthones content in the fruit hull extracts have been made using the HPLC-ESI-TOF-MS technique.

Part I Extraction and Isolation of the dried latex of *G. mangostana*

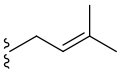
4.1 The structure elucidation of xanthones isolated from the dried fruit latex of *G. mangostana*

There have been a number of reports on the isolation of xanthone compounds from the fruit hull or the whole fruit of *G. mangostana* (Obolskiy; et al. 2009: 1047-65), however, only two reports studied on the fruit latex (Yates; & Bhat. 1968: 3770-72, Dharmaratne; et al. 2005: 239-43). It was therefore of interest to study the chemical constituent of the fruit latex. From the observation on TLC, the ethyl acetate extract of the dried fruit latex showed the green - yellow color development with anisaldehyde-H_2SO_4 agent which was recognized for the presence of prenylated xanthones. The dried fruit latex was almost dissolved in ethyl acetate, and the ethyl acetate soluble fraction was evaporated under vaccuo to give a yellow gum. Quick column chromatography of this yellow gum yielded 6 main fractions (Scheme 2). Fractions 1 and 6 did not show the presence of xanthone on TLC. Repeatedly column chromatography of fraction 2 to 5 gave a new xanthone, mangostenone H or 1,3,6-trihydroxy-7-methoxy-3-(1-methyl-ethenyl)-8-(3-methylbut-2-enyl)-furano[2,3-b]xanthone, along with twelve known xanthones, including α-mangostin, β-mangostin, γ-mangostin, a mixture of gartanin and 8-desoxygartanin, 9-hydroxycalabaxanthone, garcinone E, 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone, garcinone B, cowaxanthone D, mangostanin, structural isomer of garcinone D and cratoxylone (Figure 4 and Table 2). The structures of these

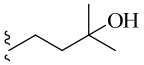
compounds were determined mainly based on their NMR data analysis and by comparison with previously reported data. This is the first report on the isolation of cowaxanthone D and cratoxylone from *G. mangostana*.



prenyl;



3-hydroxy-3-methylbutyl;



α-mangostin: $R_2 = R_4 = OH$, $R_5 = OMe$, $R_1 = R_6 =$ prenyl, $R_3 = H$

β-mangostin: $R_2 = R_5 = OMe$, $R_4 = OH$, $R_1 = R_6 =$ prenyl, $R_3 = H$

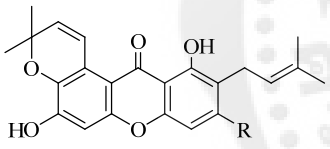
γ-mangostin: $R_2 = R_4 = R_5 = OH$, $R_1 = R_6 =$ prenyl, $R_3 = H$

garcinone D: $R_2 = R_4 = OH$, $R_5 = OMe$, $R_6 =$ 3-hydroxy-3-methylbutyl, $R_1 =$ prenyl, $R_3 = H$

cratoxylone : $R_2 = R_4 = OH$, $R_5 = OMe$, $R_6 =$ prenyl, $R_1 =$ 3-hydroxy-3-methylbutyl, $R_3 = H$

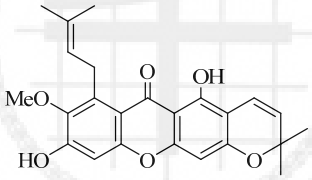
1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone: $R_2 = OMe$, $R_3 = R_4 = R_6 = H$, $R_5 = OH$, $R_1 =$ prenyl

garcinone E: $R_2 = R_4 = R_5 = OH$, $R_1 = R_3 = R_6 =$ prenyl,

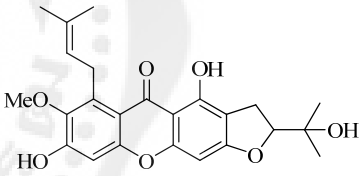


garcinone B: R = OH

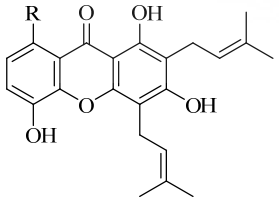
cowaxanthone D: R = OMe



9-hydroxycalabaxanthone

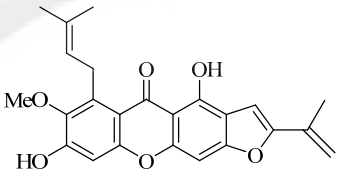


mangostanin



8-desoxygartanin: R = H

gartanin: R = OH



mangostenone H or 1,3,6-trihydroxy-7-methoxy-3-(1-methylethenyl)-8-(3-methylbut-2-enyl)-furano [2,3-b]xanthone

Figure 4 Chemical structures of isolated xanthones obtained from the fruit latex of *G. mangostana*

Table 2 Xanthones and their isolable yields obtained from the ethyl acetate extract of the *G. mangostana* fruit latex

Compound			Isolabel yields* (%)	
GM-E1	sss4924	α-mangostin	8.7 g	(21.75)
GM-E2	sss4447	β-mangostin	7.1 mg	(1.7×10^{-4})
GM-E3	sss3209	γ-mangostin	1.6 g	(4.0×10^{-5})
GM-E4	sss4460	A mixture of gartanin and 8-desoxygartanin (9:1)	7.8 mg	(1.9×10^{-4})
GM-E5	sss4597	9-hydroxycalabaxanthone	248.3 mg	(6.2×10^{-3})
GM-E6	sss4479	garcinone E	17.3 mg	(4.3×10^{-4})
GM-E7	sss4477	1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone	9.0 mg	(2.2×10^{-4})
GM-E8 New compound	sss2917	mangostenone H or 1,3,6-trihydroxy-7-methoxy-3-(1-methylethenyl)-8-(3-methylbut-2-enyl)-furano[2,3-b]xanthone	5.0 mg	(1.2×10^{-4})
GM-E9	sss3456	garcinone B	0.5 mg	(1.2×10^{-5})
GM-E10	sss3502	cowaxanthone D	2.9 mg	(7.2×10^{-4})
GM-E11	sss3458	mangostanin	4.7 mg	(1.1×10^{-4})
GM-E12	sss3301	garcinone D	119.9 mg	(2.9×10^{-4})
GM-E13	sss3300	cratoxylone	28.5 mg	(7.1×10^{-4})

* Base on the dried fruit latex (40 grams)

4.1.1 Structure determination of compound GM-E1 (α-mangostin, sss4924)

Compound GM-E1 appeared as a yellow solid with the R_f value of 0.45 (30% acetone-*n*-hexane) and gave a green coloration with anisaldehyde-H_2SO_4 reagent which was a characteristic color of prenylated xanthone. Compound GM-E1 obtained as the major compound of the extract. The ^{1}H NMR spectrum (Table 3) exhibited the resonance of a chelated hydroxy proton at δ 13.75 and two aromatic singlets at δ 6.27 (H-4) and δ 6.81 (H-5). The present of prenyl unit were observed from the resonances at δ 5.26 (t, J = 7.1 Hz), δ 3.43 (d, J = 7.1 Hz), δ 1.67 (s) and δ 1.75 (s). The other prenyl unit was shown at δ 5.26 (t, J = 6.1 Hz), δ 4.07 (d, J = 6.1 Hz), δ 1.81 (s) and δ 1.82 (s). The downfield shift of methylene proton at δ 4.07 due to an anisotropic effect of the carbonyl group (C-9) which was indicated the attachment of prenyl unit at C-8. The methoxyl group was indicated from the resonance at δ 3.78 (s). The comparison of ^{1}H NMR data of GM-E1 with authentic α-mangostin (**1**), which was isolated by our group (Suksamrarn; et al. 2003: 857-859, references cited their in Mahabusarakam; & Wirivachitra. 1987: 474-478), compound GM-E1 showed the same spectral data as α-mangostin (**1**). Thus, compound GM-E1 identified as 1,3,6-trihydroxy-7-methoxy-2,8-di-(3-methylbut-2-enyl)xanthone or α-mangostin (**1**) (Figure 5).

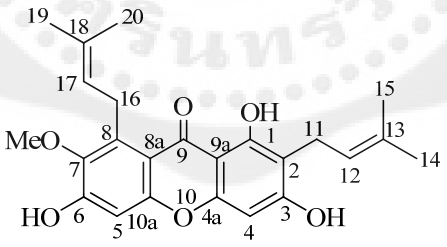


Figure 5 Structure of α-mangostin (**1**)

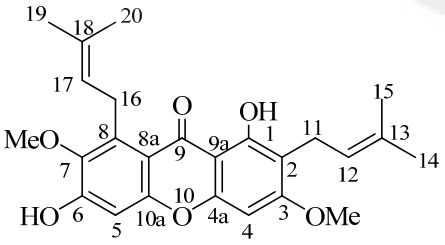
Table 3 Comparison of ^{1}H NMR data of compound GM-E1 (sss4924) with α-mangostin (**1**) in $CDCl_3$

position	α-mangostin	GM-E1 (sss4924)
	δ_H[a]	δ_H
1-OH	13.80 (1H, s)	13.75 (1H, s)
2		
3-OH	6.16 (1H, s)	
4	6.28 (1H, s)	6.27 (1H, s)
4a		
5	6.82 (1H, s)	6.81 (1H, s)
6-OH	6.31 (1H, s)	
7		
7-OMe	3.81 (3H, s)	3.78 (3H, s)
8		
8a		
9		
9a		
10a		
11	3.45 (2H, d, J = 7 Hz)	3.43 (2H, d, J = 7.1 Hz)
12	5.28 (1H, t, J = 7 Hz)	5.26 (1H, t, J = 7.1 Hz)
13		
14, 15	1.70 (3H, s)	1.67 (3H, s)
	1.78 (3H, s)	1.75 (3H, s)
16	4.11 (2H, d, J = 7 Hz)	4.07 (2H, d, J = 6.1 Hz)
17	5.28 (1H, br t, J = 7 Hz)	5.26 (1H, br t, J = 6.1 Hz)
18		
19, 20	1.82 (3H, s)	1.81 (3H, s)
	1.85 (3H, s)	1.82 (3H, s)

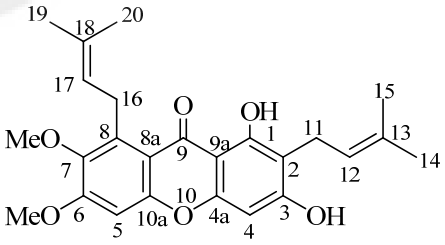
[a] Mahabusarakam; & Wirivachitra. 1987: 474-478

4.1.2 Structure determination of compound GM-E2 (β-mangostin, sss4447)

Compound GM-E2 yielded as a yellow solid and was less polar than α-mangostin with R_f value of 0.37 (20% acetone-*n*-hexane) and gave a yellow coloration with anisaldehyde-H_2SO_4 reagent. Comparison of ^{1}H NMR spectrum of GM-E2 with previous compound indicated that compound GM-E2 has similar xanthone skeleton. A 3-methylbut-2-enyl group was also observed from signals at δ 5.22 (m), δ 3.33 (d, J = 7.3 Hz), δ 1.66 (s), δ 1.77 (s). Another 3-methylbut-2-enyl group showed signals at δ 5.22 (m), δ 4.07 (d, J = 5.7 Hz), δ 1.66 (s), δ 1.81 (s). The one chelated hydroxyl group exhibited signal at δ 13.40 and two aromatic singlets showed signals at δ 6.32 (H-4) and δ 6.81 (H-5). Moreover, two singlets methoxyl signals were observed at δ 3.79 and δ 3.88. The comparison of ^{1}H NMR data with α-mangostin showed the similar ^{1}H NMR pattern, except for the GM-E2 had two signals of methoxyl group. The methoxyl group at δ 3.88 could be located at C-3 or C-6. Thus, the ^{1}H NMR spectral data of GM-E2 was compared with 1,3-dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone (**104**) which was isolated from *Cratoxylum arborescens* (Pattanaprateeb; 2004: 69-70), and β-mangostin (**2**) (Suwannapoch. 2002: 36-38, Likhitwitayawuid; Phadungcharoen; & Krungkrai. 1998: 70-72). The resulted showed that the methoxyl group of GM-E2 were at C-3 and C-7 positions same as that of β-mangostin (**2**). In addition, comparison of chromatographic pattern on TLC with authentic β-mangostin (**2**) in several solvent systems, compound GM-E2 was thus identified as 1,6-dihydroxy-3,7-dimethoxy-2,8-di-(3-methylbut-2-enyl)xanthone or β-mangostin (**2**) (Figure 6).



(**2**)



(**104**)

Figure 6 Structure of β-mangostin (**2**) and 1,3-dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone (**104**)

Table 4 NMR data of compound GM-E2 (sss4447) and β-mangostin (**2**) in $CDCl_3$

position	β-mangostin			GM-E2 (sss4447)
	δ_C[a]	δ_H[a]	δ_H[b]	δ_H
1-OH	159.8	13.39 (1H, s)	13.42 (1H, s)	13.40 (1H, s)
2	111.7			
3	163.5			
3-OMe	56.1	3.88 (3H, s)	3.87 (3H, s)	3.88 (3H, s)
4	89.0	6.32 (1H, s)	6.34 (1H, s)	6.32 (1H, s)
4a	155.7			
5	101.6	6.81 (1H, s)	6.83 (1H, br s)	6.81 (1H, s)
6-OH	155.3	6.30 (1H, s)	6.32 (1H, br s)	6.30 (1H, s)
7	142.6			
7-OMe	62.3	3.79 (3H, s)	3.81 (3H, s)	3.79 (3H, s)
8	137.1			
8a	112.6			
9	181.9			
9a	104.0			
10a	154.4			
11	21.7	3.33 (2H, br d, J = 7.0 Hz)	3.35 (2H, d, J = 7.1 Hz)	3.33 (2H, d, J = 7.3 Hz)
12	122.4	5.21 (1H, br t, J = 7.0 Hz)	5.25 (1H, m)	5.22 (1H, m)
13	131.7			
14, 15	18.1	1.78 (3H, s)	1.80 (3H, s)	1.77 (3H, s)
		1.67 (3H, s)	1.69 (3H, s)	1.66 (3H, s)
16	26.9	4.08 (2H, br d, J = 6.4 Hz)	4.09 (2H, d, J = 6.2 Hz)	4.07 (2H, d, J = 5.7 Hz)
17	123.3	5.24 (1H, br t, J = 6.4 Hz)	5.25 (1H, m)	5.22 (1H, m)
18	132.1			
19, 20	18.6	1.81 (3H, s)	1.83 (3H, s)	1.81 (3H, s)
	26.1	1.67 (3H, s)	1.69 (3H, s)	1.66 (3H, s)

[a] Suwannapoch. 2002: 36-38

[b] Likhitwitayawuid; Phadungcharoen; & Krungkrai. 1998: 70-72

4.1.3 Structure determination of compound GM-E3 (γ-mangostin, sss3209)

Compound GM-E3 appeared as a yellow solid with higher in polarity than α-mangostin (R_f 0.45 (40% acetone-*n*-hexane)) and gave a yellow coloration with anisaldehyde-H_2SO_4 reagent. The ^{1}H NMR spectrum (Table 5) obtained in acetone-d_6 showed signal for a 1,3,6,7-tetraoxygenated xanthone which included one chelated hydroxyl group at δ 13.93, two aromatic singlets at δ 6.36 (H-4) and δ 6.80 (H-5) and two prenyl unit signals at δ 5.30 (t, J = 7.2 Hz), δ 3.33 (d, J = 7.2 Hz), δ 1.62 (s) and δ 5.30 (t, J = 6.6 Hz), δ 4.17 (d, J = 6.6 Hz), δ 1.77 (s), δ 1.82 (s). Comparison of ^{1}H NMR data with α-mangostin showed a similar pattern, except for the GM-E3 does not have signal of methoxyl group. Compound GM-E3 was isolated as the second major compound from the extract. From the comparison of ^{1}H NMR data and chromatographic pattern on TLC with authentic γ-mangostin (**3**), which was isolated by our group (Suksamrarn; et al. 2003: 857-9, Mahabusarakam; & Wirivachitra. 1987: 474-478), compound GM-E3 was thus identified as 1,3,6,7-tetrahydroxy-2,8-di-(3-methylbut-2-enyl)xanthone or γ-mangostin (**3**) (Figure 7).

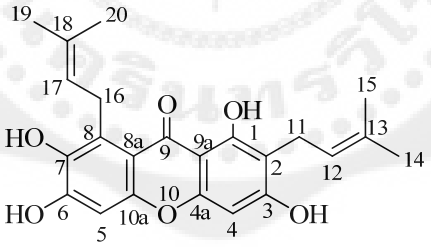


Figure 7 Structure of γ-mangostin (**3**)

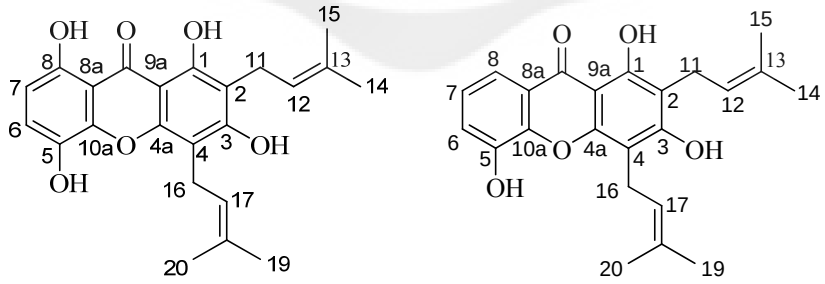
Table 5 Comparison of ^{1}H NMR data of compound GM-E3 (sss3209) with γ-mangostin (**3**) in CD_3COCD_3

position	γ-mangostin[a]	GM-E3 (sss3209)
	δ_H	δ_H
1-OH	13.93 (1H, s)	13.93 (1H, s)
2		
3-OH	6.37 (1H, s)	
4	6.37 (1H, s)	6.36 (1H, s)
4a		
5	6.81 (1H, s)	6.80 (1H, s)
6-OH	6.31 (1H, s)	
7-OH	2.91 (1H, br s)	
8		
8a		
9		
9a		
10a		
11	3.35 (2H, d, J = 7.0 Hz)	3.33 (2H, d, J = 7.2 Hz)
12	5.30 (1H, t, J = 7.0 Hz)	5.30 (1H, t, J = 7.2 Hz)
13		
14, 15	1.64 (3H, s)	1.62 (6H, s)
	1.64 (3H, s)	
16	4.19 (2H, d, J = 7.0 Hz)	4.17 (2H, d, J = 6.6 Hz)
17	5.30 (2H, br t, J = 7.0 Hz)	5.30 (2H, br t, J = 6.6 Hz)
18		
19, 20	1.78 (3H, s)	1.77 (3H, s)
	1.84 (3H, s)	1.82 (3H, s)

[a] Mahabusarakam; & Wirivachitra. 1987: 474-478

4.1.4 Structure determination of compound GM-E4 (a mixture of gartanin and 8-desoxygartanin, sss4460)

Compound GM-E4 was also a prenylated xanthone and obtained as a yellow solid. From the TLC, this compound showed only one green spot with anisaldehyde-H_2SO_4 reagent (R_f value of 0.53 (30% acetone-*n*-hexane)). The ^{1}H NMR spectrum of compound GM-E4 showed three singlets of chelated hydroxyl group at δ 11.25, δ 12.35 and δ 13.18. An AB pattern of aromatic protons H-6 and H-7 were observed at ca δ 7.23 and δ 6.65 (d, J = 8.9 Hz), respectively. Furthermore, the singnals at δ 5.25 (m), δ 3.51 (br d, J = 6.3 Hz) and δ 3.45 (br d, J = 6.6 Hz) exhibited for the presence of two 3-methylbut-2-enyl groups. This result showed that compound GM-E4 are a mixture compounds. From my previous work (Jaratrungtawee. 2007: 78-81), gartanin (**4**) and 8-desoxygartanin (**5**) were isolated from very young fruit of *G. mangostana*. The ^{1}H NMR spectrum of gartanin (**4**) exhibited two singlets chelated hydroxyl group and two prenyl unit signals whereas 8-desoxygartanin (**5**) showed one chelated hydroxyl signal. Comparison of ^{1}H NMR data of compound GM-E4 with those isolated xanthones showed that compound GM-E4 exhibited NMR data identical to those of a mixture of gartanin (**4**) and 8-desoxygartanin (**5**). Therefore, compound GM-E4 was concluded to be a mixture of gartanin (**4**) and 8-desoxygartanin (**5**) and ^{1}H NMR data showed integral of both compounds in ratio of 9:1 (Figure 8).



(**4**) (**5**)

Figure 8 Structure of gartanin (**4**) and 8-desoxygaratnin (**5**)

4.1.5 Structure determination of compound GM-E5 (9-hydroxycalabaxanthone, sss4597)

Compound GM-E5 was isolated as a yellow solid with less in polarity than α-mangostin (R_f value of 0.37 (20% acetone-*n*-hexane)) and gave a green coloration with anisaldehyde-H_2SO_4 reagent which showed characteristic of xanthone. The ^{1}H NMR spectrum (Table 6) exhibited the singlet resonance of a hydrogen bonded hydroxy proton at δ 13.67 and one methoxy group at δ 3.78. The isoprenyl unit showed signals at δ 5.24 (br t, J = 6.4 Hz), δ 4.06 (d, J = 6.4 Hz), δ 1.67 (s), δ 1.81 (s). An AB system from methine protons were observed at δ 6.70 (H-11, d, J = 10.0 Hz) and δ 5.55 (H-12, d, J = 10.0 Hz) and two methyl singlets at δ 1.46 which was characterized for the presence of a 2,2-dimethylchromene ring. The two aromatic singlets signal at δ 6.22 and δ 6.81 showed the similar chemical shift of H-4 and H-5, respectively, as that of α-mangostin. Comparison of ^{1}H NMR data with α-mangostin showed the similar ^{1}H NMR pattern, except for the one isoprenyl unit on ring A of α-mangostin was replaced with 2,2-dimethylchromene ring. From the comparison of ^{1}H NMR data and chromatographic pattern on TLC with authentic 9-hydroxycalabaxanthone (**10**) in several solvent systems (Sen; et al. 1981: 183-185, Komutiban. 2004: 44-47), the structure of GM-E5 was identified as 1,6-dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)-6′,6′-dimethylpyrano-(2′,3′:3,2)xanthone or 9-hydroxycalabaxanthone (**10**) (Figure 9).

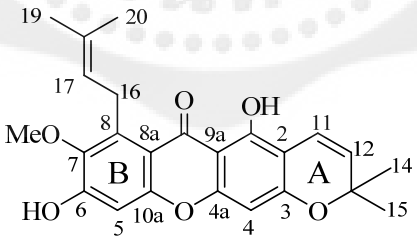


Figure 9 Structure of 9-hydroxycalabaxanthone (**10**)

Table 6 NMR data of compound GM-E5 (sss4597) and 9-hydroxycalabaxanthone (**10**) in $CDCl_3$

position	9-hydroxycalabaxanthone (**10**)			Compound GM-E4 (sss4597)
	δ_C [a]	δ_H [a]	δ_H [b]	δ_H
1-OH	157.8	13.55 (1H, s)	13.72 (1H, s)	13.67 (1H, s)
2	104.4			
3	159.8			
4	94.0	6.15 (1H, s)	6.31 (1H, s)	6.22 (1H, s)
4a	156.1			
5	101.6	6.27 (1H, s)	6.82 (1H, s)	6.81 (1H, s)
6-OH	154.5			
7	142.7			
7-OMe	61.8	3.73 (3H, s)	3.82 (3H, s)	3.78 (3H, s)
8	136.9			
8a	112.1			
9	181.8			
9a	103.6			
10a	155.6			
11	115.6	6.64 (1H, d, J = 10.0 Hz)	6.73 (1H, d, J = 10.0 Hz)	6.70 (1H, d, J = 10.0 Hz)
12	126.9	5.47 (1H, d, J = 10.0 Hz)	5.55 (1H, d, J = 10.0 Hz)	5.55 (1H, d, J = 10.0 Hz)
13	77.8			
14, 15	28.3	1.40 (2x3H, 2xs)	1.49 (2x3H, 2xs)	1.46 (2x3H, 2xs)
16	26.5	4.01 (2H, d, J = 6.5 Hz)	4.09 (2H, d, J = 6.0 Hz)	4.06 (2H, d, J = 6.4 Hz)
17	123.1	5.18 (1H, t, J = 6.5 Hz)	5.29 (1H, t, J = 6.0 Hz)	5.24 (1H, br t, J = 6.4 Hz)
18	131.8			
19, 20	18.1	1.76 (3H, s)	1.85 (3H, s)	1.81 (3H, s)
	25.6	1.62 (3H, s)	1.72 (3H, s)	1.67 (3H, s)

[a] Sen; et al. 1981: 183-185

[b] Komutiban. 2004: 44-47

4.1.6 Structure determination of compound GM-E6 (garcinone E, sss4479)

Compound GM-E6 was also a prenylated xanthone and obtained as a yellow solid with less in polarity than γ-mangostin which showed the R_f value of 0.38 (20% acetone-*n*-hexane) and gave a yellow coloration with anisaldehyde-H_2SO_4 reagent. The ^{1}H NMR spectrum (Table 7) of GM-E6 was similar to those of γ-mangostin, expect for the presence of signals for the third prenyl unit at δ 5.60 (m), δ 3.58 (d, J = 7.5 Hz), δ 1.82 (s), δ 1.85 (s) were observed. The position of this additional prenyl unit was located at C-5 due to the absence of the H-5 aromatic proton signal. From the comparison of ^{1}H NMR data and chromatographic pattern on TLC with authentic garcinone E (**26**) (Komutiban. 2004: 57-60, Sakai; et al. 1993: 958-960), compound GM-E6 was identified as 1,3,6,7-tetrahydroxy-2,5,8-tri-(3-methylbut-2-enyl)xanthone or garcinone E (**26**) (Figure 10).

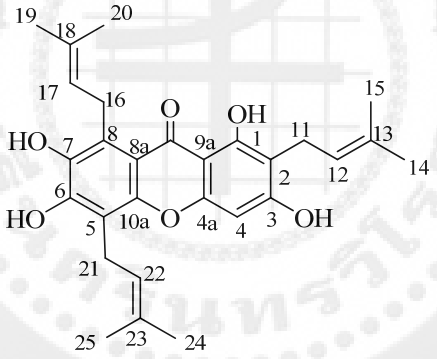


Figure 10 Structure of garcinone E (**26**)

Table 7 NMR data of compound GM-E6 (sss4479) and garcinone E (**26**) in $CDCl_3$

position	garcinone E (**26**)				Compound GM-E6 (sss4479)
	δ_C [a]	δ_H [a]	δ_C [b]	δ_H [b]	δ_H
1-OH	161.5	13.83 (1H, s)	160.5	13.84 (1H, s)	13.82 (1H, s)
2	110.7		108.2		
3-OH	162.8	6.18 (1H, s)	161.5		
4	92.9	6.32 (1H, s)	93.2	6.33 (1H, s)	6.31 (1H, s)
4a	155.6		155.1		
5	114.2		113.3		
6-OH	151.8	6.44 (1H, s)	148.7		
7-OH	140.5	5.63 (1H, s)	139.3		
8	128.2		124.6		
8a	112.2		111.3		
9	183.4		183.1		
9a	103.0		103.7		
10a	152.0		151.3		
11	21.9	3.45 (2H, d, J = 7.0 Hz)	21.5	3.45 (2H, d, J = 7.2 Hz)	3.44 (2H, d, J = 7.2 Hz)
12	123.5	5.30 (1H, m)	121.5	5.29 (1H, m)	5.60 (1H, m)
13	131.4		133.7		
14, 15	17.8	1.70 (3H, s)	25.8	1.88-1.71 (3H, s)	1.68 (3H, s)
	25.8	1.77 (3H, s)	17.9	1.88-1.71 (3H, s)	1.75 (3H, s)
16	26.3	4.29 (2H, d, J = 6.5 Hz)	25.8	4.29 (2H, d, J = 6.9 Hz)	4.28 (2H, d, J = 6.3 Hz)
17	124.4	5.30 (1H, m)	121.9	5.29 (1H, m)	5.60 (1H, m)
18	131.4		135.8		
19, 20	18.2	1.77 (3H, s)	25.9	1.88-1.71 (3H, s)	1.75 (3H, s)
	25.8	1.87 (3H, s)	18.0	1.88-1.71 (3H, s)	1.85 (3H, s)
21	23.1	3.59 (2H, d, J = 7.1 Hz)	22.6	3.60 (2H, d, J = 7.4 Hz)	3.58 (2H, d, J = 7.5 Hz)
22	122.5	5.23 (1H, m)	121.0	5.29 (1H, m)	5.60 (1H, m)
23	132.3		135.1		
24, 25	18.0	1.84 (3H, s)	25.8	1.88-1.71 (3H, s)	1.82 (3H, s)
	25.8	1.87 (3H, s)	18.0	1.88-1.71 (3H, s)	1.85 (3H, s)

[a] Komutiban. 2004: 57-60

[b] Sakai; et al. 1993: 958-960

4.1.7 Structure determination of compound GM-E7 (1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone, sss4477)

Compound GM-E7 yielded as a yellow solid with the R_f value of 0.52 (30% acetone-*n*-hexane) and gave a yellow coloration with anisaldehyde-H_2SO_4 reagent. The resonance of a chelated proton was shown at δ 12.79 (1-OH, s) in the ^{1}H NMR spectrum (Table 8). An ABX pattern of aromatic showed at δ 7.32 (d, J = 8.7 Hz), ca δ 7.24 and δ 7.46 (d, J = 3.0 Hz) whereas the isolated aromatic proton H-4 at δ 6.40 were observed. The downfield shift of aromatic proton at δ 7.46 could be assigned to locate at C-8 due to an anisotropic effect of the carbonyl group (C-9), thus the adjacent aromatic protons at δ 7.32 and ca δ 7.24 were assigned as H-5 and H-6, respectively. The presence of an isoprenyl unit was indicated from the resonances at δ 5.21 (t, J = 6.6 Hz), δ 3.35 (d, J = 6.6 Hz), δ 1.67 (s), δ 1.71 (s). One methoxyl group exhibited signal at δ 3.89 (3-OMe). Comparison of ^{1}H NMR data with authentic 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone (**12**), which was isolated by my MS work (Jaratrungtawee. 2007: 90), showed the same ^{1}H NMR pattern. Therefore, compound GM-E7 identified as 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone (**12**) (Figure 11).

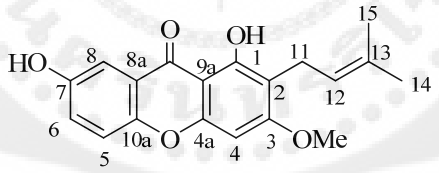


Figure 11 Structure of 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone (**12**)

Table 8 Comparison of ^{1}H NMR data of 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone with compound GM-E7 (sss4477) in $CDCl_3$

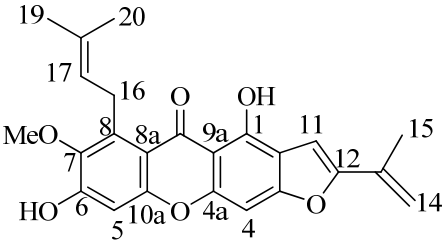
position	1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone [a]	Compound GM-E7 sss4477
	δ_H	δ_H
1-OH	12.83 (1H, s)	12.79 (1H, s)
2		
3		
3-OMe	3.92 (3H, s)	3.89 (3H, s)
4	6.42(1H, s)	6.40(1H, s)
4a		
5	7.32 (1H, d, J = 8.7 Hz)	7.32 (1H, d, J = 8.7 Hz)
6	ca 7.24 obscured signal	ca 7.24 obscured signal
7		
8	7.50 (1H, s)	7.46 (1H, d, J = 3.0 Hz)
8a		
9		
9a		
10a		
11	3.35 (2H, d, J = 6.6 Hz)	3.32 (2H, d, J = 6.6 Hz)
12	5.21 (1H, br t, J = 6.6 Hz)	5.17 (1H, br t, J = 6.6 Hz)
13		
14, 15	1.71 (3H, s)	1.75 (3H, s)
	1.67 (3H, s)	1.63 (3H, s)

[a] Jaratrungtawee. 2007: 90.

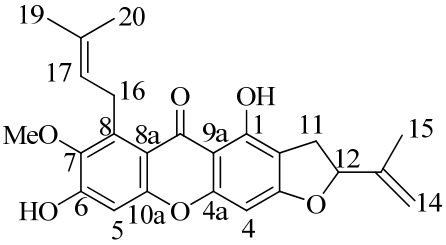
4.1.8 Structure determination of compound GM-E8 (mangostenone H or 1,3,6-trihydroxy-7-methoxy-3-(1-methylethenyl)-8-(3-methylbut-2-enyl)-furano[2,3-b]xanthone, sss2917)

Compound GM-E8 showed a pseudomolecular ion peak at *m/z* 405.1342 $[M-H]^-$ in its HRTOFMS, corresponding to the molecular formula $C_{24}H_{22}O_6$. The IR spectrum showed absorption bands at 3443 cm^{-1} for one or more hydroxyl group and 1650 cm^{-1} for a carbonyl functionality. Comparison of the UV absorption band, ^1H and ^{13}C NMR spectra of compound GM-E8 with α-mangostin indicated that compound GM-E8 has a similar xanthone skeleton. The ^1H NMR and HMQC data (Table 9, 10) showed a chelated phenolic hydroxyl group at δ 14.10, two aromatic singlets at δ 6.87 and δ 6.88, one methoxyl group signal at δ 3.80 and one prenyl group at δ 4.10 (br d, J = 10.2 Hz), δ 5.27 (t, J = 10.2 Hz), δ 1.69 (s) and δ 1.83 (s). Other series of signals could be attributed to a furano ring with an isopropenyl group at δ 6.81 (s), 5.16(br s), 5.72 (br s) and 2.10 (s). The prenyl unit was at C-8 (δ 137.1) same as that of α-mangostin which was confirmed by an NOE correlation of the methine proton resonance (H-17, δ 5.27) to the methoxy group at δ 3.80. The presence of an isopropenyl unit was confirmed from successive connectively between methylene protons at δ 5.16/5.72 (H-14a/b) and methyl proton at δ 2.10 (H-15) to C-12 (δ 156.2) in its HMBC spectrum (Table 10, Figure 13). Moreover, the methyl proton at H-15 also exhibited an NOE enhancement (Figure 13) with methylene proton H-11 (δ 6.81) in the NOSEY spectrum, confirming the placement of isoproprenyl unit to furano ring at C-12. The furano ring was placed at C-2 (δ 113.7) and C-3 (δ 159.2), as evidenced by the HMBC correlations (Figure 13) from H-11 (δ 6.81) to C-1 (δ 156.4), C-2 and C3.

The ^1H NMR and ^{13}C NMR data of compound GM-E8 were similar to those of mangostenone F (**95**), a structure related xanthone isolated from the seed case of *G. mangostana* (Ryu; et al. 2010: 6258-6264), except that the dihydrofurano ring in mangostenone F (**95**) was replaced by the furan ring in compound GM-E8. Thus, the structure of compound GM-E8 was assigned to the 1,3,6-trihydroxy-7-methoxy-3-(1-methylethenyl)-8-(3-methylbut-2-enyl)-furano[2,3-b]xanthone (**105**) (Figure 12) and named mangostenone H after its plant origin.



(**105**)



(**95**)

Figure 12 Structure of mangostenone H (**105**) and mangostenone F (**95**),

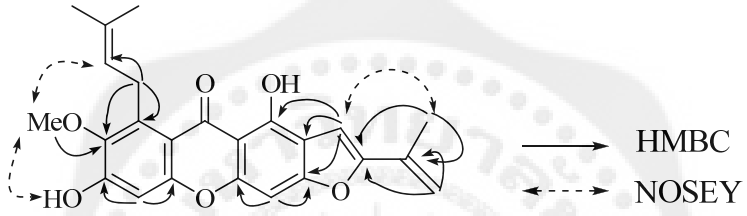


Figure 13 Selected HMBC and NOESY correlations of mangostenone H (**105**)

Table 9 NMR data of compound GM-E8 (sss2917) and mangostenone F in $CDCl_3$

position	mangostenone F[a]		Compound GM-E8 (sss2917)		
	δ_H	δ_C	δ_H	δ_C	DEPT135
1-OH	13.9 (1H, s)	162.6	14.10 (1H, s)	156.4	
2		109.4		113.7	
3		165.1		159.2	
4	6.18 (1H,s)	94.7	6.88 (1H, s)	89.4	89.45
4a		156.7		154.9	
5	6.68 (1H, s)	103.2	6.87 (1H, s)	101.5	101.5
6		156.7		154.9	
7		145.0		142.5	
7-OMe	3.66 (3H, s)	61.7	3.80 (1H, s)	62.1	62.1
8		131.8		137.1	
8a		112.2		111.72	
9		183.3		183.5	
9a		103.9		104.5	
10a		157.9		153.6	
11a	2.75 (1H, dd, 14.5, 8.0)	30.5	6.81 (1H, s)	100.3	100.3
b	2.94 (1H, dd, 14.5, 3.3)				
12	4.28 (1H, m)	77.1		156.2	
13		148.7		132.3	
14a	4.64 (1H, br s)	110.8	5.16 (1H, br s)	113.1	
b	4.82 (1H, br s)		5.72 (1H, br s)		
15	1.69 (3H, s)	18.7	2.10 (3H, s)	19.2	19.2
16	3.98 (2H, d, 6.5)	27.3	4.10 (2H, br d, J = 10.2 Hz)	26.6	
17	5.13 (1H, m)	125.2	5.27 (1H, t, J = 10.2 Hz)	123.0	123.0
18		138.5		132.3	
19, 20	1.70 (3H, s)	18.7	1.69 (3H, s)	25.8	25.8
	1.52 (3H, s)	26.3	1.83 (3H, s)	18.2	18.2

[a] Ryu; et al. 2010: 6258-6264.

Table 10 ^{1}H NMR and 2D NMR of compound GM-E8 (sss2917)

position	^{1}H NMR	COSY[a]	HMQC[b]	HMBC[c]	NOSEY
1-OH	14.10 (1H, s)			C-1, C-2, C-9a	
4	6.88 (1H, s)		C-4	C-1, C-2, C-3, C-4a, C-9a	
5	6.87 (1H, s)		C-5	C-6, C-7, C-8a, C-10a	
7-OMe	3.80 (1H, s)		C-7OMe	C-7	6-OH, H-17
11	6.81 (1H, s)		C-11	C-1, C-2, C-3, C-12	H-15
14	(a) 5.16 (1H,br s) (b) 5.72 (1H, br s)	H-14a, H-14b, H-15	C-14	C-12, C-13, C-15	H-15
15	2.10 (3H, s)	H-14a, H-14b	C-15	C-12, C-13, C-14	
16	4.10 (2H, d, J = 10.2 Hz)	H-17	C-16	C-7, C-8, C-8a, C-17, C-18	
17	5.27 (1H, t, J = 10.2 Hz)	H-16	C-17		
19, 20	1.69 (3H, s)		C-19	C-16, C-17, C-18, C-20	
	1.83 (3H, s)		C-20	C-16, C-17, C-18, C-19	

[a]proton-proton correlation

[b]1-bond heteronuclear ^{1}H-^{13}C correlation

[c]long range heteronuclear ^{1}H-^{13}C correlation

4.1.9 Structure determination of compound GM-E9 (garcinone B, sss3456)

Compound GM-E9 was also a prenylated xanthone and obtained as a pale yellow solid with higher in polarity than α-mangostin (R_f value of 0.40 (dichloromethane)). The ^{1}H NMR spectrum (Table 11) obtained in $CDCl_3$ revealed signals for a 1,3,6,7-tetraoxygenated xanthone, which include one chelated hydroxyl group at δ 13.67, two aromatic singlets at δ 6.29 (H-4) and δ 6.80 (H-5), a 3-methylbut-2-enyl group at δ 5.27 (m), δ 3.43 (d, J = 7.2 Hz), δ 1.75 (s), δ 1.82 (s) and a 2,2-dimethylchromene ring at δ 8.00 (d, J = 10.2 Hz), δ 5.80 (d, J = 10.2 Hz), δ 1.47 (s). This 2,2-dimethylchroman ring colud be attached at C-7 and C-8 due to an anisotropic effect of the carbonyl group (C-9) which caused the downfield shift of H-16 proton. The ^{1}H NMR data of GM-E9 is very similar to that of the authentic garcinone B (**14**), which was isolated by our group (Suwannapoch. 2002: 41-42), therefore, compound GM-E9 was established to be 1,3,6-trihydroxy-2-(3-methylbut-2-enyl)-6′,6′-dimethylpyrano-(2′,3′:7,8)xanthone, or named garcinone B (**14**) (Figure 14).

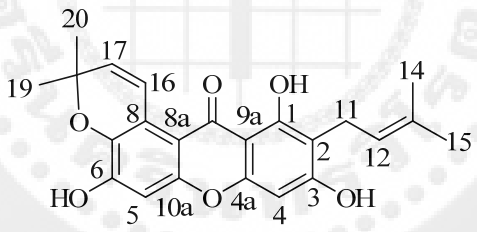


Figure 14 Structure of garcinone B (**14**)

Table 11 Comparison of ^{1}H NMR data of compound GM-E9 (sss3456) with garcinone B (**14**) in $CDCl_3$

position	garcinone B	Compound GM-E8 (sss3456)
	δ_H [a]	δ_H
1-OH	13.68 (1H, s)	13.67 (1H, s)
2		
3		
4	6.29 (1H, s)	6.29 (1H, s)
4a		
5	6.80 (1H, s)	6.80 (1H, s)
6		
7		
8		
8a		
9		
9a		
10a		
11	3.44 (2H, d, J = 7.0 Hz)	3.43 (2H, d, J = 7.2 Hz)
12	5.27 (1H, m)	5.27 (1H, m)
13		
14, 15	1.75 (3H, s)	1.75 (3H, s)
	1.82 (3H, s)	1.82 (3H, s)
16	8.01 (1H, d, J = 10.2 Hz)	8.00 (1H, d, J = 10.2 Hz)
17	5.81 (1H, d, J = 10.2 Hz)	5.80 (1H, d, J = 10.2 Hz)
18		
19, 20	1.47 (6H, s)	1.47 (6H, s)

[a] Suwannapoch. 2002: 41-42

4.1.10 Structure determination of compound GM-E10 (cowaxanthone D, sss3502)

GM-E10 was elucidated to be a close analogue of garcinone B, however, GM-E10 has less in polarity, with R_f value of 0.54 (30% acetone-*n*-hexane), than garcinone B. Its IR spectrum showed an absorption bands at 3483 cm^{-1} for one or more hydroxyl group and 1645 cm^{-1} for a carbonyl functionality. The difference was readily found from the ^{1}H NMR spectrum (Table 12). An addition of a methoxyl singlet signal at δ_H 3.84, δ_C 55.7 was observed. The location of this methoxyl group at C-3 (δ 163.4) was confirmed by its HMBC (Table 13, Figure 16) correlations with C-3. In addition, this methoxyl proton also exhibited NOE enhancement (Figure 16) with aromatic proton H-4 (δ 6.29) and methyl proton H-15 (δ 1.61) in the NOSEY spectrum, confirming the attachment of methoxy group at C-3. All of the ^{1}H and ^{13}C NMR spectroscopic data were clearly assigned as presented in Table 12 according to the detailed HMBC and HMQC correlations (Table 13). Thus, structure compound GM-E10 was assigned to the 1,6-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)-6′,6′-dimethylpyrano(2′,3′:7,8)xanthone (Figure 15). The ^{1}H and ^{13}C NMR spectral data of GM-E10 were similar to that of cowaxanthone D (**102**), a xanthone that was previously isolated from fresh fruit of *G. cowa* (Panthong; et al. 2006: 999-1004).

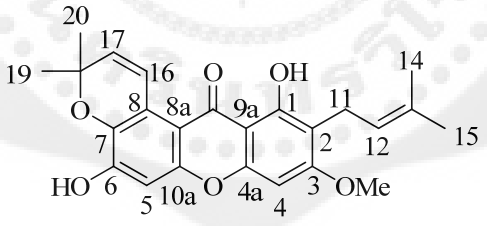


Figure 15 Structure of cowaxanthone D (**102**)

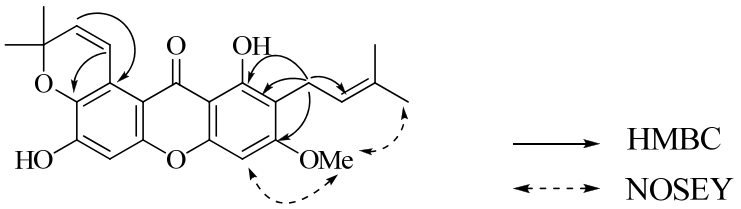


Figure 16 Selected HMBC and NOESY correlations of cowaxanthone D

Table 12 Comparison of ^{1}H and ^{13}C NMR data of compound GM-E10 (sss3502) with cowaxanthone D (**102**) in $CDCl_3$

position	cowaxanthone D[a]		Compound GM-E10 (sss3502)		
	δ_H	δ_C	δ_H	δ_C	DEPT135
1-OH	13.34 (1H, s)	159.6	13.31 (1H, s)	159.4	
2		111.0		111.2	
3		163.6		163.4	
3-OMe	3.90 (3H, s)	55.8	3.84 (3H, s)	55.7	55.7
4	6.36 (1H, s)	88.9	6.29 (1H, s)	88.9	88.9
4a		155.4		155.4	
5	6.82 (1H, s)	102.2	6.73 (1H, s)	102.3	102.3
6		150.7		151.2	
6-OH	6.20 (1H, s)				
7		136.7		137.2	
8		119.7		119.8	
8a		108.7		108.5	
9		182.4		172.2	
9a		104.0		103.8	
10a		153.0		152.9	
11	3.35 (2H, d, J = 7.2 Hz)	21.3	3.29 (2H, br d, J = 7.2 Hz)	21.2	21.2
12	5.23 (1H, m)	122.3	5.15 (1H, br t, J = 7.2 Hz)	122.1	122.1
13		131.7		131.6	
14, 15	1.80 (3H, s)	17.7	1.72 (3H, s)	17.6	17.64
	1.68 (3H, s)	25.8	1.61 (3H, s)	25.6	25.6
16	8.03 (1H, d, J = 10.2 Hz)	121.0	7.95 (1H, d, J = 10.2 Hz)	120.9	120.9
17	5.82 (1H, d, J = 10.2 Hz)	132.2	5.75 (1H, d, J = 10.2 Hz)	132.1	132.1
18		77.2		76.4	
19, 20	1.50 (3H, s)	27.3	1.43 (3H, s)	27.0	27.0
	1.50 (3H, s)	27.3	1.43 (3H, s)	27.0	27.0

[a] Pathong; et al. 2006: 999-1004

Table 13 ^{1}H NMR and 2D NMR data of compound GM-E10

position	^{1}H NMR	COSY[a]	HMQC[b]	HMBC[c]	NOSEY
1-OH	13.31 (1H, s)			C-1, C-2, C-9a	
4	6.29 (1H, s)		C-4	C-2, C-3, C-4a, C-9a	
5	6.73 (1H, s)		C-5	C-7, C-8a, C-10a	
3-OMe	3.84 (3H, s)		C-3OMe	C-3	H-4, H-15
11	3.29 (2H, br d, J = 7.2 Hz)	H-12, H14, H-15	C-11	C-1, C-2, C-3, C-12, C-13	
12	5.15 (1H, br t, J = 7.2 Hz)	H-11, H14, H-15	C-12		
14, 15	1.72 (3H, s)		C-14	C-11, C-12, C-13, C-15	
	1.61 (3H, s)		C-15	C-11, C-12, C-13, C-14	
16	7.95 (1H, d, J = 10.2 Hz)	H-17	C-16	C-7, C-8, C-17, C-18	
17	5.75 (1H, d, J = 10.2 Hz)	H-16	C-17	C-8, C-18, C-19, C-20	
19, 20	1.43 (6H, s)		C-19	C-16, C-17, C-18, C-20	
			C-20	C-16, C-17, C-18, C-19	

[a] proton-proton correlation

[b] 1-bond heteronuclear ^{1}H-^{13}C correlation

[c] long range heteronuclear ^{1}H-^{13}C correlation

4.1.11 Structure determination of compound GM-E11 (mangostanin, sss3458)

Compound GM-E11 was isolated in a very small amount as a yellow solid and also gave a yellow coloration with anisaldehyde-H_2SO_4 reagent (R_f value of 0.54 (30% acetone-*n*-hexane)). Comparison of ^{1}H and ^{13}C NMR spectra of compound GM-E11 with α-mangostin indicated that compound GM-E11 has a similar xanthone skeleton. The presence of a dihydrofurano ring with a 2-hydroxypropanyl group was showed at δ_H 3.12 (H-11a, dd, J = 9.1, 15.9 Hz); δ_H 3.03 (H-11b, dd, J = 8.5, 15.9 Hz); δ_C 26.7 (C-11), δ_H 4.72 (H-12, dt, J = 8.5, 9.1 Hz); δ_c 91.6 (C-12), δ_H 1.18 (H-14, s); δ_C 23.7 (C-14) and δ_H 1.29 (H-15, s); δ_c 25.5 (C-15). The downfield shift of methine carbon signal at δ 91.6 and quaternary carbon signal at δ 71.6 indicated that these two carbons are part of dihydrofurano system. From the comparison of ^{1}H and ^{13}C NMR data with authentic mangostanin (**63**) (Table 14), which was isolated by our group (Komutiban. 2006: 83), compound GM-E11 was thus identified as 1,6-dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)-5′-(1-hydroxy-1-methylethyl)-4′,5′-dihydrofurano(2′,3′:3,2)xanthone or mangostanin (**63**) (Figure 17).

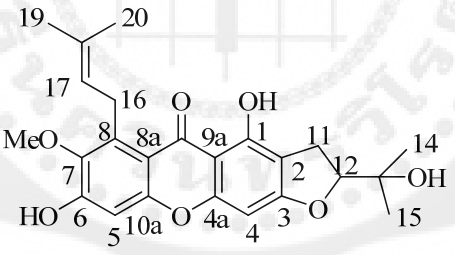


Figure 17 Structure of mangostanin (**63**)

Table 14 Comparison of ^{1}H and ^{13}C NMR data of compound GM-E11 (sss3458) in CDCl3 + 4 drop MeOD (2% CD_3OD in $CDCl_3$) with mangostanin

position	mangostanin[a]		Compound GM-E11 (sss3458)	
	δ_H	δ_C	δ_H	δ_C
1-OH	13.82 (1H, s)	157.3	13.66 (1H, s)	157.8
2		107.2		107.3
3		166.1		166.1
4	6.27 (1H, s)	87.9	6.22 (1H, s)	88.1
4a		156.9		157.0
5	6.75 (1H, s)	101.6	6.72 (1H, s)	101.7
6		156.0		155.6
7		143.2		143.0
7-OMe	3.79 (3H, s)	60.7	3.73 (3H, s)	61.4
8		137.1		137.1
8a		111.1		111.6
9		182.0		182.0
9a		103.7		104.0
10a		155.3		155.3
11	3.09 (1H, dd, J = 9.8, 15.5 Hz) 3.18 (1H, dd, J = 8.2, 15.5 Hz)	26.5	3.12 (1H, dd, J = 9.1, 15.9 Hz) 3.03 (1H, dd, J = 8.5, 15.9 Hz)	26.7
12	4.76 (1H, dd, J = 8.2, 9.8 Hz)	91.4	4.72 (1H, dd, J = 8.5, 9.1 Hz)	91.6
13		71.4		71.6
14, 15	1.25 (3H, s) 1.31 (3H, s)	23.8 24.7	1.18 (3H, s) 1.29 (3H, s)	23.7 25.5
16	4.10 (2H, d, J = 5.7 Hz)	26.0	4.04 (2H, d, J = 5.8 Hz)	26.3
17	5.26(1H, br t, J = 5.7 Hz)	123.2	5.21 (1H, d, J = 5.8 Hz)	123.2
18		131.4		131.8
19, 20	1.69 (3H, s) 1.84 (3H, s)	17.8 25.5	1.64 (3H, s) 1.78 (3H, s)	25.7 18.1

[a] Komutiban. 2006: 83 (20% CD_3OD in $CDCl_3$)

4.1.12 Structure determination of compound GM-E12 (garcinone D, sss3301)

Compound GM-E12 was also a prenylated xanthone and yielded as pale yellow solid with equal polarity to cratoxylone (**103**) (R_f value of 0.45 (30% acetone-*n*-hexane, 6 elutions) and gave a green coloration with anisaldehyde-H_2SO_4 reagent. The ^{1}H NMR spectrum of compound GM-E12 (Table 16) was similar to those of α-mangostin, except for the presence of signals for a 3-hydroxy-3-methylbutyl group at δ 3.45 (m), ca δ 1.72 and δ 1.29 (s) in ring B were observed. The identification of compound GM-E12 was done by comparing the ^{1}H NMR data and chromatographic pattern on TLC with authentic garcinone D (**16**) (Table 15), which was isolated by my previous work (Jaratrungtawee; 2007: 94-95), the structure of compound GM-E12 was deduced as 1,3,6-trihydroxy-2-(3,3-dimethylallyl)-7-methoxy-8-(3-hydroxy-3-methylbutyl)xanthone or garcinone D (**16**) (Figure 18).

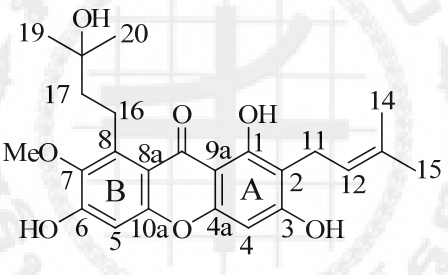


Figure 19 Structure of garcinone D (**16**)

Table 15 Comparison of ^{1}H NMR data of compound GM-E12 (sss3301) with garcinone D (**16**) in CD_3COCD_3

position	garcinone D[a]	Compound GM-E12 (sss3301)
	δ_H	δ_H
1-OH	13.81 (1H, s)	13.81 (1H, s)
2		
3		
4	6.39 (1H, s)	6.38 (1H, s)
4a		
5	6.80 (1H, s)	6.80 (1H, s)
6[e]		
7		
7-OMe	3.84 (3H, s)	3.83 (3H, s)
8		
8a		
9		
9a		
10a[f]		
11	3.33 (2H, d, J = 7.2 Hz)	3.32 (2H, d, J = 6.3 Hz)
12	5.27 (1H, br t, J = 7.2 Hz)	5.26 (1H, br t, J = 6.3 Hz)
13-OH		
14, 15	1.77 (3H, s),	1.76 (3H, s)
	1.63 (3H, s)	1.63 (3H, s)
16	3.47 (2H, m)	3.45 (2H, m)
17	ca 1.72 obscured signal	ca 1.72 obscured signal
18		
19, 20	1.30 (2x3H, 2xs)	1.29 (2x3H, 2xs)

[a] Jaratrungtawee; 2007: 94-95

4.1.13 Structure determination of compound GM-E13 (cratoxylone, sss3300)

Compound GM-E13 was isolated as yellow solid and showed a pseudomolecular ion peak at *m/z* 427.4 $[M-H]^-$ in the ESI-MS which corresponds to a molecular formula of $C_{24}H_{28}O_7$. The polarity of compound GM-E13 was equal to that of garcinone D (**14**), R_f value of 0.45 (30% acetone-*n*-hexane, 6 elutions). Its IR spectrum exhibited the same pattern as that of garcinone D (**14**). Extensive 1D and 2D NMR analysis of GM-E13 showed that this xanthone has the same substituents as garcinone D (**14**); a 3-hydroxy-3-methylbutyl, a prenyl, one chelated hydroxyl, two aromatic proton and one methoxyl signals (Table 16). Therefore, compound GM-E13 was an isomer of garcinone D (**14**). However, the ^{1}H NMR spectral data of garcinone D (**14**) and GM-E13 exhibited different chemical shifts for the 3-hydroxy-3-methylbutyl and prenyl moieties. From HMBC spectral data (Table 17, Figure 20), the methylene proton at δ 2.74 of the 3-hydroxy-3-methylbutyl showed correlations with C-1 (δ 161.6) and C-2 (δ 112.3). It was therefore apparent there that the 3-hydroxy-3-methylbutyl group was located at C-2 (δ 161.6) in ring A. The downfield shift of the methylene proton signal at δ 4.11 of a prenyl unit in compound GM-E13 showed correlation with C-7 (δ 144.4), C-8 (δ 138.0) and C-8a (δ 111.8) and the methoxyl group at δ 3.78 showed correlation with C-7 (δ 144.4). In addition, the methoxyl proton at δ 3.78 also exhibited NOE enhancement (Figure 20) with methine proton H-17 (δ 5.26) in the NOSEY spectrum. This confirmed the position of a prenyl unit and methoxyl moieties in compound GM-E13 at C-8 and C-7, respectively. From the spectroscopic evidence, the structure of compound GM-E13 was concluded to be 1,3,6-trihydroxy-2-(3-hydroxy-3-methylbutyl)-7-methoxy-8-(3,3-dimethylallyl)xanthone or cratoxylone (**103**) (Figure 19). It has been isolated from the bark of *Cratoxylum cochinchinense* (Bennett; et al. 1993: 1245-1251).

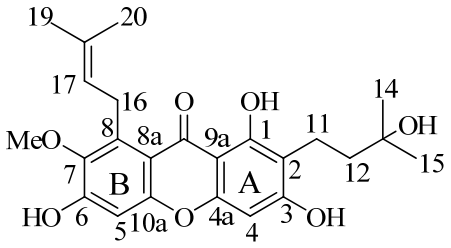


Figure 19 Structure of cratoxylone (**103**)

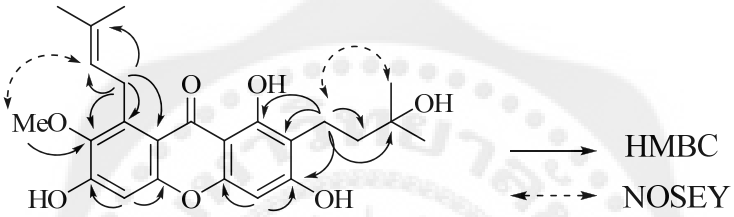


Figure 20 Selected HMBC and NOSEY correlations of cratoxylone (**103**)

Table 16 Comparison of ^{1}H and ^{13}C NMR data of compound GM-E13 (sss3300) in acetone-d_6 with cratoxylone in $CDCl_3$

position	cartoxylone		Compound GM-E13 (sss3300)	
	δ_H[a]	δ_C[a]	δ_H	δ_C
1-OH	13.83 (1H, s)	161.6	13.78 (1H, s)	161.6
2		111.8		112.3
3		163.3		163.2
4	6.38 (1H, s)	93.3	6.36 (1H, s)	93.3
4a		155.6		155.6
5	6.83 (1H, s)	102.7	6.81 (1H, s)	102.6
6[e]		157.6		157.3
7		144.5		144.4
7-OMe	3.80 (3H, s)	61.2	3.78 (3H, s)	61.2
8		138.0		138.0
8a		112.3		111.8
9		182.8		182.8
9a		103.5		103.4
10a[f]		156.2		156.1
11	2.76 (2H, m)	17.8	2.74 (2H, dt, J = 5.3, 7.9 Hz)	18.2
12	1.70 (2H, m)	43.2	1.69 (2H, dt, J = 5.3, 7.9 Hz)	43.1
13-OH		70.6		70.6
14, 15	1.26 (3H, s)	29.1	1.24 (3H, s)	29.0
	1.26 (3H, s)	29.1	1.24 (3H, s)	29.0
16	4.13 (2H, d, J = 6.5 Hz)	26.8	4.11 (2H, d, J = 6.3 Hz)	26.8
17	5.28 (1H,br t, J = 6.6 Hz)	124.8	5.26 (1H, t, J = 6.3 Hz)	124.7
18		131.3		131.3
19, 20	1.81 (3H, br s)	25.9	1.81 (3H, s)	25.8
	1.66 (3H, br s)	18.3	1.63 (3H, s)	17.8

[a] Bennett; et al. 1993: 1245-1251

Table 17 ^{1}H NMR and 2D NMR of compound GM-E13 (sss3300)

position	^{1}H NMR	COSY[a]	HMQC[b]	HMBC[c]	NOSEY
1-OH	13.78 (1H, s)			C-1, C-2, C-9a	
4	6.36 (1H, s)		C-4	C-2, C-3, C-4a, C-9, C-9a	
5	6.81 (1H, s)		C-5	C-7, C-8, C-8a, C-9, C-10a	
7-OMe	3.78 (3H, s)		C-7OMe	C-7	H-17
11	2.74 (2H, t, J = 2.42, 2.89 Hz)	H-12	C-11	C-1, C-2, C-12, C-13	H-14
12	1.69 (2H, t, J = 2.42, 2.89 Hz)	H-11	C-12	C-2, C-13, C-14, C-15	
14, 15	1.24 (3H, s)		C-14	C-12, C-13, C-15	
	1.24 (3H, s)		C-15	C-12, C-13, C-14	
16	4.11 (2H, d, J = 6.3 Hz)	H-17, H-19, H-20	C-16	C-7, C-8, C-8a, C-17, C-18	
17	5.26 (1H, t, J = 6.3 Hz)	H-16, H-19, H-20	C-17		
19, 20	1.81 (3H, s)		C-19	C-17, C-18, C-20	
	1.64 (3H, s)		C-20	C-17, C-18, C-19	

[a]proton-proton correlation

[b]1-bond heteronuclear ^{1}H-^{13}C correlation

[c]long range heteronuclear ^{1}H-^{13}C correlation

Part II Development of a method for determination of xanthone compounds in *G. mangostana*

This section describes the development of the HPLC-MS method to obtain the optimal condition for the simultaneous separation of eleven xanthone compounds. The optimum condition was then applied to determine the xanthone contents in the extract of fruit hull of *G. mangostana* obtained from the Eastern and Southern areas of Thailand.

4.2 Chromatographic study

Previous studies have shown that xanthone have various biological activities (José; et al. 2008: 3227-3239), including anticancer, antimicrobial and antimalarial. The interesting lead compounds in this work are garcinone C, garcinone D, cratoxylone, γ-mangostin, 8-desoxygartanin, gartanin, α-mangostin, garcinone E, demethyl-calabaxanthone, 9-hydroxycalabaxanthone and β-mangostin. The two major compounds, α-mangostin and γ-mangostin, and other xanthones are commonly found in *G. mangostana*, except cratoxylone which was previously isolated from *Cratoxylum cochinchinense* (Bennett; et al. 1993: 1245-1251). Cratoxylone is structural isomer with garcinone D and has not been reported before in *G. mangostana.* From our preliminary study using previous HPLC condition system (Chaivisuthangkura; et al. 2009: 315-318), cratoxylone was co eluted with garcinone D and the MS/MS analysis also showed the same MS/MS chromatographic pattern (see Appendix A). However, The HPLC system couple with ESI-TOF-MS as described in section 3.1 was employed to optimize condition for simultaneous separation of xanthone compounds in the mangosteen extracts. Thus, this is the first time for quantitative analysis of cratoxylone from this plant.

4.2.1 Effect of gradient programming

From the preliminary study, the appropriate method of the separation of xanthone compounds was reversed-phase liquid chromatography using C18 column. In this work, various mobile phase compositions of 2.00% (v/v) acetic acid and acetonitrile were studied in order to improve the resolution. The optimum gradient system was shown in Table 18. Under this chromatographic condition, the eleven xanthones were well simultaneously separated from each other with the retention times of 4.14, 4.72,

4.92, 8.47, 8.87, 10.13, 10.68, 11.85, 13.38, 13.76 and 15.06, respectively (Figure 21). From the results, this system was therefore suitable for the analysis of these xanthones with suitable analysis time.

Table 18 The optimum gradient system

Time (min)	% acetonitrile
0	45
3	55
12	75
15	95
16	45
20	45

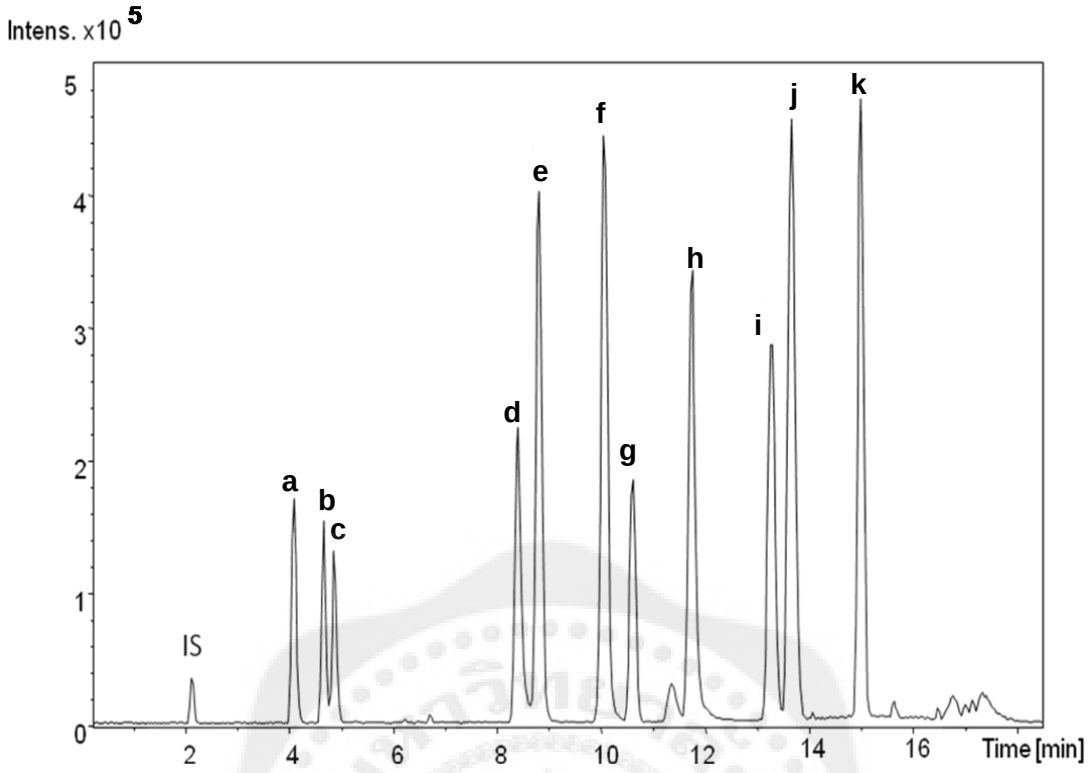


Figure 21 Typical chromatogram of eleven xanthones and internal standard (IS). Acclaim® RSLC 120 C18 column (2.1 x 50.0 mm i.d., 2.2 μm) was used with 45-95% of acetonitrile and 2.00% (v/v) acetic acid as mobile phase within 16 min. Flow rate was 0.3 mL/min and detected using mass spectrometer. The order of elution was (**IS**) internal standard, (**a**) garcinone C, (**b**) garcinone D, (**c**) cratoxylone, (**d**) γ-mangostin, (**e**) 8-desoxygartanin, (**f**) gartanin, (**g**) α-mangostin, (**h**) garcinone E, (**i**) demethylcalaba-xanthone, (**j**) 9-hydroxycalabaxanthone and (**k**) β-mangostin.

4.2.2 Effect of acetic acid concentration

The effect of acetic acid concentration was investigated for separation of xanthone compounds using the gradient system as shown in Table 18. The concentration of acetic acid at 1.50 and 2.00 %(v/v) were studied in this experiment. The results showed clearly that 2.00% (v/v) acetic acid gave shorter retention time, better peak shape and resolution than 1.50% (v/v) acetic acid, as shown in Figure 22. The result revealed that the xanthone compounds were retained in the column under less acidic condition.

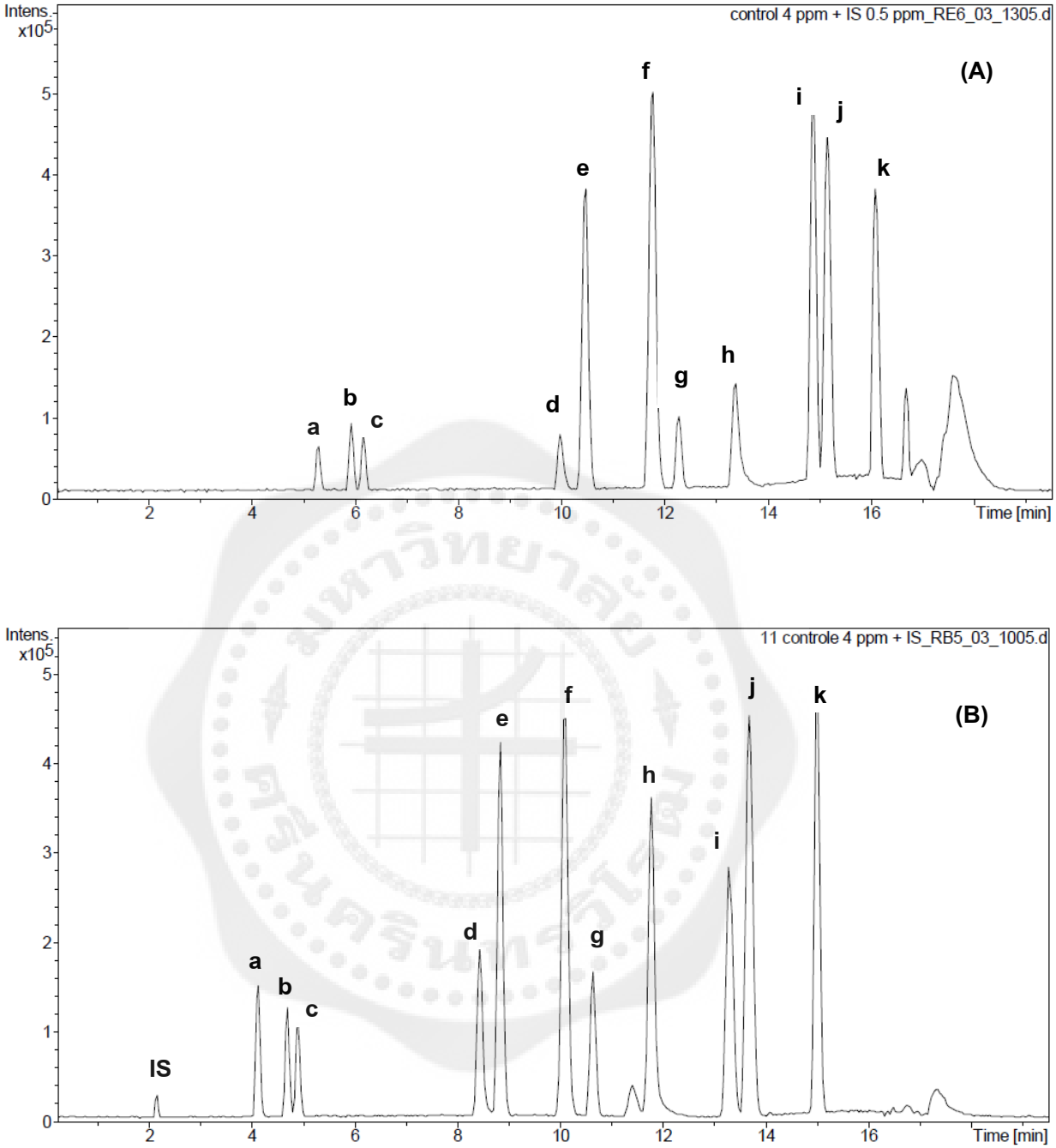


Figure 22 Chromatogram of 4 µg/mL xanthone compounds using concentration of acetic acid (A) 1.50% (v/v) and (B) 2.00% (v/v) as mobile phase. Gradient: 45-95% of acetonitrile and acetic acid within 16 min.

4.3 Analytical performance of HPLC-MS method

Various validation parameters were evaluated for HPLC-ESI-TOF-MS method using optimized chromatographic conditions (Section 4.2.1). Results for linearity, precision, limit of detection (LOD) and limit of quantification (LOQ) are given in the following sections.

4.3.1 Linearity

The linearity of the all compounds was summarized in Table 19 by using internal standard method. The ratios of peak area of the analyte and internal standard was ploted against the relative concentration of xanthones standard and internal standard solutions as previous described (Section 3.2.1). The data show that the development method exhibits a good linearity as indicated by the correlation coefficients (R^2 > 0.99). However, good linearity of 8-desoxygartanin, gartanin, demethylcalaba-xanthone, 9-hydroxycalabaxanthone and β-mangostin were achieved in the narrow concentration range of 1 to 7 μg/mL (see Appendix B). The deviation at concentration over 7 μg/mL may be due to the limitation of ionization efficiency of these compounds becoming saturation in MS system. However, this narrow linear range is in the acceptable for determination of xanthone compounds in the mangosteen extracts.

4.3.2 Precision

The precision of method was expressed as the percentage of relative standard deviation (% RSD) of the peak area ratio and retention time as described in Section 3.2.2.

The results were shown in Table 20. The precision of intra-day and inter-day for peak area ratio were in the range of 0.6 – 7.1 and 0.6 – 8.4, respectively. In addition, the results of % RSD for retention time gave good precision (< 1.2%) for both intra-day and inter-day analysis.

Table 19 Calibration curve, with correlation coefficient, for eleven xanthones using HPLC-MS method

Analyte	Concentration (μg/mL)	Calibration curve	R^2
Garcinone C	1-15	y = 0.6162 x + 0.8180	0.9991
Garcinone D	1-15	y = 0.5854 x + 0.2910	0.9999
Cratoxylone	1-15	y = 0.4581 x + 0.6403	0.9990
γ-Mangostin	1-15	y = 1.0576x + 1.7899	0.9994
8-Desoxygartanin	1-7	y = 2.3206x + 2.2925	0.9974
Gartanin	1-7	y = 3.0115 x + 4.3480	0.9968
α-Mangostin	10-150	y = 0.6405 x + 0.6774	0.9990
Garcinone E	1-15	y = 2.5763 x + 2.9496	0.9986
Demethylcalabaxanthone	1-7	y = 2.1735 x + 3.3215	0.9996
9-Hydroxycalabaxanthone	1-7	y = 3.0412x + 5.3674	0.9974
β-Mangostin	1-7	y = 1.6360 x + 5.6993	0.9924

Table 20 Precision of ten xanthones at concentration of 4 μg/mL and α-mangostin at concentration of 40 μg/mL

Analyte	Intra-day variability (n=5)		Inter-day variability (n=3)	
	RSD of Pa[a](%)	RSD of t_R(%)	RSD Pa[a](%)	RSD of t_R(%)
Garcinone C	4.2	1.1	0.6	1.1
Garcinone D	6.8	0.8	1.5	1.0
Cratoxylone	5.6	0.9	4.3	1.0
γ-Mangostin	3.8	0.2	4.1	0.8
8-Desoxygartanin	5.8	0.2	8.4	0.7
Gartanin	7.1	0.2	6.2	0.6
α-Mangostin	0.6	0.2	2.0	0.5
Garcinone E	6.3	0.4	7.4	0.5
Demethylcalabaxanthone	4.5	0.4	7.4	0.4
9-Hydroxycalabaxanthone	5.0	0.5	4.0	0.4
β-Mangostin	6.6	0.4	4.2	0.2

[a] Peak area ratios of the analytes/I.S.

4.3.3 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) of all xanthones were determined base on the signal-to-noise ratio of 3:1 and 10:1, respectively, as described in section 3.2.3. The LODs and LOQs of all xanthones were less than 50 and 170 ng/mL, respectively as shown in Table 21. The chromatograms of xanthone compounds at the limit of detection and quantification is shown in Appendix C. The data showed that this method is very sensitive for determination of xanthone compounds form *G. mangostana* and has lower detection limit than other previously reported methods.

Table 21 The Limit of Detection (LOD) and Limit of Quantification (LOQ) for eleven xanthones

Analyte	LOQ (ng/mL)	LOD (ng/mL)
Garcinone C	10.0	3.3
Garcinone D	10.0	3.3
Cratoxylone	10.0	3.3
γ-Mangostin	5.6	1.8
8-Desoxygartanin	1.4	0.4
Gartanin	2.0	0.6
α-Mangostin	50.0	16.6
Garcinone E	166.6	50.0
Demethylcalabaxanthone	3.1	1.0
9-Hydroxycalabaxanthone	2.0	0.6
β-Mangostin	1.4	0.4

4.4 Application to the extract of mangosteen

The optimized method was applied to qualitative and quantitative analyse of eleven xanthones in *G. mangostana* extracts. Base peak chromatograms (BPCs) of the extracts from different sources are shown in Figure 23. The crude extracts of all sources showed similar chromatographic patterns. The contents of eleven xanthones were calculated and the results were summarized in Table 22. The xanthone compounds in the extracts were identified on the basic of comparison of retention time and accurate mass (lower than 10 ppm) as well as their isotopic patterns obtained from

HPLC-MS analysis of the standard compounds. The result showed that α-mangostin and γ-mangostin were the major compounds in the extracts at concentration of 446.2-493.6 mg/g and 18.3-23.0 mg/g, respectively. The amount of demethylcalabaxanthone was not detected in all the extracts in this study. The result agreed well with the previously study that this compound was only found in the aril and seed (Mahabusarakam; et al. 1987: 474-478, Suksamrarn; et al. 2002: 761-763). The amounts of garcinone C, garcinone D and cratoxylone in Eastern sample were more than one fold in Southern sample. Other xanthones had same amount in each extract. Thus, the developed method is suitable determination of xanthone from the different location.

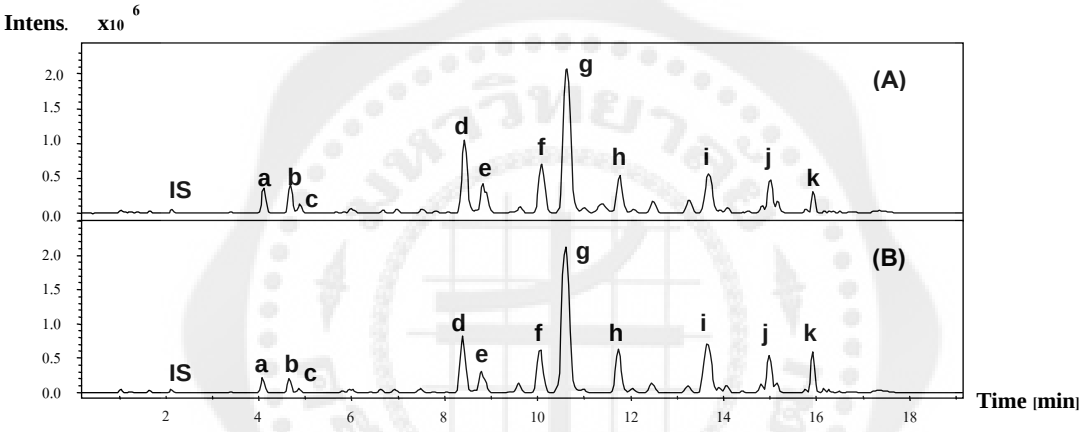


Figure 23 Base peak chromatogram of crude extracts from Southern (A), Eastern (B). The peak correspond to: (**IS**) internal standard, (**a**) garcinone C, (**b**) garcinone D, (**c**) cratoxylone, (**d**) γ-mangostin, (**e**) 8-desoxygartanin, (**f**) gartanin, (**g**) α-mangostin, (**h**) garcinone E, (**i**) demethylcalabaxanthone, (**j**) 9-hydroxycalabaxanthone and (**k**) β-mangostin.

Table 22 The xanthones contents in the extract of *G. mangostana* from Eastern and Southern part of Thailand

Analyte	Content (mg/g) (mean ± SD, n=3)	
	Eastern	Southern
Garcinone C	9.7±0.4	5.6±0.2
Garcinone D	13.0±0.4	6.4±0.1
Cratoxylone	4.5±0.4	1.8±0.1
γ-Mangostin	23.0±0.9	18.3±0.4
8-Desoxygartanin	3.2±0.2	2.4±0.1
Gartanin	5.2±0.2	4.6±0.2
α-Mangostin	446.2±9.6	493.8±1.5
Garcinone E	4.2±0.2	5.2±0.2
Demethylcalabaxanthone	ND[a]	ND[a]
9-Hydroxycalabaxanthone	4.1±0.2	6.6±0.2
β-Mangostin	3.2±0.4	4.3±0.3

[a] Not detectable.

CHAPTER 5
CONCLUSION

This study aimed at the isolation of the lead compounds from the fruit latex of *G. mangostana* and the quantitative analysis of the lead compounds using HPLC-ESI-TOF-MS method. Phytochemical study of the *G. mangostana* fruit latex led to isolation a new xanthone, mangostenone H or 1,3,6-trihydroxy-7-methoxy-3-(1-methylethenyl)-8-(3-methylbut-2-enyl)-furano[2,3-b]xanthone, along with twelve known xanthones, including α-mangostin (**1**), β-mangostin (**2**), γ-mangostin (**3**), a mixture of gartanin (**4**) and 8-desoxygartanin (**5**), 9-hydroxycalabaxanthone (**10**), garcinone E (**26**), 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone (**12**), garcinone B (**14**), cowaxanthone D, mangostanin (**63**), garcinone D (**16**) and cratoxylone. The structures of these compounds were elucidated mainly by NMR spectroscopic techniques and comparisons of their spectroscopic data with the previously reported data. This is the first report on the isolation of cowaxanthone D and cratoxylone from this plant.

Due to the interesting biological activities of the *G. mangostana* xanthones, following compounds are selected as lead compounds for development of the HPLC-ESI-TOF-MS method: garcinone C, garcinone D, cratoxylone, γ-mangostin, 8-desoxygartanin, gartanin, α-mangostin, garcinone E, demethylcalabaxanthone, 9-hydroxycalabaxanthone and β-mangostin. The HPLC-ESI-TOF-MS was successfully for developed and validated for the quantitative determination of xanthone compounds in the fruit hull extract by using Acclaim® RSLC 120 C18 column as the stationary phase and acetronitrile - 2.00% acetic acid as the mobile phase with flow rate of 0.3 mL/min in total run time of 16 min. The calibration curve of all xanthones were linear in the range of 1-15 and 10-150 μg/mL with the correlation coefficient in the range of 0.9974-0.9999 (n=3). The LOD's and LOQ's of all xanthones were less than 50 and 170 ng/mL, respectively. The %RSD of intra-day and inter-day analye were in the range of 0.6–7.1 and 0.6–8.4, respectively. These results indicated that the established HPLC-ESI-TOF-MS method was suitable for the determination of the xathone compounds in the fruit hull extract. The calculated content of xanthones in the fruit hull extract from Eastern and Southern areas of Thailand showed that α-mangostin (**1**) and γ-mangostin (**3**) were the major compounds in the extracts at 446.2-493.6 mg/g and 18.3-23.7 mg/g, respectively. The amount of demethylcalabaxanthone (**21**) was not detected in all the extract and amounts of garcinone C (**15**), garcinone D (**16**) and cratoxylone in Eastern

sample were more than one fold in Southern sample. Other xanthones had a similar amount in each extract. The HPLC-ESI-TOF-MS was established to provide benefit for the development of phytopharmaceutical from *G. mangostana*.

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APPENDIX

Appendix A

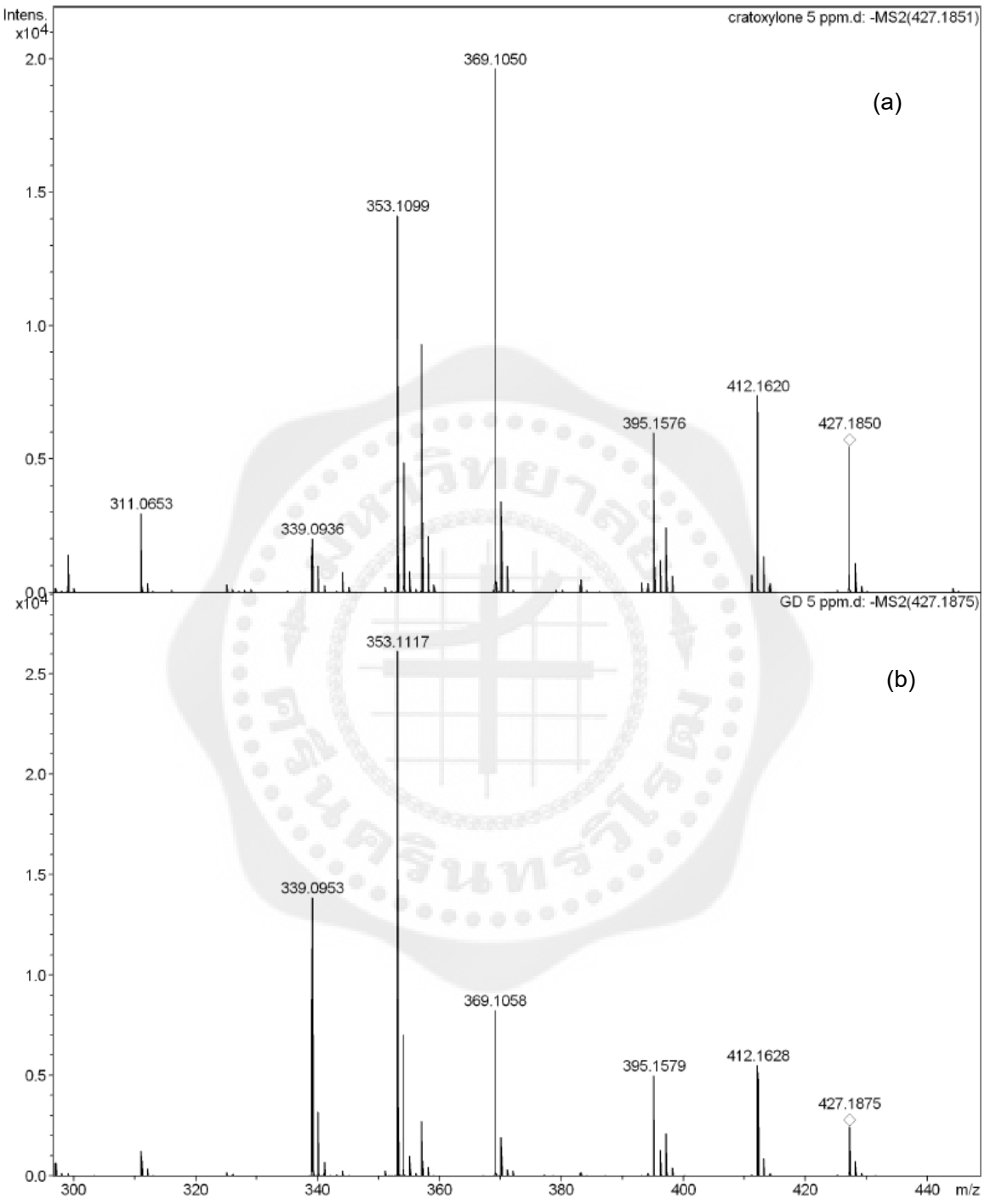
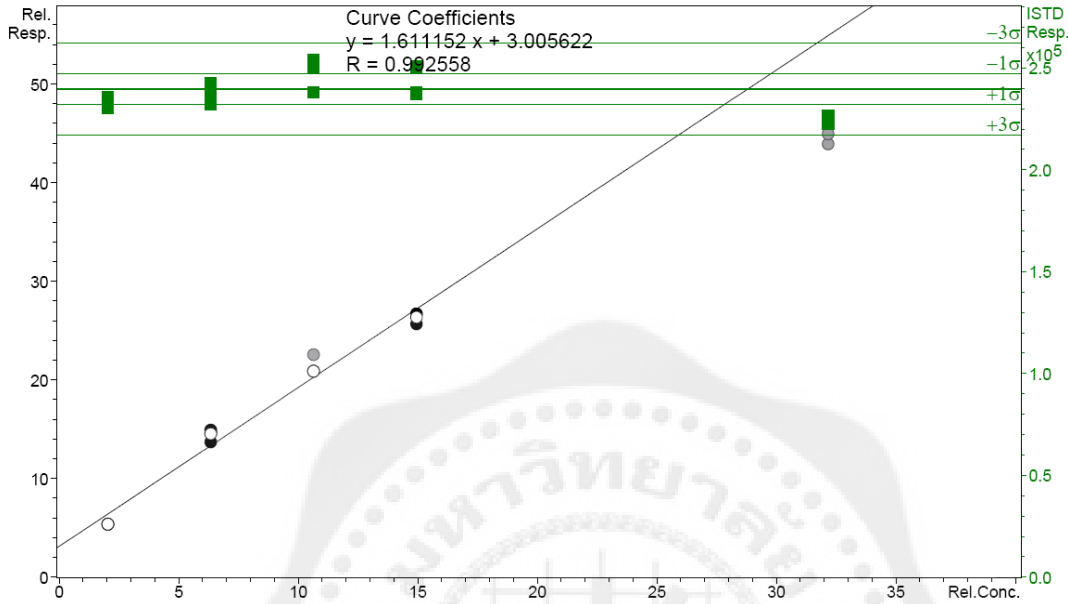


Figure 24 MS/MS spectrums of (a) cratoxylone and (b) garcinone D

APPENDIX B

8-desoxygartanin



gartanin

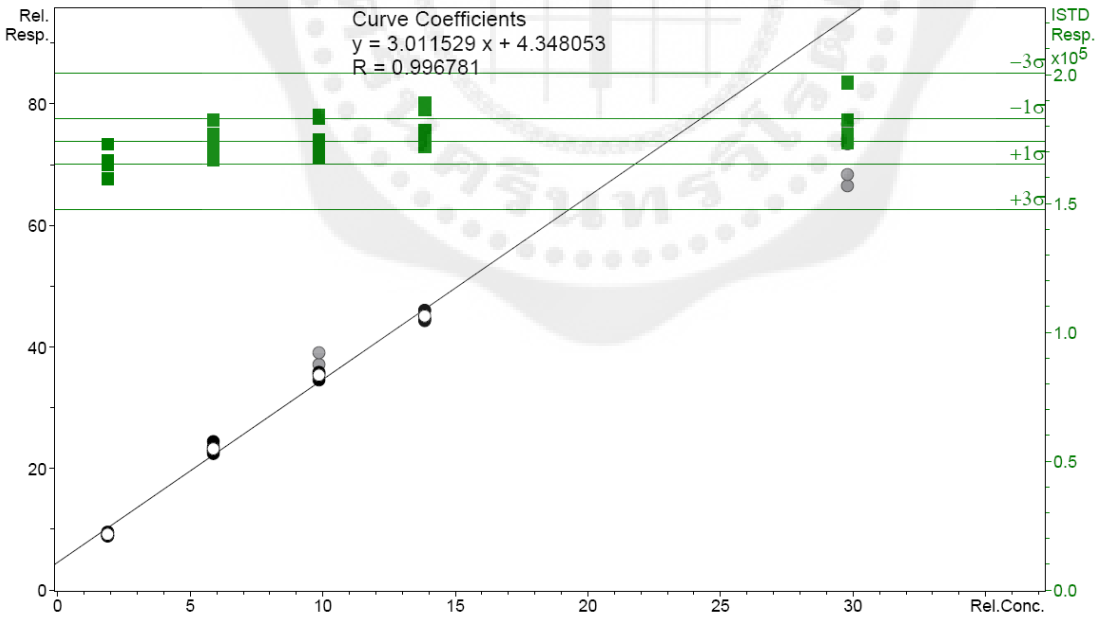
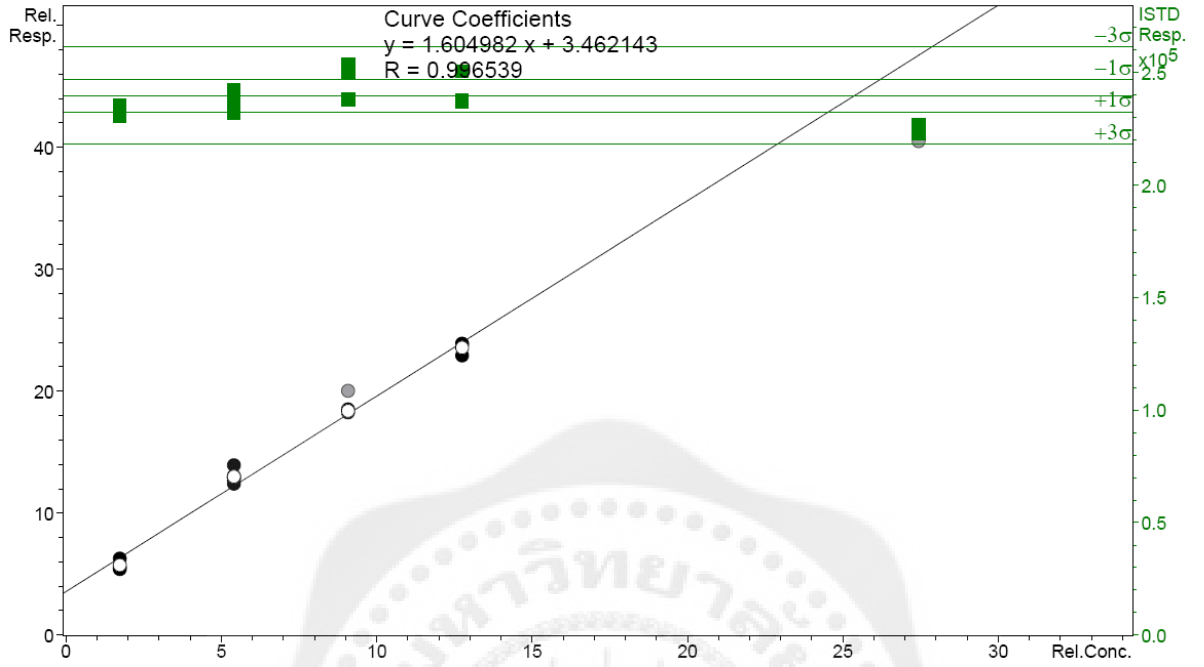


Figure 25 The linearity of standard 8-desoxygartanin, gartanin, demethylcalaba xanthone, 9-hydroxycalabaxanthone and β-mangostin using the developed HPLC-MS method

demethylcalabaxanthone



9-hydroxycalabaxanthone

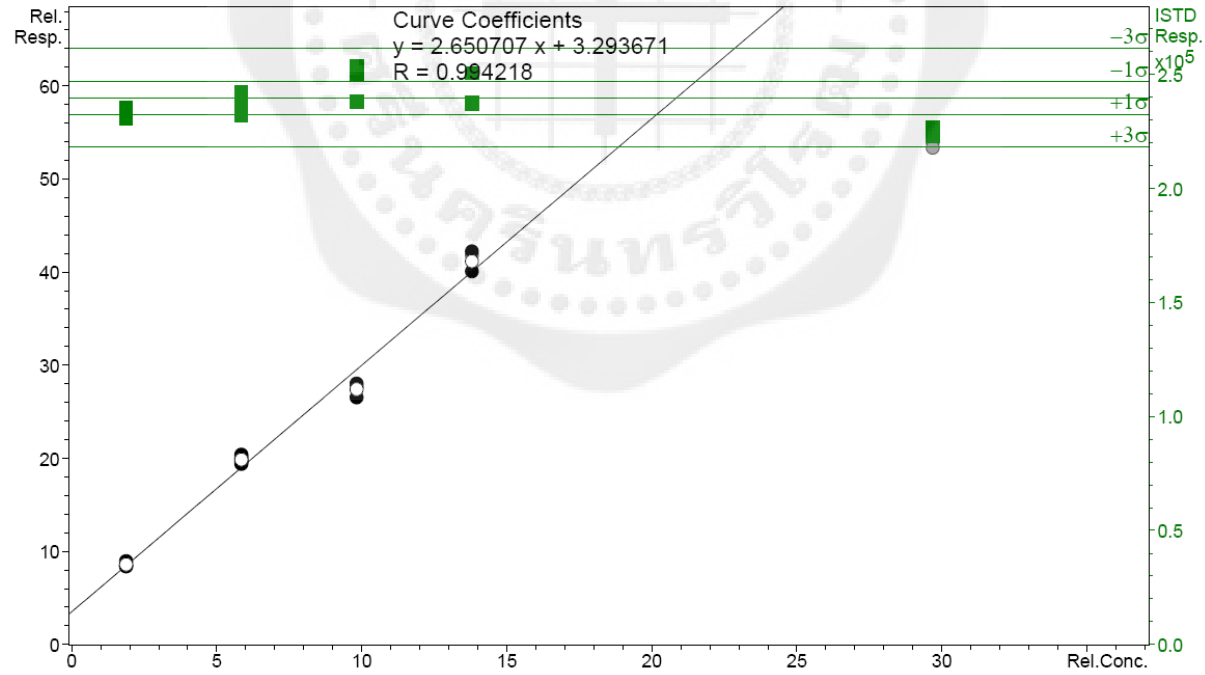


Figure 25 (continued)

β-mangostin

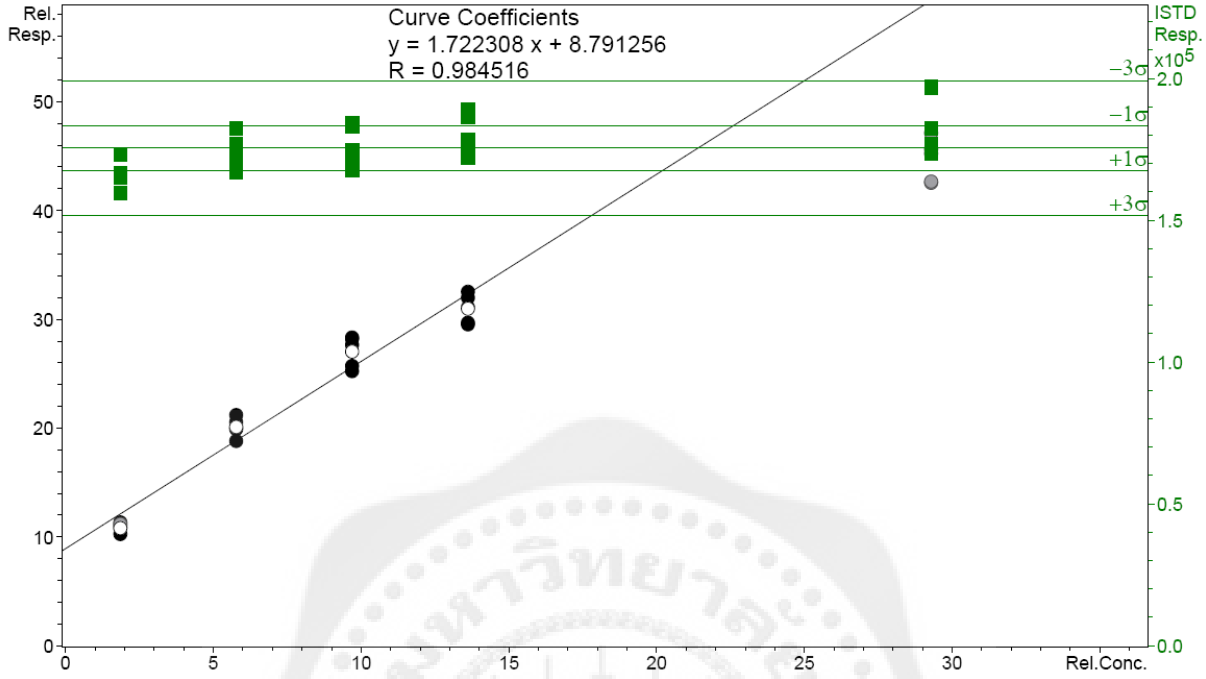


Figure 25 (continued)

APPENDIX C

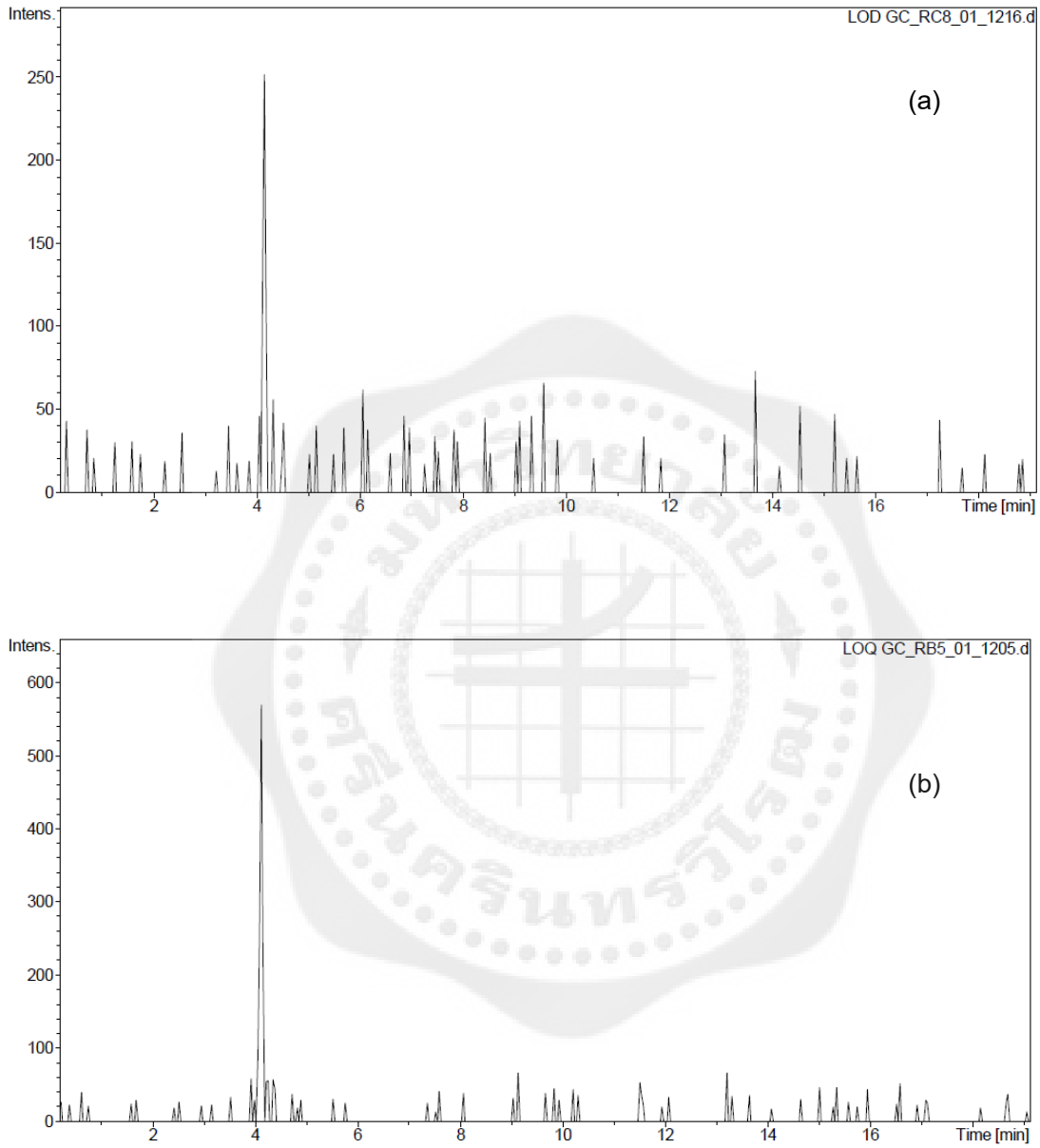


Figure 26 The chromatograms of (a) 3.3 ng/mL (b) 10.0 ng/mL garcinone C using the developed HPLC-MS method

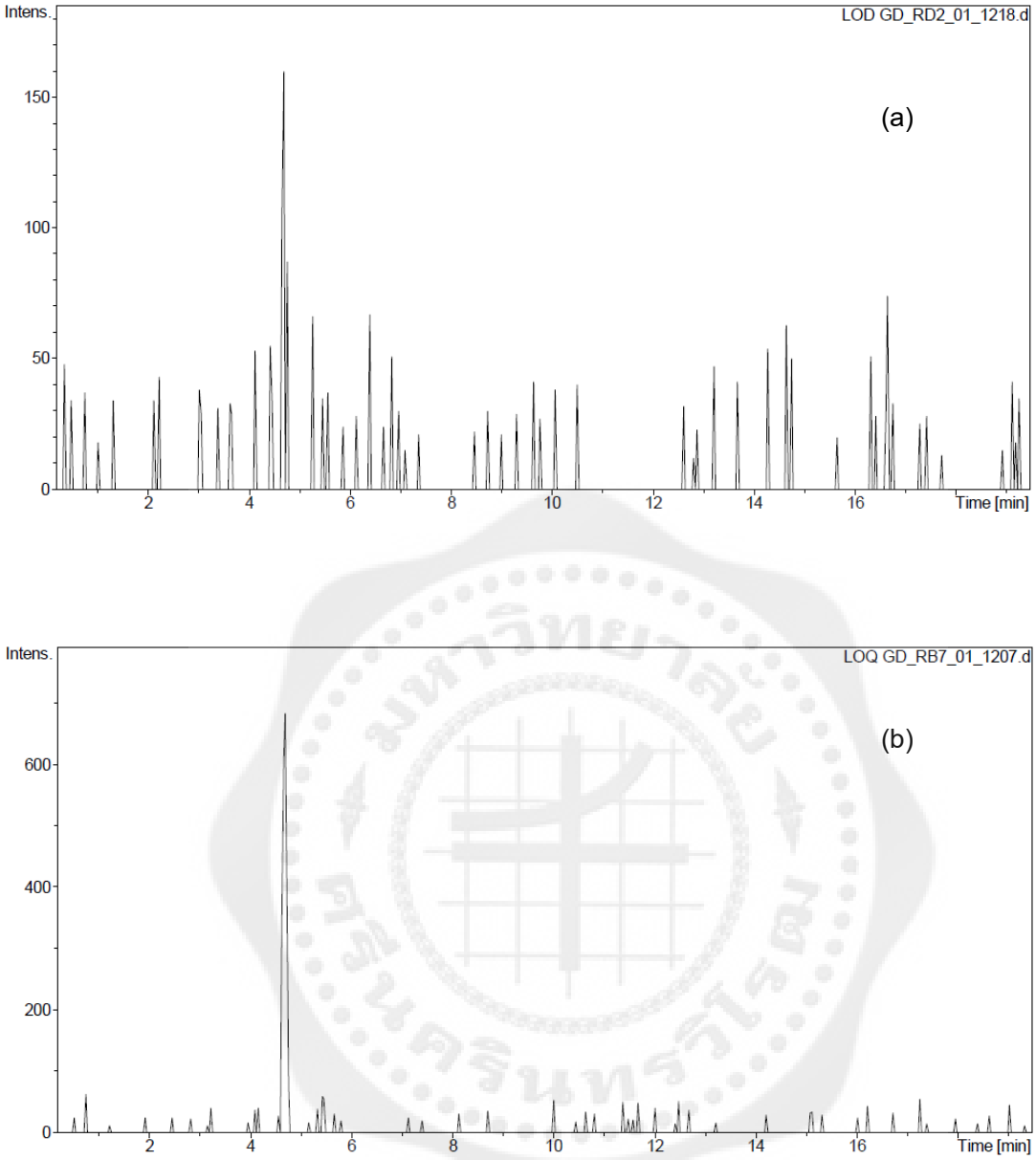


Figure 27 The chromatograms of (a) 3.3 ng/mL (b) 10.0 ng/mL garcinone D using the developed HPLC-MS method

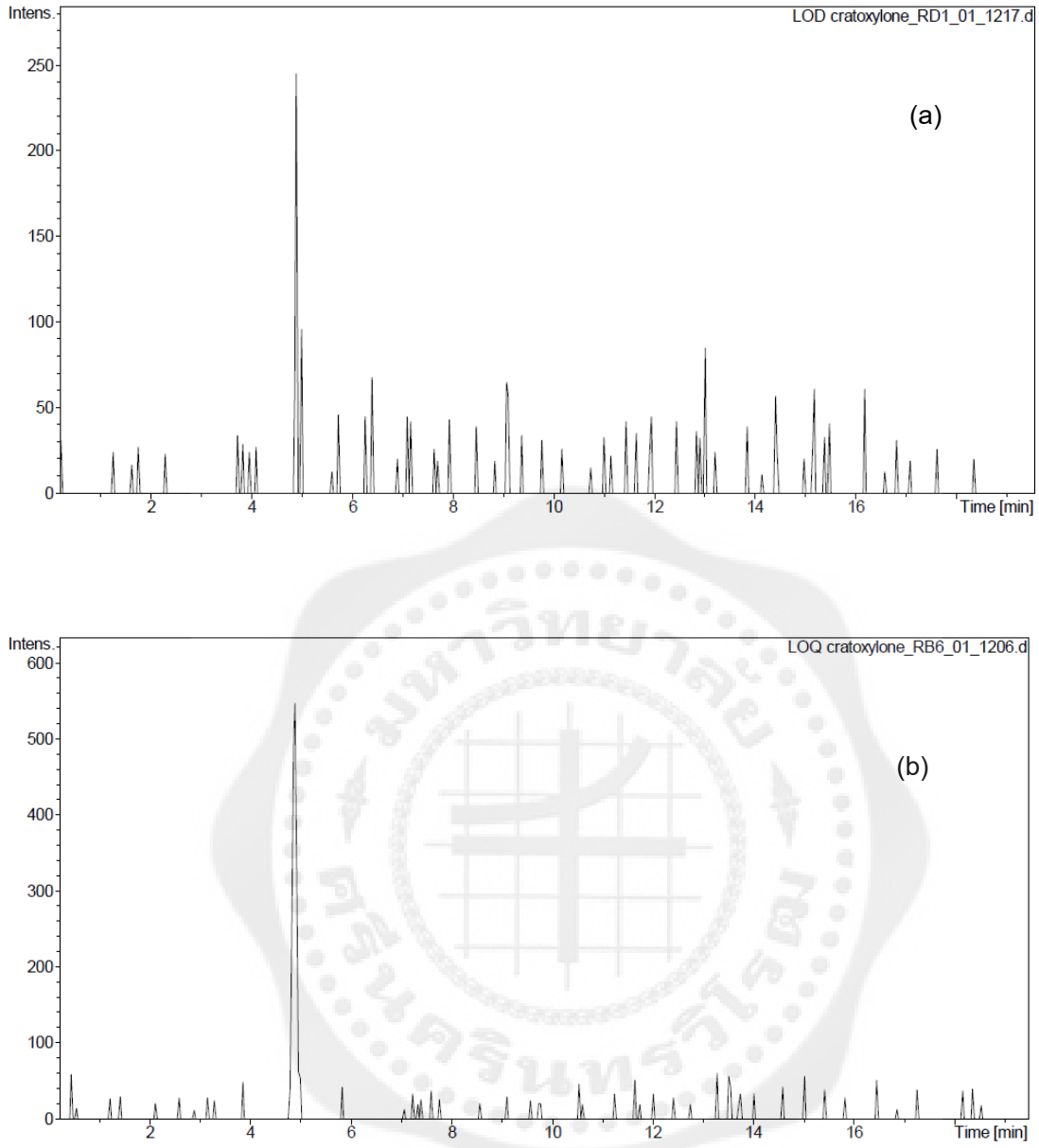


Figure 28 The chromatograms of (a) 3.3 ng/mL (b) 10.0 ng/mL cratoxylone using the developed HPLC-MS method

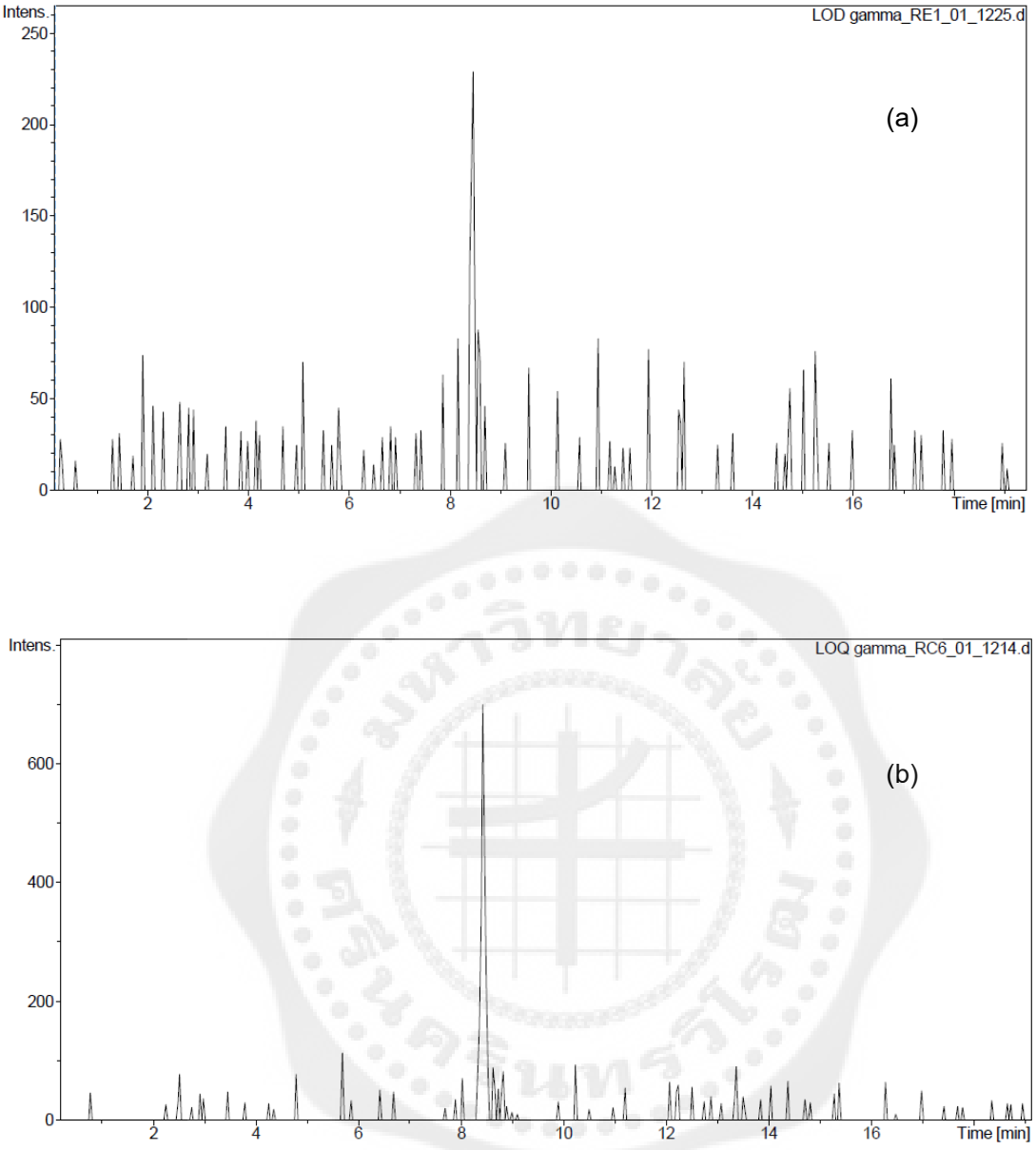


Figure 29 The chromatograms of (a) 1.8 ng/mL (b) 5.6 ng/mL γ-mangostin using the developed HPLC-MS method

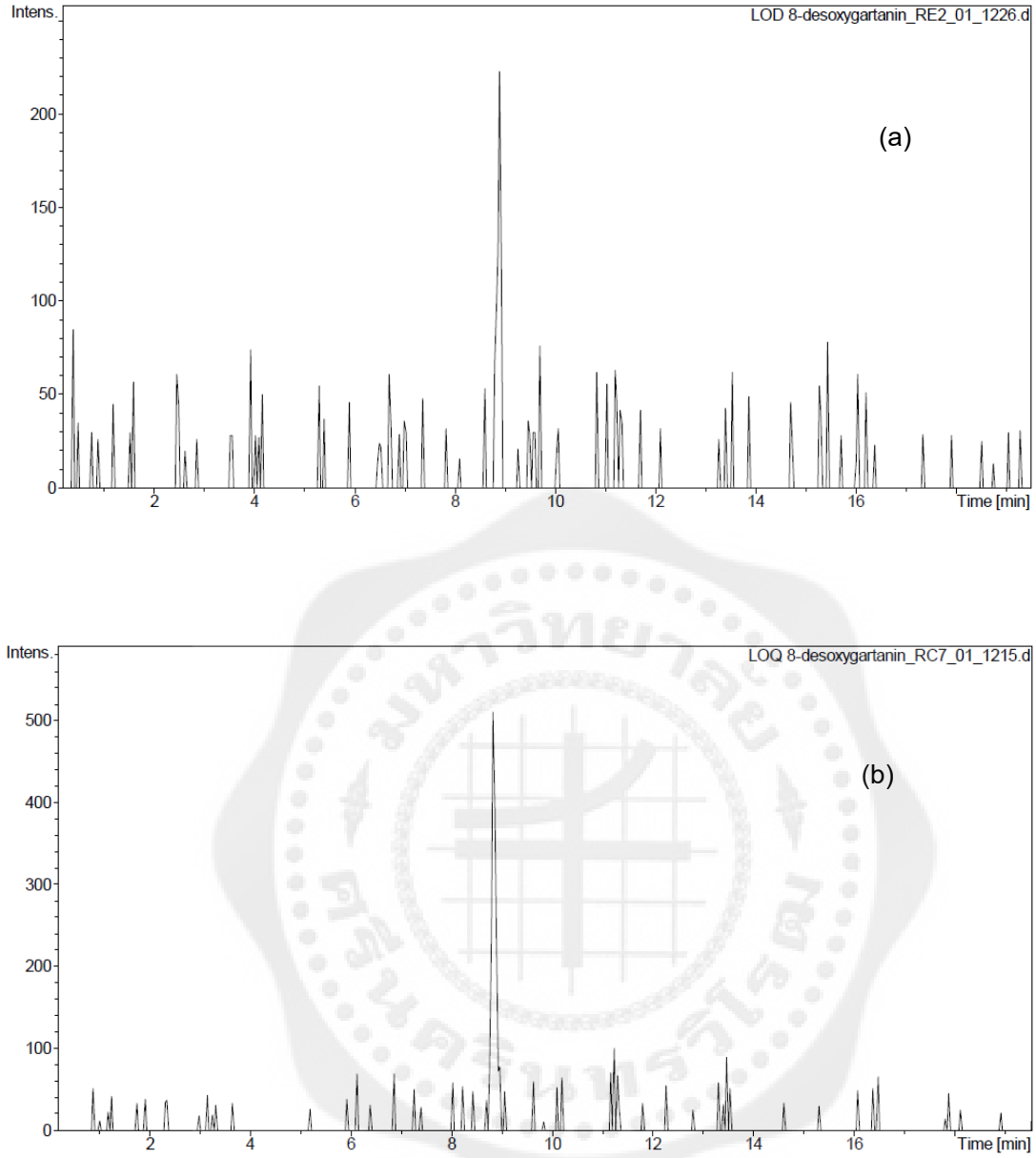


Figure 30 The chromatograms of (a) 0.4 ng/mL (b) 1.4 ng/mL 8-desoxygartanin using the developed HPLC-MS method

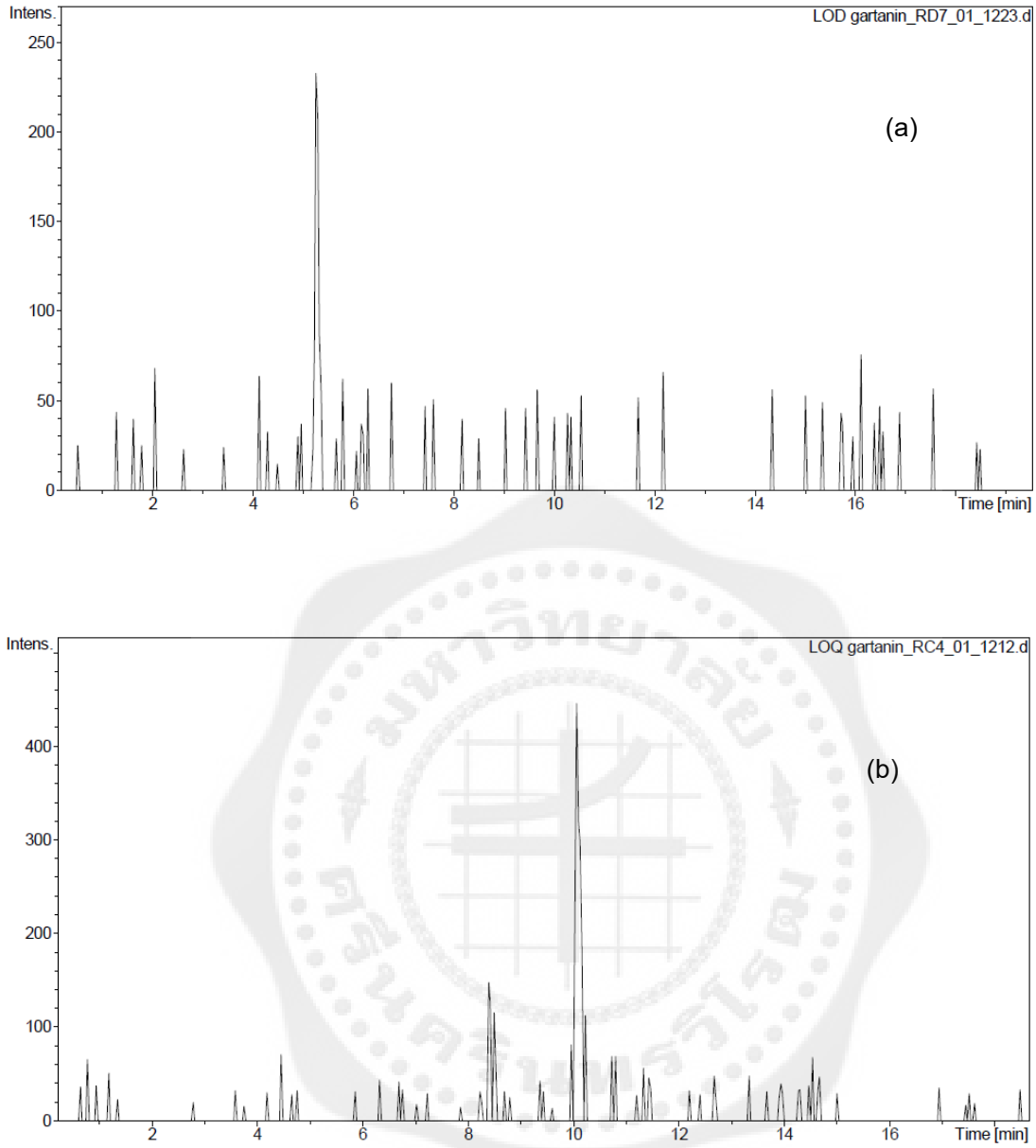


Figure 31 The chromatograms of (a) 0.6 ng/mL (b) 2.0 ng/mL gartanin using the developed HPLC-MS method

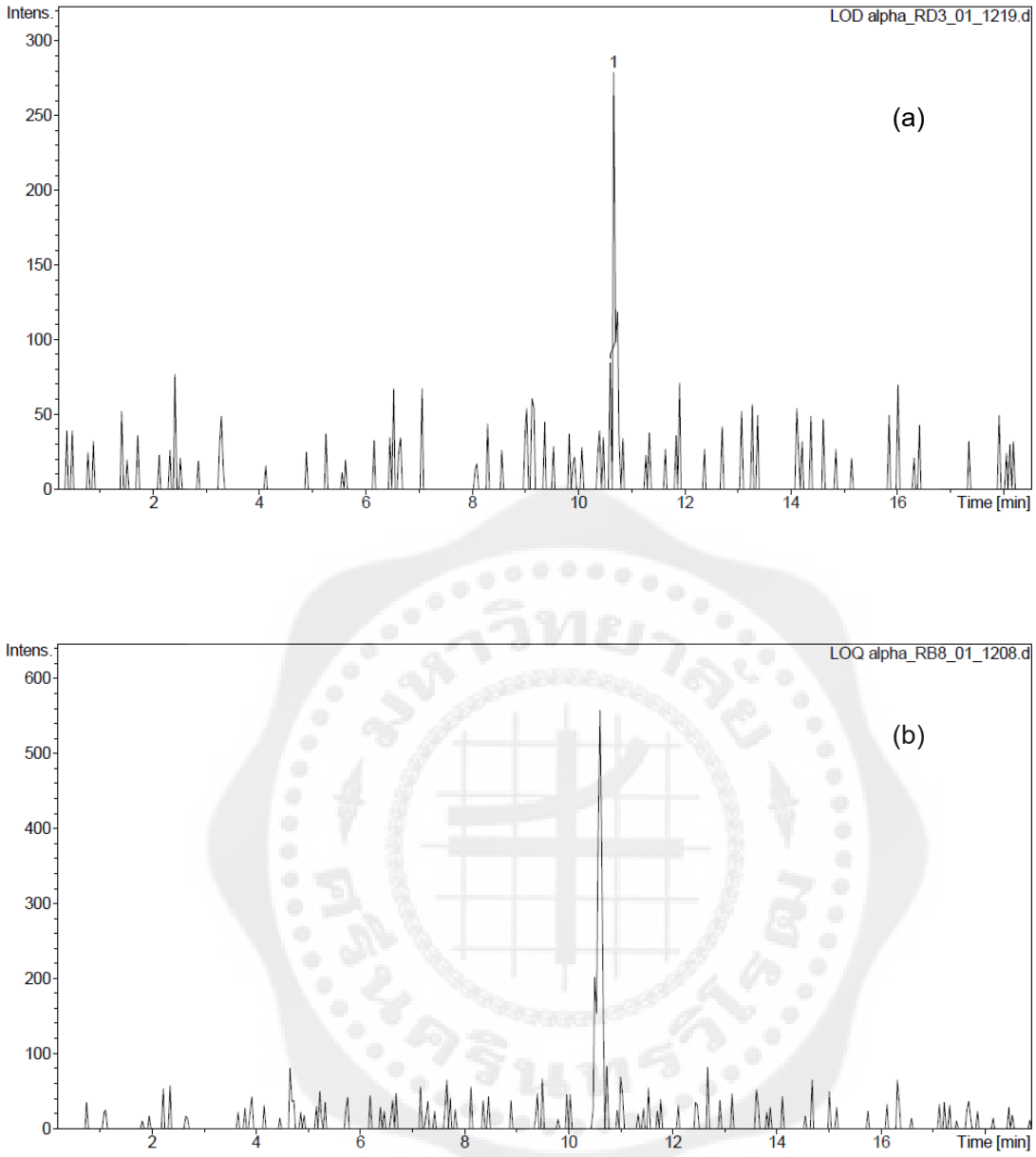


Figure 32 The chromatograms of (a) 16.6 ng/mL (b) 50.0 ng/mL α-mangostin using the developed HPLC-MS method

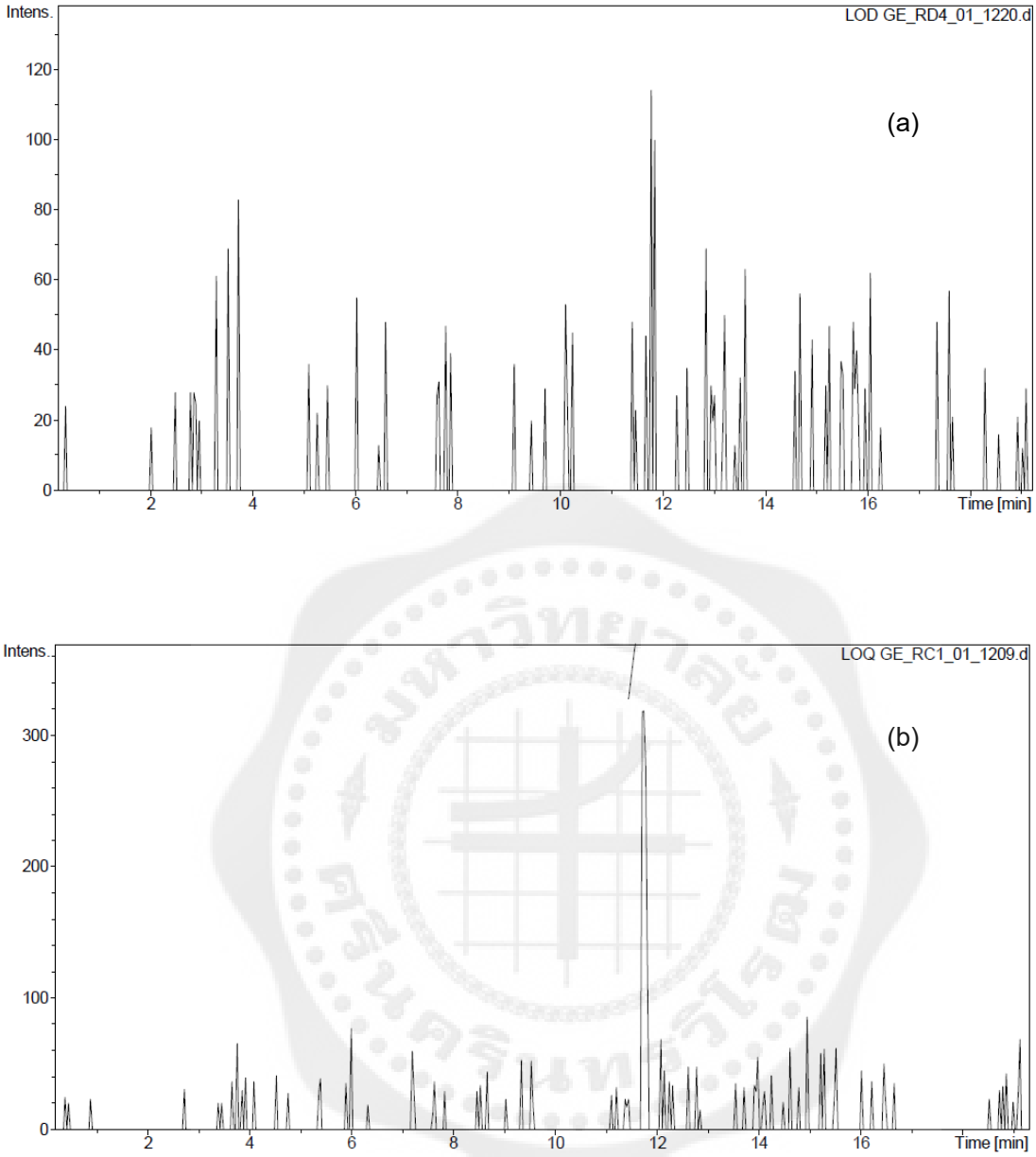


Figure 33 The chromatograms of (a) 50.0 ng/mL (b) 166.6 ng/mL garcinone E using the developed HPLC-MS method

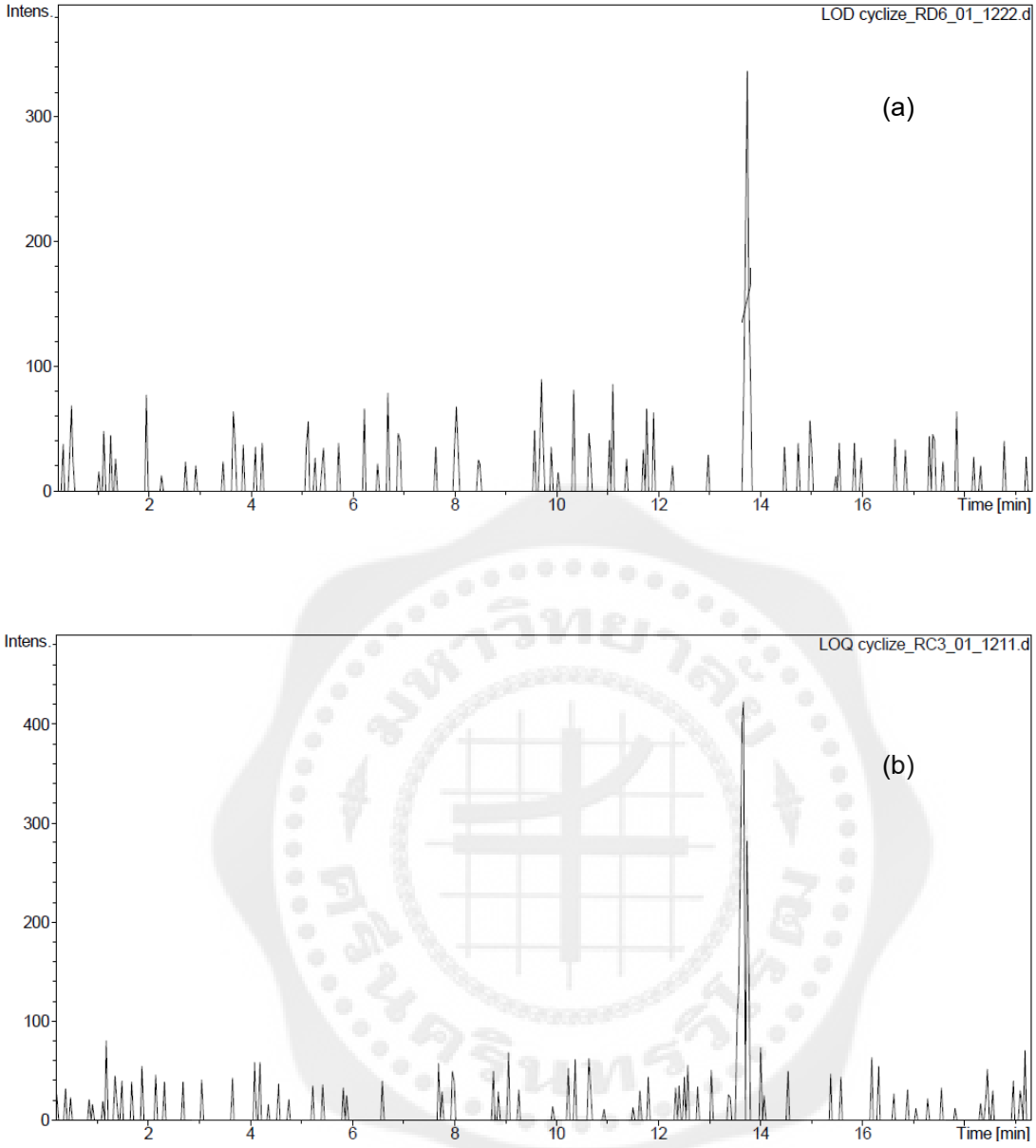


Figure 34 The chromatograms of (a) 1.0 ng/mL (b) 3.1 ng/mL demethylcalaba xanthone using the developed HPLC-MS method

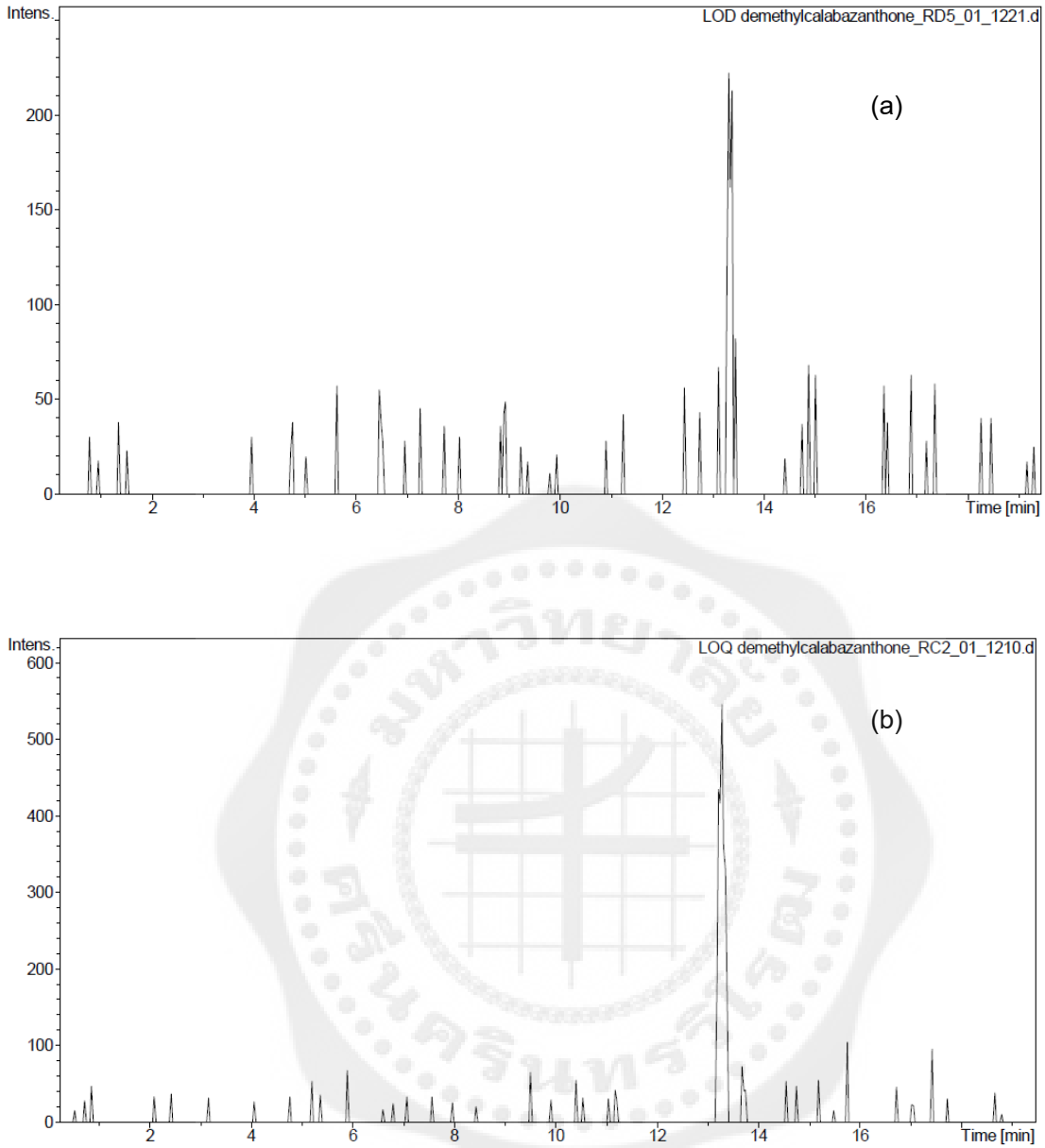


Figure 35 The chromatograms of (a) 0.6 ng/mL (b) 2.0 ng/mL 9-hydroxycalaba xanthone using the developed HPLC-MS method

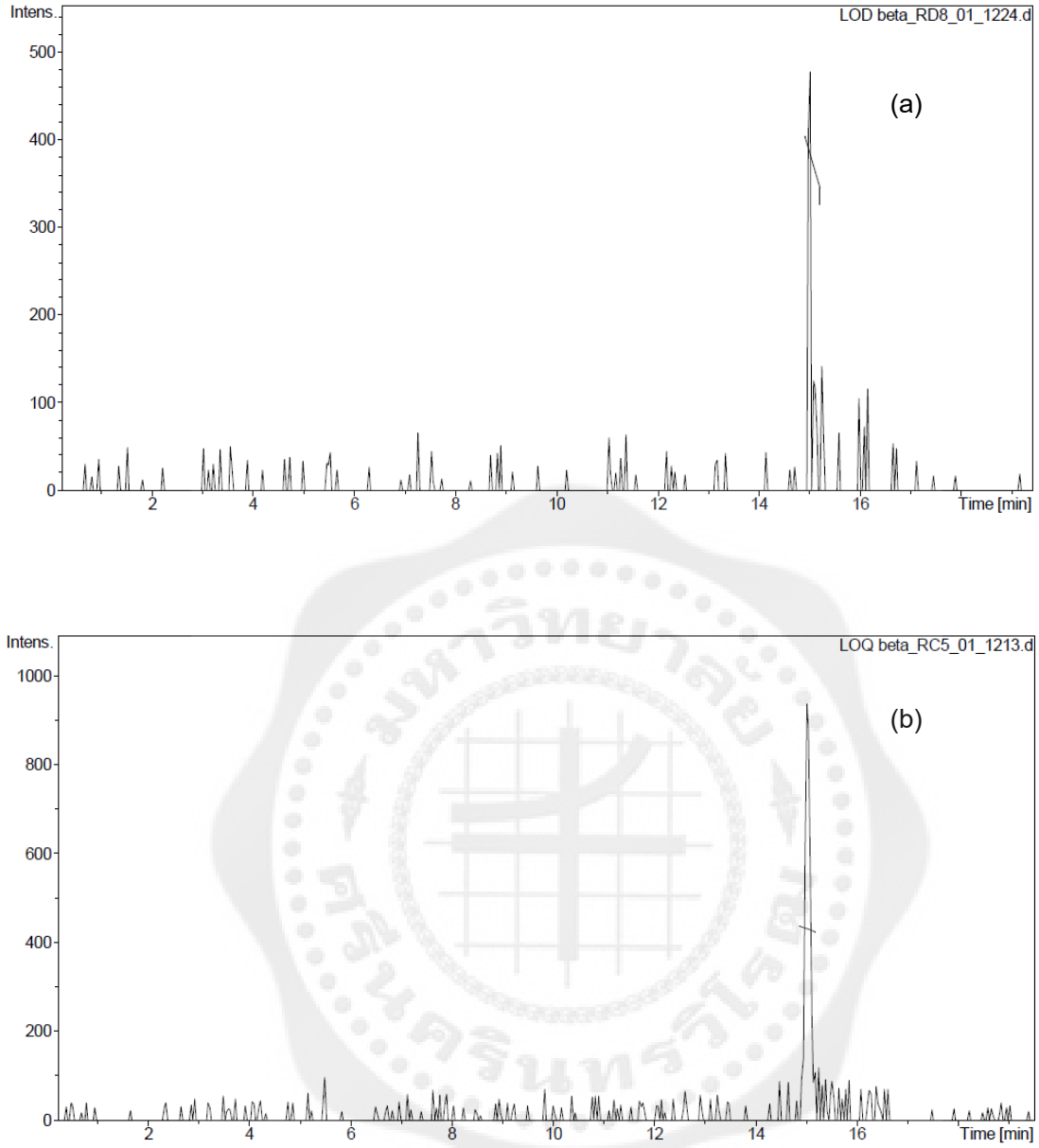


Figure 36 The chromatograms of (a) 0.4 ng/mL (b) 1.4 ng/mL β-mangostin using the developed HPLC-MS method

GLOSSARY

LIST OF ABBREVIATIONS AND SYMBOLS

α	= Alpha
APCI	= Atmospheric Pressure Chemical Ionization
CD_3COCD_3	= Acetone-d_6
β	= Beta
γ	= Gamma
br d	= Broad aoublet (for NMR data)
br t	= Broad triplet (for NMR data)
^0C	= Degree Celsius
^{13}C NMR	= Carbon-13 Nuclear Magnetic Resonance
calcd	= Calculated
cm	= Centimeter
cm^{-1}	= Reciprocal centimeter (unit of wave number)
d	= Doublet (for NMR data)
dd	= Doublet of doublets (for NMR data)
ddd	= Doublet of doublet of doublets (for NMR data)
dt	= Doublet of triplets (for NMR data)
DEPT	= Distortionless Enhancement by Polarization Transfer
$CDCl_3$	= Duterated Chloroform
δ	= Chemical shift (for NMR data)
ESIMS	= Electrospray Ionization Mass Spectrometry

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

g = Gram

hr = Hour

^{1}H-^{1}H COSY = Homonuclear (Proton-Proton) Correlation Spectroscopy

^{1}H NMR = Proton Nuclear Magnetic Resonance

HMBC = ^{1}H-Detected Heteronuclear Multiple Bond Coherence

HMQC = ^{1}H-Detected Heteronuclear Multiple Quantum Coherence

HRTOFMS = High Resolution Time of Fight Mass Spectrometry

HPLC = High Performance Liquid Chromatography

Hz = Hertz

IR = Infrared Spectrum

J = Coupling constant

KBr = Potassium bromide

Kg = Kilogram

L = Liter

lit = Literature

ε = Molar absorptivity

$[M+H]^{+}$ = Protonated molecular ion

$[M-H]^{-}$ = Deprotonated molecular ion

MHz = Mega hertz

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

m.p. = Melting Point

m = Multiplet (for NMR data)

mg = Miligram

mL = Mililiter

mm = Milimeter

min = Minute

m/z = Mass to charge ratio

MS = Mass Spectrometry

µg = Microgram

µL = Microliter

NMR = Nuclear Magnetic Resonance Spectroscopy

NOSEY = Nuclear Overhauser Effect Spectroscopy

nm = Nanometer

R_f = Retardation factor

s = Singlet (for NMR data)

H_2SO_4 = Sulfuric acid

TLC = Thin Layer Chromatography

TOF = Time of Fight

t = triplet

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

UV = Ultraviolet

V = voltage

ν_{max} = Wave number at maximal absorption

λ_{max} = Wavelength at maximal absorption

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Publication :

1. Chaivisuthangkura A, Malaikaew Y, Chaovanalikit A, **Jaratrungtawee A**, Panseeta P, Ratananukul P, Suksamrarn S. Prenylated xanthone composition of *Garcinia mangostana* (mangosteen) fruit hull. *Chromatographia*. 2009;69(3):315-8.

Oral presentation:

1. **Jaratrungtawee A**, Davies D, Walker EB. Isolation of alpha-mangostin from mangosteen fruit and subsequent glycosylation. Annual conference and centennial celebration. March 21, 2008. University of Utah. Salt Lake City, Utah.

Patent:

1. Suksamrarn S, Sudta P, Jiarwapi P, **Jaratrungtawee A**, Ratananukul P, Suksamrarn A. Xanthone with antiherpes simplex type 1 activity. *Thai Patent Application*, No. 0901004650, 16 October 2009.

Poster presentation:

1. **Jaratrungtawee A**, Ratananukul P, Suksumrarn S. Phytochemicals from the very young fruit of *Garcinia mangostana* L. The 34TH congress on science and technology Thailand (STT34). Queen Sirikit National Convention Center, Bangkok, Thailand. 31 October – 2 November 2008. p.170. Poster number C3_C0229.

2. **Jaratrungtawee A**, Komutiban O, Ratananugul P, Suksumrarn S, Walker EB. Synthesis and Subsequent identification of mangostin glycoside in mangosteen fruit. Joint NORM/RMRM. (Joint 63rd Northwest/21st Rocky Mountain Regional Meeting) American Chemical Society reginal meeting. Park city. Utah. 15-18 June 2008. p.89. Poster number 82.

3. **Jaratrungtawee A,** Komutiban O, Rattananugul P, Suksamrarn S, Pugh M, Davies D, Walker EB. HPLC analysis of xanthones and novel xanthone-*O*-glycosides in mangosteen fruit. Annual meeting and exposition, 122nd Annual meeting of the association of official analytical chemists. Dallas. Texas. 22 September 2008.

4. Bumrungpert A, Kalpravidh RW, Suksamrarn S, Chaivisuthangkura A, **Jaratrungtawee A**, Chitchumroonchokchai C, Failla ML. Bioaccessibility, biotransformation and transport of [alpha]-mangostin from *Garcinia magostana* (Mangosteen). Experimental Biology 2009, in New Orleans. Abstract Number 2968.

5. Busarakhumtragul P, Tep-areenan P, Wongsawatkul O, Suksumrarn S, **Jaratrungtawee A.** The vascular effects of alpha- and gamma-mangostins, and their analogues in the rat aorta**.** XXXVI the International Union of Physiological Sciences (IUPS). Kyoto, Japan from July 27 to August 1, 2009.

6. **Jaratrungtawee A**, Jainhuknan J, Tanglukmongkoll P, Suksamrarn A, Suksamrarn S. Quantitative analysis by HPLC-ESI-TOF-MS method of major xanthones in different parts of *Garcinia mangostana* L. Pure and Applied Chemistry International Conference (PACCON2011), Miracle Grand Hotel Bamgkok, Thailand. 5-7Jan 2011. p.162. Poster number AC_A0094.