CHEMICAL CONSTITUENTS FROM THE FRESH PERICARPS OF MATURE MANGOSTEEN FRUIT *Garcinia mangostana*

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องค์ประกอบทางเคมีจากเปลือกสดของผลมังคุดสุก *Garcinia mangostana*

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อาเหม็ด ทีจานี อาซีท : องค์ประกอบทางเคมีจากเปลือกสดของผลมังคุดสุก *Garcinia mangostana*. (CHEMICAL CONSTITUENTS FROM THE FRESH PERICARPS OF MATURE MANGOSTEEN FRUIT *Garcinia mangostana*) อ.ที่ปรึกษาหลัก : สุรชัย พรภคกุล

แอลฟา-แมงโกสติน (α-Mangostin) ถือว่าเป็นองค์ประกอบทางเคมีที่สำคัญในเปลือกของมังคุด (*Garcinia Mangostana* Linn.) เท่าที่ผ่านมา ในเอกสารอ้างอิงมีการนำเปลือกมังคุดแห้งมาศึกษากันมากมาย การศึกษานี้มีวัตถุประสงค์เพื่อทำการแยกแอลฟา-แมงโกสติน และองค์ประกอบทางเคมีอื่นๆจากเปลือกสดของผลมังคุดสุก และศึกษาสารต้านอนุมูลอิสระและฤทธิ์ต้านมะเร็งของสารเหล่านี้ การแยกสารด้วยคอลัมน์โครมาโตกราฟีแบบซ้ำซ้ำสำหรับสารสกัดเอทิลอะซีเตตและเมทานอลของเปลือกสดของมังคุดสุกทำให้ได้แอลฟา-แมงโกสตินเป็นสารหลัก (33.25% ของสารสกัดหยาบ) เบตา-แมงโกสติน (β-mangostin) (0.225%) และการ์ทานิน (gartanin) (7.57%) ทำการศึกษาฤทธิ์ต้านอนุมูลอิสระในการดักจับอนุมูล 2,2-diphenyl-1-picrylhydrazyl (DPPH) ของสารเหล่านี้ แอลฟา-แมงโกสติน, เบตา-แมงโกสติน และการ์ทานิน แสดงค่า IC_{50} ที่ 322.57, >589 และ 8.42 μM ตามลำดับ ในขณะที่ IC_{50} ของ Trolox คือ 5μM แอลฟา-แมงโกสติน, เบตา-แมงโกสติน และการ์ทานินแสดงฤทธิ์ต้านมะเร็งในการต้านเซลล์นิวโรบลาสโตมาชนิด SH-SY5Y (SH-SY5Y neuroblastoma cell line) ที่มีค่า IC_{50} 9.0, 8.4 และ 7.0 μM ตามลำดับ ทำการศึกษาเปรียบเทียบวิธีการสกัดสองวิธี (การแช่และการสกัดด้วยคลื่นอัลตราโซนิก) กับเปลือกผลแห้งและเช่นเดียวกับเปลือกผลสดโดยใช้เมทานอล เอทานอล และเอทิลอะซีเตตเป็นตัวทำละลายเพื่อเพิ่มผลในการต้านอนุมูลอิสระของสารสกัดเปลือกมังคุดแห้งและปริมาณผลิตภัณฑ์ที่ได้รับ การศึกษานี้พบว่า แอลฟา-แมงโกสติน เป็นสารต้านอนุมูลอิสระชนิดแซนโทนที่สำคัญในเปลือกสดมังคุดสุก จากทำการเปรียบเทียบฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ต้านมะเร็งของสารประกอบเหล่านี้และการทำนายเชิงเปรียบเทียบในลักษณะเดียวกันนี้พบว่าการ์ทานินที่มีอยู่ในสารสกัดด้วยตัวทำละลายทั้งสามชนิดเสริมฤทธิ์ต้านอนุมูลอิสระของแอลฟา-แมงโกสติน

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Ahmad Tijani Azeez : CHEMICAL CONSTITUENTS FROM THE FRESH PERICARPS OF MATURE MANGOSTEEN FRUIT *Garcinia mangostana*. Advisor: Assoc. Prof. SURACHAI PORNPAKAKUL, Ph.D.

α-Mangostin has been identified as a major chemical constituent in the pericarps of mangosteen (*Garcinia mangostana* Linn.) Over time, dried pericarps of mangosteen have been subjected to studies in literature. This study aimed to explore isolation of **α**-mangostin and other chemical constituents from the fresh pericarps of mature mangosteen fruits and investigate their relative antioxidant and anticancer activities. Repeated column chromatography on ethyl acetate and methanol extracts of fresh mature mangosteen pericarps led to the isolation of **α**-mangostin (33. 25% of crude extracts) as the major compound, beta-mangostin (0.225%) and gartanin (7.57%). Their antioxidant scavenging activity was investigated against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. **α**-Mangostin, **β**-mangostin and gartanin showed IC_{50} values of 322.57, >589 and 8.42 μM respectively, while the IC_{50} of Trolox was 5μM. **α**-Mangostin, **β**-mangostin and gartanin exhibited anticancer activities against SH-SY5Y neuroblastoma cell line with IC_{50} values of 9.0, 8.4 and 7.0 μM respectively. The idea of juxtaposing two extraction methods (maceration and ultrasonic-assisted extraction) with the dried pericarp, and as well as the fresh pericarps was also studied using methanol, ethanol, and ethyl acetate as solvents to maximize the antioxidant potency of the dried mangosteen pericarp extracts and their respective yields. This study established **α**-mangostin as a major antioxidant xanthone in mature mangosteen fresh pericarps, also compared the antioxidant and anticancer activities of the compounds and in the same vein predicted a synergistic antioxidant activity of **α**-mangostin and gartanin present in the three solvent extracts.

Field of Study: Chemistry Student's Signature .....

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Ahmad Tijani Azeez

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Natural products are commonly known as phytochemicals, a group of nutritive compounds concentrated in herbs, fruits, vegetables, and spices. Plants synthesize a large numbers of these metabolites for their growth and development, reproduction, defense against attack by many different kinds of organisms, and survival in often harsh and ever changing environments.(Desai et al., 2010)

Over the years, attention has been glued to the production of **α**-mangostin and other compounds from the dried pericarp of mangosteen (*Garcinia mangostana*). However, there has not been any report in literature on **α**-mangostin and/or chemical constituents (xanthones) production from the fresh pericarp of mature mangosteen fruit. This research seeks to investigate and explore the chemical constituents from the fresh pericarp of mature mangosteen fruit, evaluate their bioactivities (antioxidant and anticancer) and juxtapose the methods of extraction and production of **α**-mangostin with that of the dried pericarp.

Fruits have been a blessing to human race from time immemorial, because of the health and medicinal benefits, ranging from nutrients, treatment or suppressing diseases to strengthening of the immune system owing to various antioxidants that

most of them possess. *Garcinia Mangostana* L. commonly known as mangosteen and being referred to as "queen of fruits" is native to Southeast Asia and can be found in a few other Asian countries. Mangosteen belongs to the family Guttiferae or Clusiacae, mature mangosteen trees range from 6 to 25 m, it has an edible reddish or dark purple fruit with a juicy yet soft, edible, and attractive pulp with delectable taste. In Southeast Asia, mangosteen pericarp has been used as a traditional medicine to treat abdominal pain, diarrhea, dysentery, cholera, infectious wounds, purulence, chronic ulcers, and other diseases. It also exhibits the effect of anti-inflammatory, antibacterial, anti-malarial, lowering blood pressure, anti-oxidation, anti-HIV, immune regulation, and many other pharmacological activities(Guo et al., 2016). Nowadays, products manufactured from *G. mangostana* are being used as dietary supplements in the United States, because of their potent antioxidant potential(Jung et al., 2006). In the site of the foregoing, the dried pericarps of mangosteen have been subjected to studies reported in literatures, with successful isolation and production of myriads bioactive constituents, in which **α**-mangostin has been the most prominent amongst other xanthones. **α**-Mangostin, a yellowish compound has shown bioactivities including antiseptic, analgesic, antiallergic(Khaw et al., 2020), anti-bacterial, anti-inflammatory, anticancer activities and a promising remedy for the treatment of Alzheimer's disease(Yang et al., 2021).

Ultrasound-assisted extraction with different solvents has not been compared with maceration in the extraction from the dried pericarp, apart from Microwave-assisted extraction(Ghasemzadeh et al., 2018). Mature mangosteen pericarp contains more alpha mangosteen than the young(Pothitirat et al., 2009), alpha mangostin is an antioxidative stress[3], and it is utilized in the development of germ-free food products and cosmetics(Shibata et al., 2011).

In this study therefore, we explored the production of **α**-mangostin and other chemical constituents from the fresh pericarp of mature mangosteen fruit and investigated their relative antioxidant scavenging properties against 2,2-diphenyl-1-picrylhydrazyl radical in DPPH assay.in the same vein, we investigated their relative anticancer properties against SH-SY5Y cell line. The dried pericarp of mangosteen macerated with and without ultrasonic assistance were monitored for their respective yields and antioxidant activities and juxtaposed with those of the mature mangosteen fresh pericarps.

1.1.1 Mangosteen Pericarp

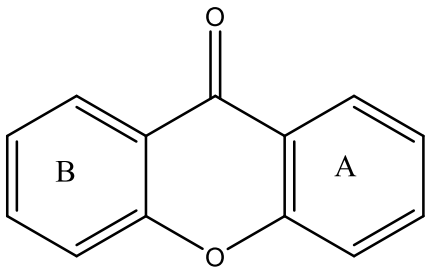
This is a reddish pulp, although inedible but is bounds with a class of isoprenylated xanthones which are referred as mangostins. Interesingly, numerous *in vitro* and *in vivo* studies have shown that mangostins and their derivatives possess diverse pharmacological activities, such as antibacterial, antifungal, antimalarial, anticarcinogenic, antiatherogenic activities as well as neuroprotective properties in

Alzheimer's disease (AD).(Ming-Hui et al., 2017) No wonder for hundreds of years, the pericarp of mangosteen has been used as a traditional medicine to treat inflammation, ulcer, skin infection, wound healing, amoebic dysentery, and diarrhea.

1.1.2 Xanthones

Xanthones are important oxygenated heterocycles found in many natural products exhibiting prominent biological and pharmacological activities. Molecules bearing a xanthone moiety exhibit several bioactivity potencies such as anticancer, antimicrobial, antimalarial, anticonvulsant, anti-cholinesterase, anti-HIV, antioxidant, anti-angiogenesis, anti-inflammatory, anti-alzheimer, and cholesterol acyltransferase inhibitory activities. In addition, some of these compounds are evaluated and utilized as major drug candidates.(Shrestha & Lee, 2018) Pinto described xanthones present in mangosteen has having a ring system that is substituted with a different forms of isoprene, phenolic and methoxy groups leading to a variety of possible structures. Apart from oxygenated xanthones, other natural xanthones classified based on the nature of substituents include but are not limited to glycosylated xanthones, prenylated xanthones and their derivatives, xanthone dimers, xanthonolignoids etc. (Pinto et al., 2005) The potential chemopreventive and chemotherapeutic activities of xanthones have been demonstrated in different stages of carcinogenesis (initiation, promotion, and progression) and are known to control cell division and growth, apoptosis, inflammation, and metastasis.(Shan et al., 2011)

Most compounds from the mangosteen pericarps are xanthones but **α**-mangostin is the most abundant and important.



Xanthone basic structure

The separation of xanthones is usually carried out by silica gel chromatography using different kinds of solvent and solvent mixtures. They are also separated and identified by comparison with standards by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The structures of all known xanthones were established mainly by ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR), Infrared Spectroscopy (IR) amongst others.(Walker, 2007) ^{1}H and ^{13}C NMR spectroscopy is adjudged the most useful method for the structural elucidation of naturally occurring xanthones. The unknown xanthones present in mangosteen require NMR Analysis of spin-spin coupling and chemical shifts for the determination of the structures of substituents and the number of aromatic protons in the compounds. Additionally, a couple of 2D NMR techniques such as COSY, NOESY, HSQC and HMBC have been used widely to elucidate the structures of more complex xanthones. (Huang et al., 2001; Nilar & Harrison, 2002; Vieira & Kijjoa, 2005) Recently, x-ray crystallography played a key role, and therefore pertinent in the

determination of the three-dimensional structure of compounds including the absolute configuration of xanthones. The crystal structures of xanthones that have been elucidated lately revealed that the three-ring system, which determines the skeleton of this class of compounds, is mainly planar. There is usually an aromatic character in a central pyranoid ring. This is based on evaluation by both bond lengths and angles. But there are some minor limitations mainly due to steric factors associated with substituents. As it was difficult to obtain diffraction quality crystals, x-ray crystallography derived structures of the main xanthones of mangosteen remained unknown for a long time. But later on the crystal structure of **α**-mangostin and 1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl) xanthone were reported.(Gales & Damas, 2005; Malathi et al., 2000)

1.1.3 **α**-Mangostin

The compound which also doubles as a Natural product with the IUPAC name 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl) xanthen-9-one and commonly known as **α**-mangostin is a well-known bioactive compound and the most abundant xanthone found in mangosteen pericarp. A member of the class of xanthones that is 9H-xanthene substituted by hydroxy group at positions 1, 3 and 6, a methoxy group at position 7, an oxo group at position 9 and prenyl groups at position 2 and 8. **α**-Mangostin is one of the most important natural xanthone derivatives. Its molecular formula $C_{24}H_{26}O_6$ has a molecular mass of 410.45 g/mol and melting point (MP) of

180-181°C. It is a naturally occurring xanthone and the most abundant active constituent isolated from mangosteen.

1.1.4 Cancer

Cancer is a deadly disease ravaging and putting burden on society in both developed and develpoing countries alike. The prevalence of cancer is due to the growth and aging of the population, as well as an increasing prevalence of established risk factors such as smoking, overweight, physical inactivity, and change of reproductive patterns especially those associating with urbanization activities and economic development. According to GLOBOCAN 2012, Lung and breast cancer are the most prevalent and the leading causes of cancer death in men and women. In developed countries, however, prostate cancer is the most prevalent cancer among men, while lung cancer is the leading cause of cancer death among women. Other frequently diagnosed cancers worldwide include those of the liver, stomach, and colorectum in males population and those of the stomach, cervix uteri, and colorectum in females population. In more developed countries, bladder cancer among males and uterine cancer are the most prevalent among females while in less developed countries, liver and stomach cancer among men are the second and third most prevalent, respectively, and leading causes of cancer death.(Torre et al., 2015)

In the cancer promotion phase, cells with a defect in key cellular proliferation control and apoptosis regulatory proteins tend to persist, actively replicate, and

accumulate to originate a focus of preneoplastic cells. Consequently, the preneoplastic cells transform into neoplastic ones and gain angiogenic properties as well as invasive and metastatic potential, entering the stage of cancer progression. Therefore, the strategies that can induce cell cycle arrest and/or apoptosis, disrupt angiogenesis, or disallow tumor cells from escaping from the original location and invading other tissues are pertinent for cancer prevention. **α**-Mangostin has been shown to possess varied bioactivities including antiproliferative, proapoptotic, antiangiogenic and antimetastatic activities against a wide range of cancer cell types by modulating various aberrant molecules and signal transduction pathways.(Zhang et al., 2017)

1.1.5 Neuroblastoma (SH-SY5Y)

Neuroblastomas are an heterogeneous group of tumours which is the most common extracranial solid tumour of childhood, with 90% of cases occurring in children less than 5 years of age. It occurs at an average of 2 years of age at diagnosis. The development of neuroblastoma proves an aberration in developmental processes.(Qiu & Matthay, 2022)

1.1.6 Free Radicals (Oxidants)

Numerous investigations have indicated that free radicals cause oxidative damage to lipids, proteins, and nucleic acids. Antioxidants seem to be very important in the prevention of these diseases because they can inhibit or delay the formation

of oxidizable substrate chain reactions. gnized that consumption of fruits and vegetables can reduce the incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction, and cataracts. These protective effects are considered mainly to be due to the presence of various antioxidants in fruits and vegetables.(Jung et al., 2006) Cui et al. isolated two compounds, α-mangostin and γ-mangostin, in which they both not only exhibited analgesic effects, but also possess the ability of scavenging Reactive Oxygen Species (ROS) in each dose-dependently.(Cui et al., 2010) Furthermore, they explored its ability of free radical scavenger and analyzed its monoanion through the corresponding theory. Vy *et. al* (2021) argued that α-mangostin (α-MG) and its derivatives could be free radical inhibitors due to the hydroxyl groups at C-3 and C-6. These antioxidant functions may be credited to their hydrogen-donating capability. Since free radicals induce the autoxidation of unsaturated lipids. Antioxidants inhibit the free oxidation radical chain reaction and block the transferring of hydrogen from the phenolic hydroxyl groups, thereby creating a stable end compound.(Tran et al., 2021) Generally, xanthones from mangosteen possess potent scavenging activities on nitric oxide, hydroxyl and superoxide radicals.[24] Also, the water-soluble partition of methanol-extract of mangosteen pericarp has been confirmed to exert a neuroprotective effect against oxidative stress in various neuronal cells *in vitro* and *in vivo*.(Moongkarndi et al., 2010)

1.1.7 Wound healing

There have been reports on the roles of mangosteen pericarps and **α**-mangostin on wound healing. Wound healing is one of the biological effects of xanthones found in the mangosteen pericarp (PATRICK et al., 2022). The process of wound healing is intricate and carefully controlled, playing a crucial role in maintaining tissue function. In chronic wounds, the series of events involved in wound healing becomes prolonged, resulting in considerable discomfort and distress for the individual affected (Han & Ceilley, 2017). A chronic wound is characterized by a delayed and disorganized healing process, leading to the loss of structural and functional integrity (Yao et al., 2020). The healing of chronic ulcers is hindered as it extends beyond a few weeks from the start of treatment due to factors like infection and the presence of foreign objects. These conditions contribute to the development of persistent ulcers. Common types of chronic ulcers include diabetic foot ulcers, pressure ulcers, arterial ulcers, venous ulcers, and wounds infected with fungi (Liu et al., 2017). Within the United States, there is a widespread and often overlooked issue of chronic wounds, which affects approximately 6.5 million individuals. This can be considered a silent pandemic, as it poses a significant health burden to a substantial number of people (Sen et al., 2009). According to a study conducted, alpha-mangostin possessed anti-inflammatory properties and enhanced the rate of wound healing by promoting the regeneration of damaged cells and

tissues. The presence of antioxidants in alpha-mangostin is believed to facilitate and expedite the overall process of wound healing (Tantra et al., 2021).

1.2 Objective(s)

1. To investigate the chemical constituents from the fresh pericarp of mature mangosteen fruit and their bioactivities
2. To explore the production of α-mangostin and other constituents from the fresh and dried pericarp of mangosteen fruit
3. To juxtapose the extraction and production processes of α-mangostin and other constituents from the fresh pericarp of mature mangosteen fruit with that of the dried pericarp of mangosteen

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

Fig. 2.1 *Garcinia mangostana* L.

Garcinia mangostana L. known as mangosteen is a source of xanthones including **α**-mangostin, possess various bioactivity including anticancer have only been gotten from dried parts of the plant including the trunk, peels, seed, and the pericarp. A yield of **α**-mangostin (5.2%) was obtained by extraction from dried mangosteen pericarps (Sultan et al., 2022). The production of **α**-mangostin and other xanthones from the fresh pericarp of Mangosteen is unprecedented.

Mangosteen (*Garcinia mangostana* L.) belongs to the Garcinia genus, which mainly grows in Thailand, Vietnam, Malaysia, Indonesia, and other Southeast Asian countries. It is also widely cultivated in few provinces in other Asian countries as well

including a few parts of China. The mangosteen fruit is reddish/dark purple with a juicy, soft, edible pulp and delectable taste. Generally, the genus Garcinia includes more than 300 distinct species from which several classes of bioactive compounds such as xanthones, flavonoids, triterpenoids, and benzophenones have been isolated and characterized. Although many Garcinia species including *G. mangostana*, *G. schomburgkiana*, *G. dulcis*, *G. cowa*, *G. atroviridis*, *G. hanburyi*, *G. bancana*, *G. xanthochymus*, *G. thorelii*, *G. hombroniana*, and *G. speciosa*, bear edible fruits, mangosteen has captured the most attention in the market (Gutierrez-Orozco & Failla). An important part of the fruit is the pericarps. Mangosteen rind has been used as a traditional medicine in Southeast Asia to treat abdominal pain, diarrhea, dysentery, cholera, infectious wounds, purulence, chronic ulcers, and other diseases. In addition, it also exhibits the effect of anti-inflammatory, antibacterial, anti-malarial, lowering blood pressure, anti-oxidation, anti-HIV, immune regulation, and many other pharmacological activities (Guo et al., 2016).

Xanthones are the major active and prominent compounds in mangosteen, including α-mangostin with the IUPAC name of 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)-9*H*-xanthen-9-one). Various studies have taken different dried parts of *G. mangostana* into consideration. Results from the young fruit peel showed the presence of 1,6-dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)6’6,6’-dimethylpyrano(2’,3’:3,2)xanthone, demethylcalabaxanthone, 8- desoxygartanin,

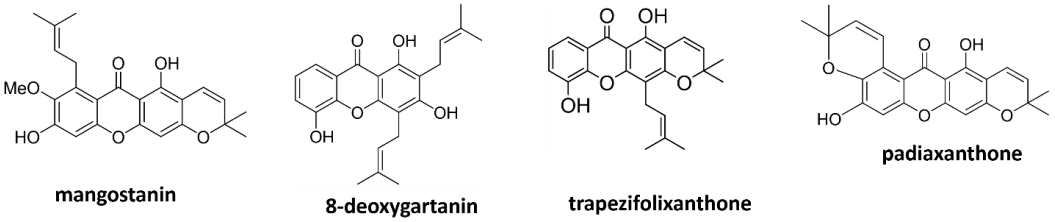
gartanin, α-mangostin, β-mangostin, γ-mangostin, garcinone B, garcinone C, garcinone D, garcinone E, mangostenone C, mangostenone D, mangostenone E, mangostanol, 11-hydroxy-1- isomangostin, mangostinone, thwaitesixanthone and mangostanin. [27] Also, from stem bark, α-mangostin, β-mangostin, γ-mangostin, from root bark found α-mangostin, β-mangostin, 3-hydroxy-4-geranyl-5-methoxybiphenyl and β-sitosterol and from young fruit latex, α-mangostin, β-mangostin, γ-mangostin, methoxy-β-mangostin and garcinon E were gotten. [27] From seeds and fruit pulp, 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone, 2-isoprenyl-1,7-dihydroxy-3-methoxyxanthone, 1,7-dihydroxy-8-(3-methylbut-2-enyl)-6',6'dimethylpyrano (2',3':3,2)-xanthone and trapezifolixanthone (Anggia et al., 2015). The structures of all the known compounds were demonstrated in Fig. 2.2.

The pericarp of *G. mangostana* was used as a cure for chronic intestinal catarrh and dysentery, as a lotion, as a treatment of respiratory disorders, to heal skin infections and relieve diarrhea, and as an astringent. α-Mangostin is the most abundant compound found in mangosteen pericarp, other prenylated xanthone commonly found are β-mangostin, γ-mangostin, gartanin, 8-deoxygartanin, garcinone C and garcinone D (Khaw et al., 2020).

Mohammad *et. al* (2019) reported Wittenauer et al. 2012 that Xanthones are polyphenol compound that are found abundantly in mangosteen pericarp which contain α-mangostin (69.01%) as the major xanthones compound followed by γ-

mangostin (17.86%), while the minor xanthones compounds (13.13%) include gartanin, 8-deoxygartanin, garcinon E, 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone, and 1,3,7-tri-hydroxy-2,8-di (3-methylbut-2-enyl)-xanthone.(Mohammad et al., 2019)

α-Mangostin, a yellowish coloring matter has shown bioactivities including antioxidant, anti-bacterial, anti-inflammatory, and anticancer activities and shown multiple activities of **α**-M related to Alzheimer's Disease therapy, including anti-cholinesterase, anti-amyloid-cascade, anti-inflammation, anti-oxidative stress. It shows that **α**-M is a promising candidate for the treatment of AD. Interestingly, the isolation of the ethanol extract of naturally dried fresh fruit pericarps of *G. mangostana* through a repeated column chromatography led to 14 known compounds as shown in figure 2.2– but **α**-mangostin not among. (Yang et al., 2021)



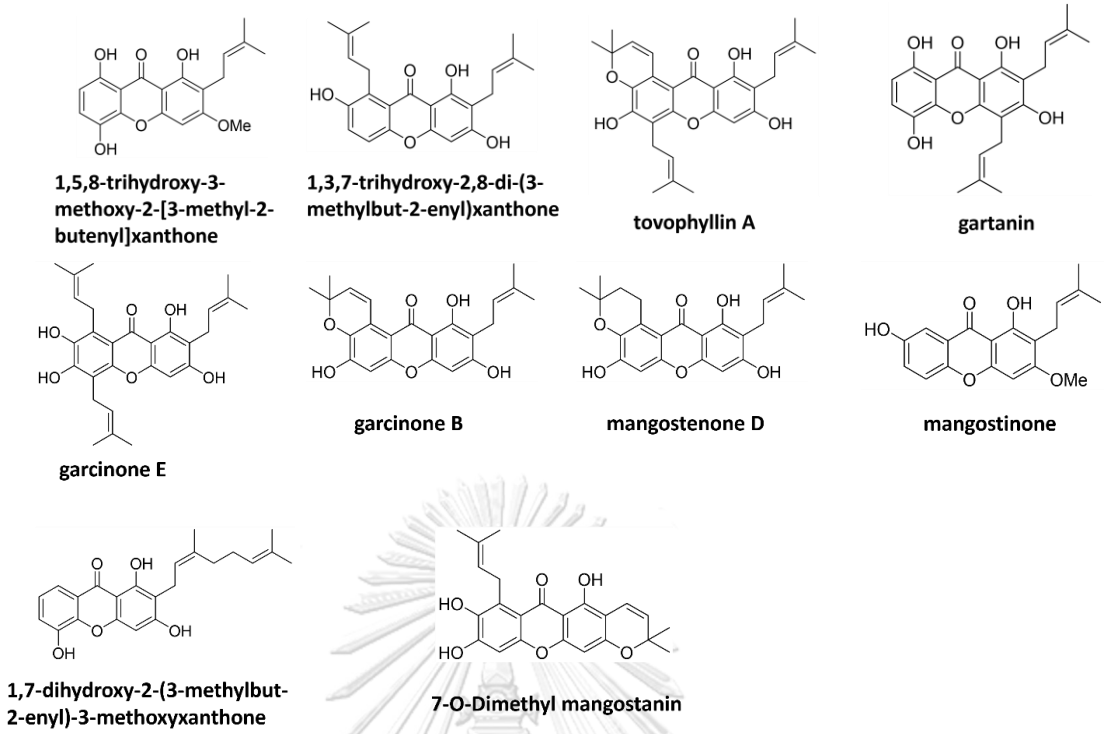


Fig. 2.2. compounds isolated from *Garcinia mangostana* fruit.

Purification on HPD-400 macroporous resin gave high purity of α-mangostin (95.6%) in 5.2% yield in which binding to human serum albumin (HSA) or transferrin (TRF) was explored by combining spectral experiments with molecular modeling. The results showed that α-mangostin binds to HSA or TRF as static complexes, but the binding affinities were different in different systems (Guo et al., 2016).

Again, the dried peel was extracted to obtain α-mangostin, which was then used to form (α-MG) derivatives at positions of C-3 and C-6, and then investigated, in which they show the antioxidant and anticancer activities (Tran et al., 2021). To obtain the optimum antioxidant activity in mangosteen peel extract, a sound preparation for the condition and method of extraction must be well considered

(Kusmayadi et al., 2018). 5.0 g of dried mangosteen pericarp sample were loaded into extraction vessel where the pressure vessel was sealed tightly. High-pressure CO_2 was supplied from the CO_2 cylinder to the extractor equipped with a back-pressure regulator unit. Samples were collected from collector valve and excess solvent was removed using rotary evaporator (Panichayupakaranant, 2015).

In a rapid method of extraction, dried pericarp of mangosteen was extracted with analytical grade methanol, after characterization, 0.92 % of DW of α-mangostin was obtained(Khaw et al., 2020).

Also, α-mangostin, β-mangostin and gartanin were isolated from the trunk latex of Mangosteen and investigated for their antimicrobial activities against *Bacillus subtilis*, *Enterococcus faecalis*, *Micrococcus luteus*, *Salmonella typhosa*, *Staphylococcus epidermidis*, *Streptococcus mutans and Vibrio cholerae*. α-Mangostin gave significant activity to inhibit the growth of *B. subtilis, E. faecalis, S. typhosa, S. epidermidis and V. cholerae*. While gartanin gave significant activities against *V. cholerae* and moderate activities against *B. subtilis, S. typhosa* and *S. epidermidis*. β-mangostin gave significant activities against *B. subtilis, E. faecalis and V. cholera* and moderate activities against S. epidermidis, but there was no inhibition toward *M. luteus* and *S. mutans*. (Anggia et al., 2015)

In a microwave assisted extraction specifically, 2g of powdered mangosteen pericarp was extracted with 20 mL of solvents. The optimal microwave power, time,

and ethyl acetate percentage of extraction to maximize the α-mangostin extract from mangosteen pericarp (120.68 mg/g DM) were 189.20 W, 3.16 min, and 72.40% (v/v), respectively (Ghasemzadeh et al., 2018).

Furthermore, the yield of α-mangostin was 9.2 g/kg of dry weight (DW) in a recent study (Khaw et al., 2020). The highest yield of an extract and the highest content of α-mangostin from the pericarp of mangosteen was 26.60% dry weight and 46.21% w/w of crude extract respectively (Panichayupakaranant, 2015). Dried mangosteen rind gave 86g crude extract resulting to α-mangostin (8.92 g), β-mangostin (11.1 mg) and gartanin (16 mg) (Anggia et al., 2015).

α-Mangostin may be beneficial as an agent against specific infections in human beings as well as in other applications such as the development of germ-free food products and cosmetics (Yang et al., 2017). The mature fruit rind extract contained higher contents of flavonoids and α-mangostin xanthone and gave higher anti-acne producing bacteria activity than the young fruit rind extract. The mature fruit rind extract contained α-mangostin (13.63 ± 0.06% w/w) about 2 times higher than the young fruit rind extract (8.07 ± 0.11% w/w), even though the young fruit rind extract contained much higher content of phenolic compounds (42.57 ± 0.11 g GAE/100 g extract) than the mature fruit rind extract (Pothitirat et al., 2009).

α-Mangostin is a yellowish powder with a tricyclic aromatic planar system and side chains with isoprene, methoxyl and hydroxyl groups. A lot of groups have researched on its activities, which include anti-oxidant (Ding et al., 2020) anti-inflammatory, anti-bacterial and anti-tumor (Mohammad et al., 2019). In a series of experiments reported in recent years, it has been found that compound **α**-mangostin has a potential to slow down cognitive decline and shows a delaying effect on AD. **α**-Mangostin is being identified as MAN and it was stressed that it is a bioactive compound isolated from the inedible pericarp of an edible fruit popularly known as mangosteen. They revealed a unique anti-tumor mechanism of MAN by provoking ROS production through downregulation of NAMPT/NAD signaling and further validated MAN as a potential therapeutic reagent for lung cancer treatment (Ding et al., 2020). Sometimes, patients under chemotherapy take juice from mangosteen extract while unaware of the amazing effect that may result from combining them with their therapy (Pérez-Rojas et al., 2016).

2.2 Botanical description

The genus Garcinia which is also widely known as Clusiaceae, includes about 50-300 species of evergreen trees and shrubs that are native to Asia, Australia, tropical and southern Africa. A good number of the species bear edible fruits, including *G. mangostana* which is known as mangosteen. Mangosteen is native to

Malaysia Australia, Brazil, Central America, Hawaii, Southern India, Indonesia, Thailand and other tropical countries. While several Garcinia species, such as *G. mangostana*, *G. schomburgkiana*, *G. dulcis*, *G. cowa*, *G. atroviridis*, *G. hanburyi*, *G. bancana*, *G. xanthochymus*, *G. thorelii*, *G. hombroniana*, and *G. speciosa*, produce edible fruits, it is the mangosteen that has garnered the most significant interest in the market (Yapwattanaphun et al., 2000). *G. mangostana* is best described as an erect slow-growing tree with a pyramidal crown. It attains a height range between 6-25 m and possesses dark-brown or faintly black bark. Also, the inside of the bark contains yellow, gummy but bitter latex. Leaves are opposite, and short-stalked. Shape is simply elliptic while 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 in texture, dark green in colour and somewhat glossy above but yellowish-green beneath, having about 9-25 cm lenght and 4.5-11 cm wide. The leaves' petiole is usually 1.2-2.5 cm long. The flowers are 4-5 cm wide and may be either male or hermaphrodite on the same tree. The flowers align as clusters of about 3-9 pieces at the branch tips. There are four sepals and four ovate, thick, fleshy petals. The petals' colour is green having a touch of red spots on the outside, but yellowish-green inside. The flowers carry a lot of stamens without pollens as they possess no anthers. Hermaphrodite flowers are situated singly or in pairs at the tips of young branchlets. 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, with about 3.4-7.5 cm diameter. The colour is usually from dark-purple to red-purple. The rind better known as the pericarp is 6-10 mm thick, red in cross-section but purplish-white on the inside. The pericarp (rind) contains a bitter yellow latex and a purple, staining juice. The fruit may be seedless or has a 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 flavour and is classically very luscious and delicious. (Obolskiy et al., 2009)

2.3 Drying methods for mangosteen pericarps

Often, mangosteen rind or peel are dried prior to extraction of the active compounds. However, the effects of selected drying methods and conditions on the retention of xanthones in mangosteen were studied. Mangosteen rind was abruptly subjected to hot-air drying, vacuum drying at 60, 75 and 90°C. The results obtained showed that the drying methods significantly affected degradation of xanthones (i.e., α-mangostin and 8-desoxygartanin) and their antioxidant activity.(Suvarnakuta et al., 2011) Furthermore, 50% ethanol extract from mangosteen pericarp was freeze-dried (Ibrahim et al., 2016).

2.4 Extraction methods of mangosteen pericarps

Traditionally, extraction of natural products from various plants uses various techniques such as steam distillation (SD), Soxhlet extraction (SE), amongst others. These techniques however usually consume more energy, have long extraction time, and use large volume of solvents which eventually create environmental issue. Furthermore, due to increasing need for sustainable extraction techniques, that will result in shortened extraction time, reduced solvent consumption, automation, and energy saving. Eventually, a number of techniques including supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE), have been created and developed (Desai et al., 2010). The microwave assisted extraction was carried out in a study to determine the total phenolic content (TPC) and properties of xanthone extract from mangosteen pericarp, result showed a higher extraction of α-mangostin, as compared to the conventional water bath-maceration technique.(Mohammad et al., 2019)

Advantages of an extraction method over the other have been reported, for instance, Microwave-Assisted Extraction (MAE) method has advantages over the conventional maceration method such as low solvent consumption, less time consuming, and fast energy transfer through the irradiation that permit well diffusion of solvent within the extraction medium (Nayak et al., 2015). Even the advantages spread across in terms of getting antioxidant rich extracts, it has been confirmed that

MAE and ultrasound-assisted extraction (UAE) could extract the highest value of total phenolic content (TPC) and antioxidant from orange peel which led to the most prevailing extraction techniques compared to other extraction methods (M'hiri et al., 2015). Factors that normally affect extractions vary, in the case of MAE method for instance, several primary factors could affect the extraction, such as solvent types, solvent volume, power, temperature, irradiation time, and size of raw material amongst others. These factors convincingly affect the extracted yield, as well as the amount of total phenolic content (TPC) (Desai et al., 2010).

Apart from report from Kusmayadi *et al*. that also confirmed it (Kusmayadi et al., 2018), extract yields and amount of total xanthone in mangosteen pericarp are strongly affected by the solvent employed in the extraction (Suttirak & Manurakchinakorn, 2014).

As this study stands as the first to juxtapose the extraction procedures of maceration and ultrasonic-assited extraction in the case of fresh and dried mangosteen, there is need to mention that Mariani *et. al* (Hamid et al., 2018) has the first report focusing on optimisation using supercritical fluid CO_2 (Hamid et al., 2018). Natural Deep Eutectic Solvents (NADES) have been applied as a green solvents for the extraction of bioactive compounds, mainly **α**-mangostin, from mangosteen pericarp. The NADES consisted of choline chloride, a quarternary ammonium salt, and four hydrogen bond donors: 1,2- propanediol, citric acid, glycerol, and glucose.

The highest **α**-mangostin extraction yield of 2.6 % (w/w) in dried pericarp was obtained using a mixture of choline chloride and 1,2-propanediol in 1:3 mole ratio (Mulia et al., 2015). To round off, the extraction procedure and method can have effect on the efficacy of mangosteen extracts (Pothitirat et al., 2010).

2.5 Traditional medical use

Almost all parts of the mangosteen especially the fruit hull, bark, and roots, have been used for hundreds of years have been used in Southeast Asia countries as a medicine for a great variety of medical conditions. In few Asian countries, the dried and powdered fruit hull is used for the antiparasitic treatments in dysentery and also employed as antimicrobial agents (Ji et al., 2007). Again, it also includes uses externally for healing wounds, suppurations, and chronic ulcers (Farnsworth & Bunyapraphatsara, 1992). Furthermore, mangosteen leaves and bark also possess a strong anti-inflammatory property which allows an ointment extracted from them to be used for the treatment of not only eczema and hyperkeratosis but also other skin disorders including psoriasis (Matsumoto et al., 2003; Sakagami et al., 2005; Sato et al., 2004). Also, the astringent qualities of mangosteen are also employed for prevention of dehydration and the loss of essential nutrients from the gastrointestinal tract to combat diarrhea. In Thai folk medicine the fruit hulls (pericarps) of mangosteen have been used for the treatment of skin infections, wounds, and diarrhea relief (Jung et al., 2006; Suksamrarn et al., 2003; Suksamrarn et

al., 2002). A tea made from the rind (pericarp) and boiling of the leaves and bark is used not only as an antipyretic medicine but also in the treatment of ailments such as diarrhea, dysentery, and a wide range of urinary disorders in the Philippines and Malaya. Boiling of the root is used by women with menstrual cramps. In fact, a bark extract of mangosteen regionally called 'amibiasine' has been used for the treatment of amoebic dysentery (Moongkarndi et al., 2004; Nakatani et al., 2002). Mangosteen has also been used medically in the Caribbean and Latin America, for instance, a tea made from mangosteen fruits served widely as a tonic against fatigue and low energy states. Even, Brazilians take similar tea from mangosteen as a digestive aid, as they also in Venezuela used the poultices of the fruit pericarp for parasitic skin infections treatment (Chairungsrilerd et al., 1996; Gopalakrishnan et al., 1997).

2.6 Chemical constituents from mangosteen

The major bioactive secondary metabolites of *G. mangostana* are xanthone derivatives which occur commonly in but a few higher plant families, fungi, and lichens. The pharmacological activities of xanthones are responsible for the great interest to this class of substances. The major constituents from the xanthone fraction of *G. mangostana* were discovered to be α-mangostin and γ-mangostin but also at least sixty (60) other xanthones have been isolated from different mangosteen parts which amongst others include β-mangostin, 1-isomangostin, 3-

isomangostin, 9-hydroxycalabaxanthone, 8-deoxygartanin, demethylcalabaxanthone, garcinone B, garcinone D, garcinone E, gartanin, mangostanol, mangostanin and mangostinone. (Obolskiy et al., 2009)

2.7 Biological Activities

Major xanthones from the pericarp of mangosteen of myriads *in vitro* studies have shown that they possess biological activities such as antioxidant, anti-proliferative, pro-apoptotic, anti-inflammatory, and anti-carcinogenic activities. This is evident in the fact that there was aggressive marketing of such health promoting which led to sales of mangosteen containing beverages in USA alone exceeding $200 million in 2008 despite very limited animal and human studies. This earned mangosteen the benefit to be classified as a "superfruit" (Gutierrez-Orozco & Failla). α-Mangostin (α-MG), the major xanthone in the pericarp of mangosteen possess a wide range of biological activities, with anti-inflammatory, anti-tumor, cardioprotective, antidiabetic, antibacterial, antifungal, antiparasitic, antioxidant and anti-obesity (Ibrahim et al., 2016).

It was previously reported that intravenously injected α-MG (2 mg/kg) in rats was slowly eliminated from blood and rapidly distributed to tissues with a maximum concentration of 17.9 μg/mL. The bioavailability of orally administered α-MG (20 mg/kg dose) dissolved in an aqueous solution containing 2% ethanol and 2% Tween 80 was estimated as only 0.4% (Gutierrez-Orozco & Failla, 2013). In a similar study, α-

MG (40 mg/kg) dissolved in corn oil was orally administered to rats. The maximum plasma concentration (4.8 μg/mL) was reached within 63 minutes (Rahayu, 2010). The biological activities of natural synthetic xanthone derivatives(Pinto et al., 2005), xanthones from mangosteen pericarps, whole fruit, trunk, leaves and branches(Pedraza-Chaverri et al., 2008), aril, seeds, heartwood(Chin & Kinghorn, 2008; Obolskiy et al., 2010) were investigated.(Obolskiy et al., 2009)

Table 2.1. Properties and bioactivities of xanthones in mangosteen

Properties	**Biological activities**
Natural and synthetic derivatives of xanthone	enzyme modulation, anti-tumor activity, anti-microbial, central nervous system (CNS) depressants, CNS stimulants, neurological disorders, anti-convulsant, analgesic, anti-arrhythmic, anti-hypertensive, anti-inflammatory, anti-allergic and immunomodulatory activities.
Xanthones isolated from pericarp, whole fruit, trunk, leaves, and branches.	antioxidant, anti-tumor, anti-inflammatory, anti-allergic, anti-bacterial, anti-fungal, anti-viral and anti-malarial activities.
Chemical constituents and methods of isolation from pericarp, whole fruit, stem, aril, seeds, heartwood, leaves.	antioxidant, anti-fungal, anti-bacterial, cytotoxic, anti-histamine, anti-HIV, CNS-depressant, cardiovascular, anti-inflammatory and anti-ulcerative activities.
Structural characterization of mangosteen xanthones in whole fruit, stem, aril, seeds, heartwood, leaves.	antioxidant, anti-bacterial, anti-fungal, anti-malarial, anti-HIV, cytotoxic, aromatase inhibitory, anti-cancer and anti-inflammatory activities

2.7.1 Anti-inflammatory of xanthones

The *in vitro* Anti-inflammatory activity of xanthones from mangosteen is tested on Human U397 macrophage-like cells and primary adipocytes (Bumrungpert et al., 2010), as well as Murine RAW 264.7 macrophage-like having LPS at 100 μg/mL (Tewtrakul et al., 2009) and 0.5-1 μg/mL (Chen et al., 2008). The results are illustrated below.

Table 2.2. *In vitro* anti-inflammatory activities of mangosteen xanthones.

Cell type	Pro-Inflam.	Tested compound	Dose	Results
Human U397 macrophage-like cells and primary adipocytes	LPS (100 μg/L) for 3 h	α- and γ-MG	α and γ-MG (2 h-pretreatment) with 10 or 30 μmol/L	α- and γ-MG decreased expression of IL-6, TNF-α, IFN-γ-inducible protein (IP)-10 in macrophage-like cells; decreased phosphorylation of MEK, JNK, ERK and p38; inflammation and insulin resistance in adipocytes.
Murine RAW 264.7 macrophage-like	LPS (100 μg/mL)	pericarp ethanol extract, α- and γ-MG	pericarp ethanol extract, (3–100 μg/mL), α- and γ-MG (3–100	α-MG and γ-MG inhibited NO and PGE2 production with moderate inhibitory effects on secretion of TNF-α and IL-4.

In a recent study, the impact of α-MG on the release of pro-inflammatory substances by both transformed and primary human cells was investigated. It was found that α-MG effectively suppressed the secretion of IL-8 or TNF-α by human cell lines originating from different tissues when exposed to an inflammatory stimulus. However, unexpectedly, α-MG enhanced the secretion of basal and LPS-stimulated TNF-α in primary cultures of human monocyte-derived macrophages cells (Gutierrez-Orozco et al., 2013).

2.7.2 Antioxidant Properties

Several human diseases have been linked to oxidative stress, which include diabetes, cardiovascular diseases, and neurodegenerative disorders especially. As a result, a lot of interest is given towards researching naturally occurring protective antioxidants. In this regard, it has been revealed that a few plant extracts possess powerful antioxidant activity and the ability to protect from oxidant-induced damages (Ibrahim et al., 2016).

Oxidative stress may result from the generation of excess reactive oxygen species (ROS). When these ROS accumulate in neuronal cells, then without doubt induces mitochondrial damage which automatically changes the membrane permeability, and finally activates the caspase cascade. Mostly, Antioxidant mechanism are achieved via either quenching the reactive oxygen species, preventing lipid

peroxidation, or stimulating cellular antioxidant defense systems (Ibrahim et al., 2016).

In essence, it was observed that **α**-mangostin reduces copper- or peroxyl radicals-induced oxidation of the human low-density lipoproteins (LDL). In the findings, a-mangostin prolonged the lag time of conjugated dienes at 234 nm dose-dependently, it also decreases the production of thiobarbituric reactive substances (TBARS), and as well diminishes the consumption of **α**-tocopherol that is induced by LDL oxidation (Ibrahim et al., 2016).

The safety of the polar fraction of mangosteen extract was thoroughly investigated in humans. The safety of oral administration of the polar fraction of mangosteen extract in 11 healthy Thai volunteers was investigated. For a whole 24-week period of the study, only minor and tolerable side effects were reported; no serious side effects were recorded. Also, blood chemistry studies confirmed no liver damage or kidney dysfunction in all subjects. In addition, polar fraction of ME both *in vitro* and *in vivo* study confirmed that oral administration of the polar fraction of mangosteen extract not only enhanced the antioxidant capability of red blood cells but also decreased the oxidative damage to proteins within red blood cells and as well as the whole blood (Suthammarak et al., 2016). In a study, it was demonstrated that the water-soluble partition of mangosteen extract not only protects the SK-N-SH neuroblastoma cell line from beta-amyloid-induced oxidative stress but also alters

the proteomic profile of the cells (Moongkarndi et al., 2010). It was in another study that exposed vivid evidence for the antioxidant effect of mangosteen extract as SK-N-SH cells were protected from cytotoxicity caused by endogenous hydrogen peroxide and exogenous polychlorinated biphenyl, both of which induce the production of reactive oxygen species (ROS) (Sattayasai et al., 2013).

2.7.3 Anticancer Properties

Cancer is a major deadly disease worldwide. The American Cancer Society reported that nearly 577,190 cancer patients didn't survive and again more than 1.6 million new cases occurred in the year 2012 in the US alone. These numbers seem big but small compared to what is in play worldwide, in that about 70% of all cancer deaths occurred in low- and middle-income countries according to the World Health Organization (WHO). α-Mangostin is considered one of the most studied chemo-preventive agents. It interferes with the initiation, promotion, and progression stages of carcinogenesis(Zhang et al., 2017).

Cancer is conspicuously a multifactorial disease in that a lot of factors and stages assemble to create this terminal disease or illness, occurring over an extended period starting with the initiation phase followed by promotion phase and then the progression phase. In the carcinogenic process, a cell acquires significant alterations yielding the progressive transformation from being normal into a cancerous cell. The

cellular alterations include blocking growth suppressors and apoptosis, self-sufficiency in growth signals, uncontrolled replicating potential as well as sustained angiogenesis, tissue invasion, and metastasis.(Zhang et al., 2017). Apparently, since necessity is the mother of invention, there is an increasing need to develop alternative treatment for cancer. The use of nontoxic chemical entities to block, reverse, or retard carcinogenesis otherwise known as chemoprevention, has emerged as a rescue for the management of cancer.

Chemotherapy is a highly effective method for reducing the occurrence of cancer. To combat cancer and other diseases that pose a threat to human health, scientists are continuously exploring plant-derived compounds to develop new drugs. In recent times, the potential of **α**-MG as an anticancer agent has been increasingly established. Promising results have been obtained from studies focusing on the treatment of common types of cancer, including breast cancer, lung cancer, colon cancer, stomach cancer, prostate cancer, pancreatic cancer, and skin cancer. These investigations have shed light on the anticancer and cytotoxic properties of mangosteen (Mohamed et al., 2017). Recently, **α**-mangostin, which is isolated from the pericarp of the fruit, was shown to induce cell death in various types of cancer cells in *in vitro* studies. It induces cell-cycle arrest and apoptosis in various types of human cancer cells. It was reported that the inhibitory effect of **α**-MG on cell viability in five HCC cell lines was studied. The healthy primary hepatocyte cells were

treated with α-MG (0–40 μM) for 24 and 48 h, the 50% growth suppression concentration (IC_{50}) values have cytotoxic effects. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay showed α-MG in both dose- and time-dependent ways to growth inhibition of SK-Hep-1 cells (Chen et al., 2018). Without mincing words, α-MG is a promising drug, and therefore worthy of production in myriad ways.

A study was designed to further explore the mechanisms involved in cytotoxicity of MAN on A549 cells. Apoptosis and cell cycle distribution were analyzed by flow cytometry methods. The results obtained indicated that MAN caused significant apoptosis and cell cycle arrest in A549 cells, which eventually resulted in inhibition on cell proliferation in vitro (Ding et al., 2020).

In vivo studies examining the anti-tumorigenic activities of mangosteen xanthones with cancer cell lines BJMC 3879 (Doi et al., 2009), GBM8401 (Chao et al., 2011) and NL-17 (Kosem et al., 2013) are summarized in Table 2.3

Table 2.3. In vivo anti-tumorigenic activities of mangosteen xanthones.

Cancer cell	Animal model	Compound	Delivery route	Dose	Results
BJMC3879 (murine mammary adenocarcinoma)	Balb/c	panaxanthone (75%–85% α-MG, 5%–15% γ-MG)	diet	5,000 ppm	suppression of tumor volume and lung metastasis; also, reduction in micro vessel
GBM8401 (human malignant glioblastoma)	nude Balb/cA-V (V/V)	α-MG	intraperitoneal	2 mg/kg/day	Inhibition of tumor growth by half; increased phosphorylation of AMPK; induction of autophagy
NL-17 (murine colon adenocarcinoma)	Balb/c	pericarp methanolic extract (25% α-MG)	intraperitoneal	100–200 mg/kg	50%–70% reduction in tumor mass.

Aside being used in mono-therapy, mangostins can also be used in combination with other chemotherapeutic agents either to increase therapeutic efficacy or to minimize the chemotherapy-induced toxicity (Shan et al., 2011).

Importantly, α-Mangostin exhibits synergistic effect on 5-fluorouracil (5-FU)-induced growth inhibition with another drug in human colon cancer DLD-1 cells at low concentrations (< 5 μmol·L–1) (Nakagawa et al., 2007). Sometimes, patients under chemotherapy use such products, unaware of the effect that may result from combining them with their treatment.

In an animal cancer model conducted in vivo, the consumption of crude α-mangostin (consisting of 78% α-mangostin and 16% γ-mangostin) through the diet had a significant suppressive effect on the formation of aberrant crypt foci, which are considered preneoplastic lesions in rat colon carcinogenesis (Nabandith et al., 2004). Moreover, in more recent findings, we reported that the dietary administration of panaxanthone, a compound consisting of approximately 75% to 85% α-mangostin and 5% to 15% γ-mangostin, with a combined content of both exceeding 90%, effectively inhibited both tumor growth and metastasis in a mouse model of mammary cancer (Doi et al., 2009).

2.7.4 Gastric cancer

Gastric cancer (GC) is the third leading cause of cancer deaths worldwide, with the highest incidence and mortality rates in East Asia. The antitumor effects of α-M

regulate the proliferation and apoptosis of GC cells and have been implicated in diverse activities, including modulation of autophagy (Li & Zeng, 2021).

2.7.5 Pancreatic cancer

Pancreatic cancer is a highly lethal and aggressive form of cancer that lacks effective treatment options. To explore the potential inhibitory impact of α-MG on the viability of pancreatic cancer cells. Xu et al. (Xu et al., 2014) conducted an experiment in which pancreatic cancer cells, specifically BxPc-3 and Panc-1, were exposed to varying concentrations of α-MG (ranging from 0-32 μM) for different durations (6, 12, 24, and 48 hours). The viability of the cells was evaluated using the MTT assay, while apoptosis induction was measured through flow cytometry. Additionally, cell cycle analysis was performed using PI/flow cytometry. Treatment with 32 μM, α-MG resulted in a reduction of cell viability by more than 80% in both types of pancreatic cancer cells. Furthermore, treatment with 16 μM α-MG led to an increase in the population of apoptotic cells by more than 30% in both cell lines. Moreover, when exposed to 8 μM α-MG, the pancreatic cancer cells exhibited significant accumulation in the G1/G0 phase, and treatment with 16 μM α-MG caused the cells to become larger within the G1/G0 phase. These findings indicated that α-MG not only effectively suppressed the activity of pancreatic cancer cells but also preserved the cells' original biological characteristics, which holds significant clinical relevance. In a separate study, the researchers developed mangostin-

encapsulated PLGA nanoparticles (Mang-NPs) and administered them intraperitoneally to KPC mice (around 4 weeks old, males and females). After approximately 10 weeks, the effects of Mang-NPs on stem cell markers (CD24 and CD133), pluripotency maintaining factors (c-Myc, Nanog, and Oct4), components of the Shh pathway, and downstream targets in tumor tissues derived from KPC mice were examined. The results revealed that the populations of stem cell markers and pluripotency maintaining factors were downregulated by Mang-NPs. Additionally, Mang-NPs suppressed the expression of Gli1, Gli2, Patched-1, and Patched-2 in KPC mice. These findings suggested that Mang-NPs hold promise as a potential treatment and/or preventive measure for pancreatic cancer by targeting cancer stem cells (CSCs). The Mang-NPs exhibited the ability to inhibit carcinogenesis by targeting the CSC population and inhibiting the self-renewal capacity of CSCs obtained from human pancreata and KrasG12D mice (Xu et al., 2014).

The research efforts focused on the treatment and prevention of pancreatic cancer have been ongoing. In a recent study, human pancreatic cancer cells TMIA PaCa-2 and PANC-1 were cultured and exposed to different concentrations of **α**-MG or **γ**-MG. After 48 or 72 hours, the cells were subjected to TUNEL assay, Western blotting, and miRNA assay to evaluate the anticancer effects of **α**-MG and **γ**-MG. The findings demonstrated that both **α**-MG and **γ**-MG were capable of reducing the viability of MIA PaCa-2 and PANC-1 cells, inducing apoptosis in these cells, and stimulating

autophagy in pancreatic cancer. Moreover, when MIA PaCa-2 or PANC-1 cells were treated with gemcitabine alone or in combination with α-MG or γ-MG at the IC_{50} concentration for 72 hours, it was observed that α-MG and γ-MG exhibited synergistic effects with gemcitabine, leading to a greater reduction in cell viability of MIA PaCa-2 cells compared to treatment with gemcitabine alone (Kim et al., 2017).

2.7.6 Skin Cancer

For the first time, the researchers investigated the impact of α-MG on DMBA/TPA-induced skin cancer in mice. In the experiment, the mice were administered two doses of α-MG. After 20 weeks, it was observed that the model group exhibited a significant development of skin tumors, whereas no tumors formed in the group treated with α-MG. These findings indicated that α-MG effectively inhibited the formation of skin tumors, resulting in a reduction in tumor incidence rate and multiplicity. Subsequently, the expression of inflammatory factors in both the skin tumors and blood samples using the enzyme-linked immunosorbent assay (ELISA) was analyzed. The results demonstrated a decrease in the expression of pro-inflammatory factors and a significant upregulation of anti-inflammatory factors. Overall, the results indicated that α-MG protected the mice from DMBA/TPA-induced skin tumorigenesis by suppressing inflammation (Wang et al., 2017).

2.7.7 Breast and prostate cancers activity

Mangostins inhibit proliferation of both estrogen receptor (ER)-negative human breast cancer MDA-MB-231 cells and ER-positive human breast cancer MCF-7 cells at micromolar concentrations, resulting in cell cycle arrest in correlation with an increase in reactive oxygen species (ROS) and apoptosis (Ibrahim et al., 2014). Johnson *et al*. (Johnson et al., 2012) have shown that **α**-mangostin significantly reduces the cell viability of human prostate cancer LNCaP, 22Rv1, DU145, and PC3 cells in a concentration-dependent manner with IC_{50} of 5.9, 6.9, 22.5 and 12.7 **μ**M respectively. It also inhibits the growth of 22Rv1 cells in athymic nude mice without obvious toxicity.

The efficacy of **α**-MG as an anticancer compound has been well established for various cancer types. However, its effects and mode of action against lung cancer have not been documented and necessitate additional research. Recent studies have highlighted the growing prevalence of non-small cell lung cancer (NSCLC) in China and South Asian nations. To delve deeper into the mechanism by which **α**-MG acts against lung cancer, Zhang *et al.* (Zhang et al., 2018) conducted further investigations. The non-small cell lung cancer cells (A549) were cultured in DMEM complete medium supplemented with 10% FBS. Various concentrations of **α**-MG (ranging from 0 to 10 **μ**M) were applied to the cultured A549 cells for a duration of 24 hours. A portion of the treated cells was extracted to assess apoptosis induction using

Annexin V-FITC apoptosis kit and flow cytometry. The analysis using Annexin V/PI staining revealed that treatment with 5 μM α-MG resulted in an increase of approximately 18% in apoptotic A549 cells. Furthermore, treatment with 10 μM α-MG led to an apoptosis rate of 38%, accompanied by an elevation in reactive oxygen species (ROS) levels. These findings confirmed the ability of α-MG to induce apoptosis in A549 cells, with ROS playing a crucial role. The remaining α-MG-treated A549 cells were placed in the upper compartment of a Boyden chamber containing serum-free medium. The cells were exposed to a culture medium containing FBS, stained with crystal violet to form a complex, and subsequently dissolved in 10% acetic acid. The absorbance at 600 nm was measured to determine the extent of migration based on the relationship between absorbance and migration. It was observed that treatment with 5 μM α-MG inhibited cellular migration by approximately 33%, while the presence of 10 μM α-MG inhibited migration by 60%. In conclusion, these results suggest that ROS plays a significant role in the cytotoxicity mediated by α-MG in non-small cell lung cancer cells (NSCLC). (Zhang et al., 2018)

2.8 Pharmacological properties of mangostins from mangosteen

Fruits are a fundamental part of our daily diet and are widely recognized as beneficial for health. There is a common belief that consuming more fruits can help lower the risk of certain cancers, such as those affecting the oral cavity, pharynx,

larynx, esophagus, stomach, and lungs. This belief stems from the notion that fruits contain specific active substances that can exert protective effects against cancer when consumed regularly (Grundy et al., 2016). Highlight of pharmacological activities of mangostins and their derivatives from mangosteen are revised. Mahabusarakam *et al*. (Mahabusarakam et al., 2000) have synthesized several **α**-mangostin derivatives and tested their *in vitro* antioxidant activity. They suggested that structural modifications significantly affect their antioxidant activity: For instance, the derivatization of the C-3 and C-6 hydroxyl groups with methyl, acetate, nitrile, or propane-diol significantly decreases antioxidant activity. Also derivatization of C-3 and C-6 with aminoethyl enhances antioxidant activity, in which di-ethylaminoethoxy derivatives exhibit the most potent antioxidant activity. As well as cyclisation of prenyl side chains has little or no influence on antioxidant activity.

2.8.1 Anti-infectious property

Mangostins have been reported to exhibit outstanding inhibitory activities against various pathogenic microorganisms, including multi-drug resistant (MDR) strains, by distinctly different modes of action (Fairhurst et al., 2012).

2.8.2 Antibacterial activity

Sivaranjani et al. investigated the rapid killing efficacy of **α**-mangostin on planktonic cells of S. epidermidis by performing time kill curve assay. Expectedly, **α**-

MG exert rapid concentration-dependent killing of *S. epidermidis* cells at concentrations above 4× MIC (5 g/mL) and 2× MIC (2.5 μg/mL) of α-MG, achieving 6 and 4-log reduction of viable count within 5 min of exposure time, respectively. Less than 2- log reduction of viable counts was achieved while treating with 1× MIC (1.25 g/mL) of α-MG (Sivaranjani et al., 2017). *Staphylococcus epidermidis*, known as a commensal flora on human skin, extends beyond its local homeostasis characteristics and is recognized as a frequent cause of health-related issues. The extracts derived from *Staphylococcus epidermidis* were found to target multiple metabolic pathways, particularly glucose and TCA (tricarboxylic acid) metabolisms. Detecting malate, a metabolite, in the culture medium of the exposed parasite was challenging. The difficulty in detecting malate indirectly suggests that mangosteen blocks TCA metabolism. Furthermore, other researchers evaluated not only the antimalarial activity of mangosteen rind extract but also its interaction with artemisinin against *Plasmodium falciparum* 3D7 in vitro. They diluted these substances with DMSO and examined their antimalarial activity individually or in combination with artemisinin against the *Plasmodium falciparum* 3D7 clone. The conclusions drawn from these studies indicated promising antimalarial activity of the extract and its synergistic effect with artemisinin (Tjahjani, 2017). The antibiofilm activity of α-MG against three strains of *Staphylococcus aureus* was examined. A 96-well plate model was utilized to facilitate biofilm formation at 37°C for 24 hours.

Among the tested strains, one was methicillin-resistant *S. aureus*, while the other two were methicillin-sensitive *S. aureus*. Crystal violet staining to quantify the biomass of the biofilm and utilized confocal microscopy to observe cell viability was employed. The results revealed a significant reduction in biofilm biomass. Additionally, assays using human red blood cells demonstrated that **α**-MG caused significant damage to the cell membranes, resulting in approximately 50% cell lysis at a concentration of approximately 36 mmol/L. These findings indicate that **α**-MG not only exhibited sterilization properties but also inhibited the formation of biofilm (Phuong et al., 2017).

2.8.3 Anti-obesity of **α**-MG

Taher et al. observed that **α**-mangostin could be a potential prevention for metabolic diseases such as obesity particularly the type 2 diabetics. On one hand, **α**-MG decreased intracellular fat accumulation significantly (up to 44.4% relative to MDI-treated cells) via decreasing PPAR**γ** expression (Taher et al., 2015). Additionally, the ability of **α**-MG to combat obesity further confirms its potential in ameliorating metabolic disorders (Choi et al., 2015).

2.8.4 Inhibition of angiogenesis

Wihastuti et al. (Wihastuti et al., 2014) demonstrated that extract of mangosteen pericarp promotes the formation of anti-angiogenesis through H_2O_2, HIF-1**α**, and NF-**κ**B and that inhibition of iNOS in rats gives a high-fat diet. **α**-MG in a

study inhibited the proliferation, migration, and tubule formation of human umbilical vein endothelial cells (HUVECs) (Shiozaki et al., 2013). α-Mangostin mitigates oxidative stress and restricts VEGF-induced angiogenesis by interfering with the activation of VEGFR2 and ERK1/2-MAPK pathways (Jittiporn et al., 2014).

2.8.5 Antimalarial of α-mangostin

Chaijaroenkul *et al*. (Chaijaroenkul et al., 2014) investigated the antimalarial activities of crude ethanolic extract of mangosteen together with α-MG and β-MG. They found that the extracts targeted several metabolic pathways, especially glucose and TCA metabolisms. Again, antimalarial activity of mangosteen peel extract was evaluated together with the interaction with artemisinin against the *Plasmodium falciparum* 3D7 *in vitro* and then examined the antimalarial activity, either singly or in combination with artemisinin *in vitro* against *Plasmodium falciparum* 3D7 clone. The results concluded that the antimalarial activity of the extract and its synergistic effect with artemisinin is greatly promising (Tjahjani, 2017).

2.8.6 Antiviral of α-mangostin

The treatment of Dengue virus - DENV-infected cells with varying concentrations of α-MG was carried out. After some period, as compared to the untreated DENV-infected cells, treatment of α-mangostin (10–20 μM) significantly

reduces the number of infected cells. At least, 87% cell viability was observed in all the conditions used (Tarasuk et al., 2017).

2.8.7 The Efficacy of α-mangostin on Microbes

Expectedly, in a study by Phitaktim et al. (2016) (Phitaktim et al., 2016), α-mangostin put up synergistic effect with an antibiotic- oxacillin, tested on oxacillin-resistant *S. saprophyticus*. It was observed that the growth of the bacterial strain was restricted by the isolated α-mangostin in conjugation with the antibiotics. Nittayananta *et al.* (2018) (Nittayananta et al., 2018) extended the use of α-mangostin, a common antimicrobial agent used against oral pathogens in oral sprays. The compound was evaluated against *Candida albicans*, *Streptococcus mutans*, and *Porphyromonas gingivalis*, α-mangostin restricted the growth of these microbes without causing cytotoxicity. Chokpaisarn et al. (2019) (Chokpaisarn et al., 2019) reported the use of Ya-Samarn-Phlae, a traditional Thai medicine which contains mangosteen, together with *Curcuma longa*, *Oryza sativa*, and *Areca catechu* against *Pseudomonas aeruginosa*. It showed a high level of inhibition of the bacteria. Five xanthones were isolated from *C. cochinchinense* and mangosteen and they were tested for antibacterial activity against MRSA and *P. aeruginosa,* α-mangostin exerted the highest antibacterial activity, even though showed poor pharmacokinetic properties (Boonnak et al., 2020). Since there can be little or no effect cases for all

drugs, It was suggested that at times α-mangosteen should be used in a conjunction with any commercial antibiotic agent to maximize its antimicrobial property and potency (Sakagami et al., 2005).

2.8.8 Anti-inflammatory activity of α-MG

Liu et al. investigated the anti-inflammatory activity of α-MG by treating the U937 and EL4 cells in the lipopolysaccharide (LPS) with different concentrations of α-MG sample for 4 hours. The anti-inflammatory effects were observed by the levels of tumor necrosis factor (TNF)-α and interleukin (IL)-4 in cell culture media that were determined with ELISA kits. The results showed that α-MG did not only diminishes the LPS expression of the inflammatory cytokines TNF-α and IL-4 but also decreases the gene expressions in oncostatin M signaling through the MAPK pathways.(Liu et al., 2012)

2.8.9 Modulation of Pro-Apoptotic, Anti-Proliferative and Anti-Metastatic Signaling Pathways by Xanthones

Recent reports have delved into the mechanisms underlying the anti-proliferative and pro-apoptotic effects of xanthones in cultured cells. These findings reveal that mangosteen xanthones exert their pro-apoptotic effects by activating the caspase cascade signaling in various cell types. Additionally, they have been found to disrupt the mitochondrial membrane potential and induce the release of cytochrome c from mitochondria into the cytoplasm. The effects of xanthones on

other signaling pathways, such as ERK1/2 and JNK1/2, have yielded somewhat controversial and cell type-dependent results, either stimulating or inhibiting their activation. In relation to cell survival, α-MG has been observed to downregulate the levels of p-Akt, a protein kinase associated with cell survival. Furthermore, the anti-proliferative activity of α-MG in colorectal cancer cells has been attributed to its inhibition of TCF/β-catenin transcriptional activity mediated by xanthones. While less is known about the effects of mangosteen xanthones on the cell cycle, several studies have demonstrated cell cycle arrest at the G1 phase and downregulation of cyclins. Regarding anti-metastatic potential, mangosteen xanthones have been shown to inhibit the activities of matrix metalloproteinases (MMPs), leading to reduced adhesion, invasion, and migration of cancer cells treated with α-MG. This suppressive effect is associated with the inhibition of IκBα degradation and activation of the αvβ3 integrin/FAK/ERK pathway, which serves as a major upstream regulator of NF-κB and impedes its nuclear translocation (Gutierrez-Orozco & Failla, 2013).

2.9 Mangosteen as a healthy food supplement

In 2004, following a significant decline in the popularity of food supplements in the US market, there was a notable surge in the sales growth of botanical products known as "superfruits." This led to the emergence of a new generation of health products. Network marketing companies played a key role in establishing a large global niche market for liquid botanical supplements, which are now being sold in

more traditional beverage forms through retail channels. The term "liquid botanical supplements" was coined to describe convenient products, primarily juice blends made from exotic fruits, that were believed to offer health benefits based on the traditional usage of the original plants. This term was used to convey the alleged health claims associated with these juice products. This approach was influenced in part by the previous trend in the US supplement market, where antioxidant supplements like green and black tea, grape seed extracts, and polyphenol-rich juices gained popularity. The success of incorporating traditional Western fruits such as elderberries, blueberries, and black currants led to an increasing influx of exotic and foreign fruits into the herbal and botanical supplements market, particularly in 2004 and 2005. This period witnessed a strong rise in the sales of liquid supplements such as noni, mangosteen, and goji juice. In 2006, among the best-selling supplements in the US market, mangosteen juice claimed the 22nd position and surpassed the sales figures of green tea, as reported by NBJ's Supplement Business Report in 2007. The growth rate for mangosteen juice was remarkable, with a 200% increase in 2004, followed by 73.5% in 2005, and a more modest 18% in 2006, resulting in a total sales volume of 147 million USD. The dissemination of "health messages" surrounding these "novel superfruits" was predominantly carried out by direct sales and multi-level marketing companies, which played a crucial role in driving the substantial turnover of this market category. The network structure of these companies allowed them to command premium pricing for their products.

Mangosteen juice was initially introduced to the global market in 2003 by a food supplement manufacturer, which has since maintained a significant market share of 80% within the mangosteen juice category, despite increasing competition from other companies. In 2006, the company expanded its export operations to Europe and Asia, in addition to the established markets in the USA, Japan, Canada, Mexico, and Australia. As a result, mangosteen juice is now available in 16 international markets.(Obolskiy et al., 2009)

Again, **α**-mangostin compound derived from the Garcinia genus, when included in the diet, exhibited anticancer and antiproliferative characteristics in various types of cancers including leukemia, prostate cancer, breast cancer, colorectal cancer, and brain cancer (Chao et al., 2011).

2.10 Toxicity of *G. mangostana*

In a study conducted by Pongphasuk et al. (2005) (Pongphasuk et al., 2003), they examined the toxicity effects of G. mangostana by administering plant extracts orally to mice. Their findings revealed that a dose of 9.37 g/kg of mouse weight was required to achieve a lethal dose for 50% of the mice (LD_{50}). Surprisingly, when the extract was administered at a concentration of 20 g/kg, no mortality was observed in the mice. However, when examining the chronic toxicity of the extract in mice, the study discovered that at concentrations of 2, 4, and 8 g/kg/day, it resulted in mortality rates of 15%, 17%, and 43%, respectively. Although, the results of this

study appear to be inconclusive. Following the administration of mangostin, obtained from the extract of the pericarp of the mangosteen fruit, into mice at a dosage of 200 mg/kg, a reduction in the levels of the enzymes glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) was observed after a 12-hour interval.(Sunarjo et al., 2017) In a research investigating the possible mutagenic properties of G. mangostana extract dissolved in 50% methanol, it was determined that the extract did not exhibit any mutagenic effects in Salmonella typhimurium strains TA98 and TA100. However, intriguingly, the study also revealed that the methanol extract of mangosteen demonstrated antimutagenic activity in Salmonella typhimurium strains TA98 and TA100 (Obolskiy et al., 2009). However, the hot-water extract of mangosteen showed no antimutagenicity in TA98 at all. (Chanprechakul, 2000)

2.11 DPPH Assay

Briefly, isolated band A from TLC separation was dissolved in methanol to various concentrations (0, 2.5, 5, 10, 20, 50, and 100 μg/mL). To 100 μL of extract solution, 100 μL of fresh 0.4 mM DPPH solution in methanol was added. The experiment was conducted in a 96-well plate (n=3 independent samples). The reaction was incubated in darkness at room temperature for 30 min. The absorbance was measured at 517 nm by microplate reader. Vitamin C was used as positive control. The degree of discoloration indicates the scavenging potential of the antioxidant

property. The scavenging activity was calculated by [1 – ($\square_1$ – blank)/$\square_0$ – blank] × 100, where $\square_1$ was the absorbance of DPPH in the presence of the extracts, $\square_0$ represented the absorbance of DPPH solution without extract addition, and blank was the absorbance without DPPH solution (Suthammarak et al., 2016).

CHAPTER 3

METHODOLOGY

3.1 General experimental procedures

Crude extracts of both EtOAc and MeOH were subjected to column chromatography using a range of solvent systems of hexane, ethyl acetate and methanol. The fractions collected were grouped and combined based on their TLC profiles for subsequent isolation. ^{1}HNMR spectra, and the ^{13}C NMR spectra were recorded at 500 MHz on a JEOL JNM-ECZ500R/S1 spectrometer in which the chemical shift values in ppm were recorded with respect to the deuterated solvent shift ($CDCl_3$, δ 7.26 for the proton resonance and δ 77.0 for the carbon). Silica gel (200–300 mesh) (Merck) was used as solid phase for column chromatography. Trolox was from EMD chemicals, San Diego, CA, USA. DPPH was from Sigma-Aldrich, Germany, D9132-1G, Pcode: 102015127. All the solvents for column chromatography were distilled prior to use.

3.2 Plant Materials

Fresh pericarps of mature mangosteen fruit (*Garcinia mangostana*) were obtained from organic farm in Chantaburi province, Thailand. The mangosteen pericarps were extracted fresh (no form of drying). The dried mangosteen pericarps were purchased from TPC herb, Thailand.

3.3 Extraction of the fresh pericarps of mangosteen

The fresh pericarps of mature mangosteen fruit (2 kg) were thoroughly washed and crushed into smaller form. The crushed pericarps were then extracted by maceration with ethyl acetate (5 L x 3) three times by maceration for 96 hours and then with methanol (3 L x 3). The extracts were filtered and evaporated under reduced pressure using rotary evaporator to get crude EtOAc extract (40 g) and MeOH extract (5 g).

3.4 Isolation & purification of chemical constituents

The ethyl acetate extracts (40 g) were subjected to silica gel column chromatography eluted with hexane: ethyl acetate in stepwise fashion from 9:1 to 7:3 ratio, while the fraction F_3 was subjected to further isolation eluting with 7:3 ratio of Hex:EtOAc. Similarly, further isolation of F_7 to F_{10} on silica gel column chromatography eluting with 7:3 ratio of Hex:EtOAc. Further purification of F_{10} (8-9) using TLC separation developing with 7:3 ratio of Hex:EtOAc. Similarly, the MeOH extracts (5 g) were subjected to silica gel column chromatography eluted with hexane: ethyl acetate in 7:3 ratio. All compounds were kept under reduced pressure, in a clean container containing silica gel desiccant at room temperature. Dimethyl Sulfone was used as an internal standard in checking the purity of the compounds and quantifying the amount of compound(s) in both fresh and dried mangosteen pericarp.

3.5 Extraction of the dried pericarps of mangosteen

Two separate extraction methods (ultrasonic-assisted extraction & maceration), were employed to extract the dried pericarps using MeOH, EtOH & EtOAc as solvents. 1g each of dried pericarp powder was macerated separately with 20 mL (twice) of each of the solvents to get extracts. Also, 1g each of dried pericarp powder was macerated with ultrasonic assistance separately with 20 mL (twice) of each of the solvent to get extracts. The separate extractions were carried out two times to optimize the extraction yield. The percentage yield was calculated as follows.

$$\text{Percent yied} = \frac{\text{mass of compounds}}{\text{mass of crude mangosteen extract}} \times 100$$

3.6 Juxtaposition of extractions of the fresh and the dried pericarps of mangosteen

Two separate extraction methods (ultrasonic-assisted extraction & maceration), were employed to extract the fresh pericarps using MeOH, EtOH & EtOAc. Then, 1g each of fresh pericarp powder was macerated separately with 20 mL (twice) of each of the solvents to get extracts. Also, 1g each of fresh pericarp powder was macerated with ultrasonic assistance separately with 20 mL (twice) of each of the solvent to get extracts.

The separate extractions were carried out two times to optimize the extraction yield. In which the results of extraction were recorded as an amount (in mg) of 1g of dried and fresh mangosteen pericarp.

3.7 The extraction of moisturized dried pericarps (imitated-fresh)

The dried pericarp powder (10 g) was moisturized by soaking with 20 mL, 50 mL and 90 mL water for 24 hr and then macerated separately with EtOAc (20 ml x 2) for 18 hr to yield crude extract of moisturized-dried mangosteen pericarps. This is done to imitate the fresh pericarps, to juxtapose the effect of soaking the dried mangosteen pericarps in different measure of water content. The results gotten from this extraction were subjected to qNMR analysis together with those of the fresh and dried mangosteen pericarps.

3.8 q-NMR Analysis

The qNMR analysis was done in quantifying the contents of the crude extracts using Dimethyl sulfone as the internal standard. An appropriate ration of the internal standard (IS) and sample was weighed and checked for their ^{1}HNMR for analysis. The chemical shift of the methyl on the of the internal standard at about 2.98 ppm was employed. The formula used for the calculation is as follows.

$$\%P = \frac{I_t}{I_{is}} \times \frac{N_{is}}{N_t} \times \frac{MW_t}{MW_{is}} \times \frac{m_{is}}{m_t} \times 100$$

For the determination and juxtaposition of the α-mangostin and gartanin contents in fresh, imitated-fresh and dried mangosteen pericarps since β-mangostin was negligible. The integration of the δ_H of the methoxy at C-7(OMe) in α-mangostin and hydroxyl at C-1(OH) in gartanin were employed to estimate the amount of α-mangostin and gartanin in both fresh and dried mangosteen pericarp. The result gotten gotten for the fresh pericarp extract is compared with that of the dried pericarp. Instrument set up was relaxation delay (T_1) 2.4 s and D_1 is 16.8 s.

3.9 DPPH Radical Scavenging Activity

This was carried out as described by Susy *et al.*(Tjahjani et al., 2014) The radical scavenging activity of each extract and the purified alpha-mangostin, beta-mangostin and gartanin were determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. DPPH solution was prepared by dissolving 7.6 mg of DPPH in methanol (50ml), and subsequent dilution to get 40 μM. Trolox was used as the positive control in this assay. The concentration of Trolox used in the assay, are 20, 10, 5, 2.5 & 1.25 μM. The radical scavenging activity of the compounds and extracts was determined by the ratio of DPPH absorption decrease against the absorption of DPPH solution in the absence of test sample (negative control) and consequently the IC_{50} of each sample determined. To obtain the IC_{50} value, 50 μL of each solvent extract and the three purified compounds in MeOH at final concentrations 250, 50,

10, 2, and 0.4 μg/mL were added into 96 well plates, after which 200 μL of 40 μM DPPH (Sigma Aldrich) in methanol was added into the test solutions, each reaction mixtures were shaken and kept in the dark for 30 minutes at room temperature. Eventually, DPPH scavenging activity was determined on microplate reader at 517nm by giving corresponding absorbance.

$$Scaveninge(\%) = \frac{Ac - As}{AC} x100$$

A_S: absorbance of samples, A_C: negative control absorbance (without sample)

3.10 MTT Assay for anticancer activities

The test for anticancer activity of the isolated pure compounds was carried out by employing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) assay method as done by Ahung *et. al* (Aung et al., 2021). The neuroblastoma SH-SY5Y Cancer cell line was cultured in a 96 well plate at 2.13 x 10^4 cell/well density. The stock solution of each of the pure compounds were prepared dissolving in 0.1 M of DMSO, after which 5 times serial dilutions to get 5, 1 and 0.2 μg/ml). The 100 ml were treated with the cell at 37°C in a CO_2 incubator for 24 hours. Purple formazan crystals were formed after 100 ml of MTT reagent (50 mg in 10 ml phosphate buffered saline) was filled in each of the wells and incubated in CO_2 incubator for 2-4h. The formed formazan was proportional to the total number of viable cells that

read its absorbance value at the wavelength 560 nm using ELISA (Enzyme-Linked Immunosorbent Assay) reader.

The percentage cell viability and IC_{50} value were calculated and determined using Microsoft excel and equation of a straight line (y=mx+c) respectively. The formula used is given below:

$$\% \text{ Cell viability} = \frac{\text{Absorbance of treatment} - \text{Absorbance of media control}}{\text{Absorbance of negative control} - \text{Absorbance of media control}} \times 100\%$$

CHAPTER 4

Results & Discussion

4.1 Compounds from fresh mangosteen pericarps

The fresh pericarp of mature mangosteen pericarp is a relatively new idea of producing xanthones, mostly mangostins. The ethyl acetate extracts (40 g) was subjected to silica gel column chromatography eluted with hexane: ethyl acetate in stepwise fashion from 9:1 to 7:3 ratio to give 15 combined fractions (F_1 to F_{15}). The fraction F_3 was subjected to further isolation eluting with 7:3 ratio of Hex:EtOAc to afford gartanin (**3**) (3.03 g), while further isolation of F_7 to F_{10} on silica gel column chromatography eluting with 7:3 ratio of Hex:EtOAc gave **α**-mangostin (**1**) (13.3 g). Further purification of F_{10} (8-9) using TLC separation developing with 7:3 ratio of Hex:EtOAc gave **β**-Mangostin (**2**) (90.1 mg). In the same vein, MeOH extracts (5 g) was subjected to silica gel column chromatography eluted with hexane:ethyl acetate in 7:3 ratio to give **α**-mangostin (**1**) (1.62 g). All compounds were kept under reduced pressure, in a clean container containing silica gel desiccant at room temperature.

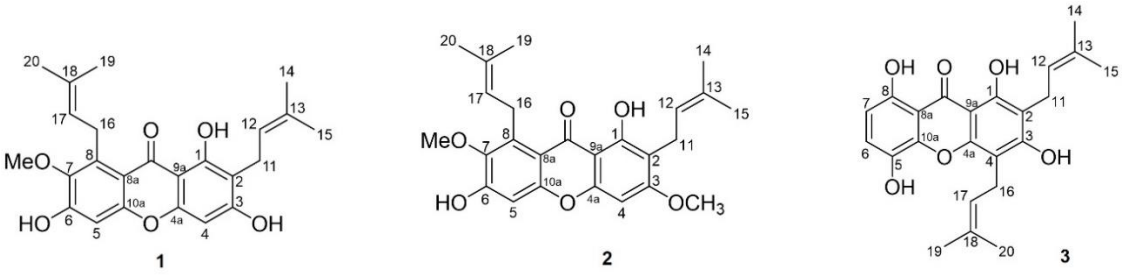


Figure 4.1. Structures of alpha-mangostin (1), beta-mangostin (2) and gartanin (3) isolated from the fresh pericarp of *G. mangostana.*

The identification of the compounds was solemnly based on the comparison of their spectroscopic data to those of reported data in literature particularly ^{1}H and ^{13}C NMR. For compound (1), it was identified as the well-known α-mangostin. The ^{1}H-NMR spectrum of compound 2 was just similar to that of α-mangostin (1), except for the fact that instead of having a single methoxyl group (OCH_3) it showed two signals of methoxy at δ 3.78 (3H, s) and 3.83 (3H, s) (d, ppm, multiplicity). The ^{1}H-NMR spectrum of compound 2 showed four singlet signals of methyl protons as shown in *appendix L*, which is corresponding to those of prenyl group having carbon signals at C14 (25.9, 3H, s), C15 (, 3H, s), C19 (, 3H, s) and C20 (25.9, 3H, s) (d, ppm, multiplicity), as well as its 2 aromatic protons δ 6.52 (1H, s, H4) and δ 6.87 (1H, s, H5) similar to those of α-mangostin (1). In essence, compound (2) was identified as the known β-mangostin. The ^{1}H-NMR of compound 3 showed the presence of two aromatic protons at δ 7.22 (1H, d, J 7 Hz, H7) and 6.63 (1H, d, J 7 Hz, H8) which coupled to each other with coupling constant 7 Hz of both signals indicated the presence of ortho-coupling of H6 and H7. Also, the presence of two prenyl groups were observed by the signals of 4 methyl groups at δ 1.76 (3H, s), 1.79 (3H, s). 1.86 (3H, s) and 1.86 (3H, s). There was no presence of methoxy signal observed. Additionally, with their ^{13}C chemical shifts, the compounds 1, 2 and 3 were confirmed as α-mangostin, β-mangostin, and gartanin respectively. All the signals were consistent with chemical

shifts of each signal in literature for all compounds as shown in appendix L (Anggia et al., 2015).

4.2 Ultrasonic-assisted extraction and Maceration of the dried and fresh mangosteen pericarps

The extraction of the dried pericarp of mangosteen was carried out with MeOH, EtOH and EtOAc as solvents using maceration with and without ultrasonic assistance. The variation that was recorded in the yield of extracts from these solvents could be attributed to the contrasting polarity and solubility characteristics of the pericarp and the solvents used (Bundeesomchok et al., 2016). Extraction yield was determined as the quantity from 1g of mangosteen pericarp extracted by each solvent for both extraction methods.

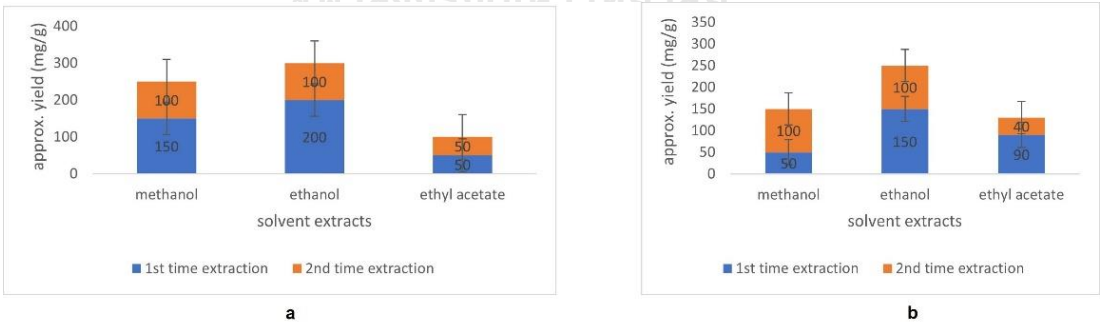


Figure 4.2. Crude extract yield from the dried pericarp of *G. mangostana* using (a) Maceration (b) Ultrasonic-assisted extraction.

However, the extraction in duplicate with MeOH, EtOH and EtOAc separately for the dried pericarp and their average yields resulted in 14.05%, 17.6%, 8.2% for maceration and 9.3%, 14.9% 9.6% for ultrasonic-assisted extraction, respectively. Similarly, two separate extraction methods (ultrasonic-assisted extraction & maceration) of the fresh pericarps using MeOH, EtOH and EtOAc, resulted in average yields of 13.5%, 13.0%, 9.75% for maceration, and 13.1%, 14.9%, 9.6% for ultrasonic-assisted extraction, respectively.

The three non-toxic solvents used in the extraction of the dried and the fresh pericarps were able to extract bioactive substances with high yields. Additionally, those solvents possess relatively low volatility factor and do not leave excess residue in the extracts. Two times extraction was necessary in order to improve the extraction yields. The solvents resulted in a high tendency to extract mangostin faster, that of ethanol has been confirmed than NADES ChCl-1,2- propanediol (1:2) (Mulia et al., 2015). The three solvents have a relative low viscosity, therefore, with the kinetic energy resulted from the ultrasonic, intermolecular bonding within the three solvents active groups were more easily broken to form new bonds with mangostin active groups since low viscosity solvents normally have higher diffusion coefficients which will consequently increases the rate of extraction. In essence, xanthones generally are naturally insoluble in water and therefore difficult to extract with water. Xanthones were far soluble in organic solvents with a moderate polarity such as acetone, ethanol, methanol, and ethyl acetate.(Chhouk et al., 2016).

4.3 Extraction of moisturized dried pericarps (imitated-fresh)

Vividly, the moisture content influenced the extraction of the dried mangosteen pericarp. The yield increased with an increment in moisture content available. The highest percentage yield at 15% obtained by moisturizing 10 g of dried pericarp with 90mL moisture followed by extraction with EtOAc (50ml). Consequently, the **α**-mangostin and gartanin contents of the extracts was approximately 8.32% and 1.36% respectively. This may also informed the reason the decoction of pericarps of *G. mangostana* was reported to be active against *Escherichia coli*, *Vibrio cholerae* and *Salmonella typhi*, the crude water extract was also active against *Streptococcus faecalis*, and *Vibrio cholerae* (Suksamrarn et al., 2003).

4.4 **α**-Mangostin and gartanin contents

All solvents extract of the fresh and those of the dried pericarp were dried in dessicator for 48 hr after which there was utilization of a recommended internal standard (Dimethyl sulfone) for calibration in qNMR to check the **α**-mangostin and by extension gartanin content in the extract of the fresh, and dried mangosteen pericarps including the moisturized dried (otherwise called immitated-fresh). To determine the specific amounts of extract and internal standard to be weighed and added together, the ^{1}HNMR spectra of the extracts were first investigated. It is important to note that the exact amounts of extract and internal standard may vary

for each analysis, and it is advisable to optimize the sample preparation accordingly with care. The α-mangostin and gartanin in the extract of the fresh, moisturized dried (otherwise called immitated-fresh), and dried mangosteen pericarps were shown in Table 4.1

Table 4.1. The α-mangostin and gartanin content (in % w/w) of the fresh and dried mangosteen pericarps in MeOH, EtOH and EtOAc using UAE and maceration.

pericarp	method	MeOH		EtOH		EtOAc	
		α-MG	gartanin	α-MG	gartanin	α-MG	gartanin
Fresh	UAE	16.6%	-	18.56%	-	19.38%	-
	maceration	10.84%	-	12.86%	-	11.70%	-
Dried	UAE	14.39%	0.73	14.4%	0.68%	18.4%	0.96%
	maceration	8.7%	-	7%	0.37%	11.15%	0.78%

Presented as % w/w of the extracts.

The chemical shift of the internal standard (dimethyl sulfone) at 2.98 ppm was key in estimating the α-mangostin and gartanin content of the extracts through equation shown in page 66.

The variation in the extracts of the fresh and dried mangosteen pericarps could arise due to the methods and condition of drying which induced chemical reactions that probably affected the concentration of α-mangostin. Since if care is not taken, prolonged exposure to high temperatures while drying can lead to the degradation of α-mangostin, and other compounds or an entirely new compound can even be formed.

Table 4.2. The α-mangostin and gartanin content (in mg/g) of the fresh and dried mangosteen pericarps in MeOH, EtOH and EtOAc using UAE and maceration.

pericarp	method	MeOH		EtOH		EtOAc	
		α-MG	gartanin	α-MG	gartanin	α-MG	gartanin
Fresh	UAE	13.6 mg	-	21.9 mg	-	15.31 mg	-
	maceration	13 mg	-	12.86 mg	-	7.02 mg	-
Dried	UAE	5.18 mg	0.26 mg	6.9 mg	0.32 mg	11.4 mg	0.59 mg
	maceration	2.68 mg	-	3.64 mg	0.19 mg	7.14 mg	0.5 mg

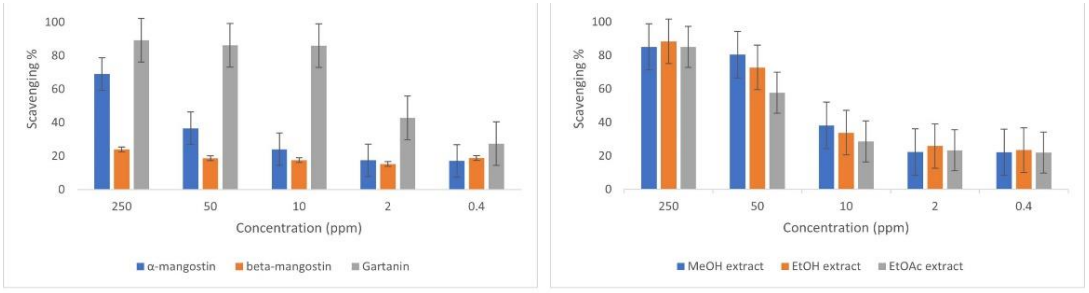
Presented as mg/g of the fresh and dried pericarps.

The amount of **α**-mangostin in mg per 1 g of the fresh and dried mangosteen pericarps was presented in Table 4.2. The amount of **α**-mangostin revealed that the extracts from the EtOAc of both the fresh and dried pericarps contains significant quantity of **α**-mangostin for both fresh and dried mangosteen pericarps, as well as those of EtOH in fresh pericarp. The fresh pericarps extract in all the three solvents generally showed more contents of **α**-mangostin than those of their dried counterparts. However, the amount of gartanin in the extracts of the fresh is infinitesimally small.

The availability of **α**-mangostin from the fresh mangosteen pericarps in small scale system is being favored as compared to its production in large-scale system of isolation. Since the former has **α**-mangostin content in a range of 7 mg/g as the lowest and about 15 mg/g as the highest in EtOAc, while the latter **α**-mangostin content was about 7.5 g/kg.

4.5 Antioxidant Scavenging Activity

The antioxidant scavenging activities of the compounds and extracts were determined in DPPH assay which is considered one of the most widely used methods for measuring the antioxidant activity of various chemical compounds. Radical scavenging activity is one of the recognized mechanisms of action of antioxidant compounds, it occurs that the reactive free radical abstracts the H atom from the antioxidant(Hawash et al., 2022) and IC_{50} shown in Table 4.3.



a b

Figure 4.3. Antioxidant scavenging activities in DPPH assay (a) α-mangostin, β-mangostin and gartanin (b) The extracts.

There is appreciable antioxidant scavenging activities for compound (1) and compound (2) in which can be faintly regarded as α-Mangostin (**1**), β-mangostin (**2**) and gartanin (**3**) isolated from the fresh pericarp of mature mangosteen (*Garcinia mangostana* Linn.) were investigated for their relative antioxidant activities in DPPH assay. Significant antioxidant activity was shown by α-mangostin (**1**), as well as gartanin (**3**) in DPPH assay. In the interaction of an antioxidant with DPPH, in this case being a phenolic compound or even other free radical scavenging compound, it undergoes a reduction reaction. The antioxidant substance donates an electron or a hydrogen atom to the DPPH radical, which in turn neutralizes its unpaired electron and converts it into a stable, non-radical form usually yellowish in color. Precisely, the reduction was visible with a color change from deep violet to light yellow. This color change is easily measurable using spectrophotometry, and the degree of discoloration is directly proportional to the antioxidant activity of the substance

being tested. The more effective an antioxidant is at scavenging free radicals, the greater the reduction of DPPH and the stronger the discoloration observed.

4.6 Antioxidant scavenging activity in DPPH assay.

The compounds isolated from the fresh pericarps of mature mangosteen were investigated for their relative antioxidant scavenging activities to reduce 2,2-diphenyl-1-picrylhydrazyl DPPH radical, which will be evident by the change in colour from purple to yellow and consequent reading on microplate reader at 517 nm wavelength to record the absorbance. Table 4.3 shows the relative antioxidant scavenging inhibition at 50% concentration better known as IC_{50} for each compound, which are IC_{50} of 322.57, >589 and 8.42 μM respectively for α-mangostin, β-mangostin and gartanin.

The dried pericarp crude extracts from the MeOH, EtOH & EtOAc solvents were also investigated for their antioxidant scavenging activities, they have IC_{50} of 21.142, 26.59 and 39.38 μg/ml respectively as equally shown in Table 4.3.

The relatively lower IC_{50} values exhibited by the solvent extracts which indicates more activity are intriguing and could possibly predict a synergistic effect of α-mangostin and gartanin, since both are present in each solvent extract.

Table 4.3. 50% Inhibitory Concentrations (IC_{50}) of positive control (Trolox), compounds and extracts.

sample	IC_{50} (μg/ml)	IC_{50} (μM)
Trolox	1.32	5
α-mangostin	132.40	322.57
β-mangostin	>250	>589
gartanin	3.34	3.34
MeOH extract	21.14	-
EtOH extract	26.59	-
EtOAc extract	39.38	-

The occurrence of two hydroxyl groups at the C-5 and C-8 positions in compound (3) was consistent with its potent antioxidant scavenging effect while that of compound (1) could be assigned to the fact that it possesses hydroxyl groups at positions C-1, C-3, and C-6.

4.7 Anticancer activities

Mangosteen has been reported to exhibit a wide range of cytotoxic activity in various cancer cell lines. The underlying mechanisms of cell cytotoxicity included the induction of cell apoptosis, necrosis, autophagy, cell cycle arrest, cell mobility alterations, and modulation of ER stress(Tahir et al., 2022). Equally, the three compounds were investigated for their anticancer activities against SH-SY5Y neuroblastoma cancer cell lines and were evaluated in MTT (3-(4,5-dimethylthiazol-

2-yl)-2,5- diphenyltetrazolium bromide) assay. The formation of purple formazan crystals indicates the activity of the compounds. While the anticancer activities of the compounds have IC_{50} of 9.0, 8.4 and 7.0 μM respectively. The anticancer activities in MTT assay using medium control are shown in Figure 4.4.

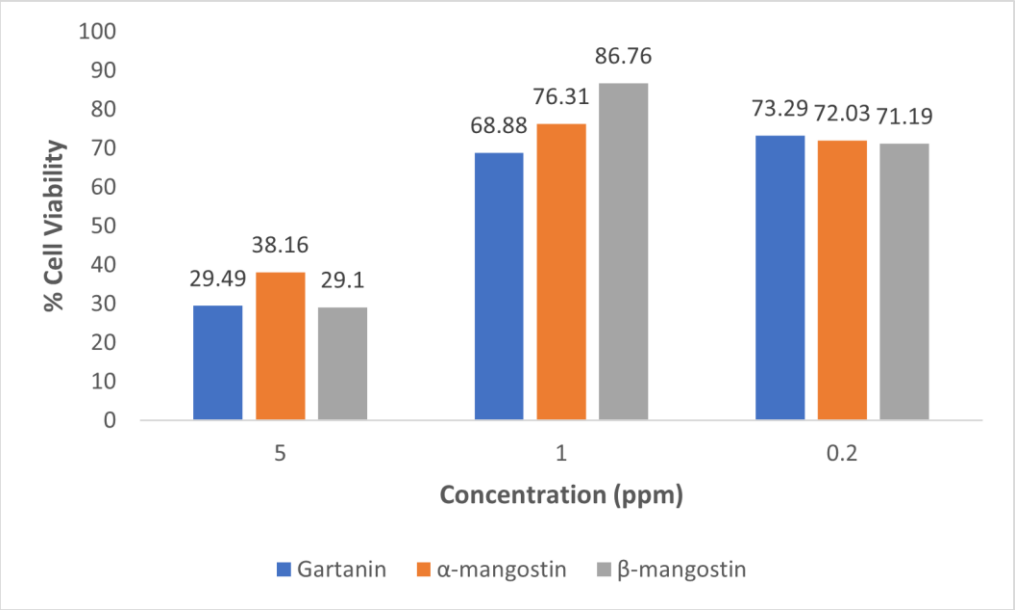


Figure 4.4. Anticancer activities in MTT assay for α-mangostin, β-mangostin and gartanin.

4.8 Cell viability and IC_{50}

The neuroblastoma cell line viability after treatment in MTT assay is given in fig 4.5.

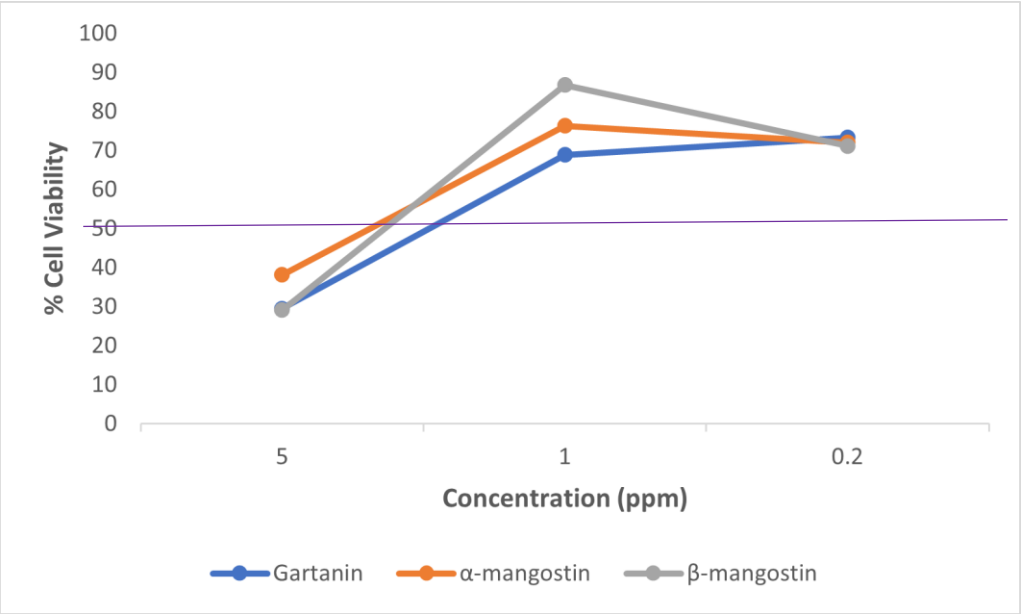


Figure 4.5. Graph of anticancer activities in MTT assay for isolated pure compounds.

The neuroblastoma cancer activities of the compounds were investigated with SH-SY5Y cancer cell line since there is restriction in employing embryonic central nervous system tissue-derived primary mammalian neurons lies in the fact that these cells, once fully developed into mature neurons, cannot be further reproduced. It has the capacity to differentiate into a neuron-like cell. α-Mangostin, β-mangostin and gartanin showed appreciable activity against the neuroblastoma SH-SY5Y cancer cell lines.

Table 4.4. 50% Inhibitory Concentrations (IC_{50}) of isolated pure compounds.

Sample	IC_{50} (μg/ml)	IC_{50} (μM)
Gartanin	2.91	7.0
α-mangostin	3.758	9.0
β-mangostin	3.55	8.4

In a nutshell, we isolated α-mangostin (**1**) (13.3g) as the major compound, together with β-mangostin (**2**) (90.1mg) and gartanin (**3**) (3.03g) from the fresh pericarp of mangosteen extract (40g). Extraction yield from the dried and even the fresh pericarp of mangosteen is partly dependent on the solvent, and on the method of extraction as shown by both maceration and ultrasonic-assisted extraction yields for the three solvents (MeOH, EtOH and EtOAc). The estimated α-mangostin content in fresh and dried pericarps varied in different solvents and methods with ultrasonic-assisted extraction of EtOAc having the highest value of 19.38%. Gartanin (3) showed the most antioxidant scavenging activity in DPPH assay. There seems to be a synergistic antioxidant activity of α-mangostin and gartanin in each of the solvent extract as their IC_{50} values are much lower than that of α-mangostin only which is the major

compound of the extracts. The chemical constituents isolated are α-mangostin (33.25% w/w of crude extract), β-mangostin (0.225% w/w of crude extract), and gartanin (7.57% w/w of crude extract). The compounds were active against neuroblastoma SH-SY5Y cancer cell line. Structures of these compounds were identified by spectroscopic data and compared with those in literature.

CHAPTER 5

CONCLUSION

This study examined the isolation of chemical constituents from the fresh pericarps of mangosteen fruit. We examined the antioxidant scavenging activities of α-mangostin (1), β-mangostin (2) and gartanin (3) in DPPH assay having IC_{50} of 322.57, >589 and 8.42 μM respectively, including those of solvent extracts from the dried mangosteen pericarp. And the anticancer activities of three compounds having IC_{50} of 9.0, 8.4 and 7.0 μM respectively. This study as well, juxtaposed the crude extract yields from the fresh and dried mangosteen pericarps by two distinct methods. By maceration and ultrasonic-assisted extraction in MeOH, EtOH & EtOAc solvents. Consequently, portraying fresh pericarp as a better option for isolation as its extract contains more content of the isolated compounds. Since the highest yield of an extract and the highest content of α-mangostin from the dried pericarp of mangosteen is 26.60% dry weight and 46.21% w/w of crude extract respectively (Ghasemzadeh et al., 2018). The estimated extraction average percentage yield from the fresh and dried pericarps in MeOH, EtOH and EtOAc solvents are 13.5%, 13.0%, 9.75% and 14.05%, 17.6%, 8.2% respectively for maceration while those of Ultrasonic assisted are 13.1%, 15.9%, 11.45% and 9.3%, 14.9%, 9.6% respectively as an average of two replicates, which was determined by the amount in mg in 1g of dried and fresh mangosteen pericarp for two-times extractions. The solvent extracts of the fresh pericarps as well as those of the dried pericarps were investigated most

especially for their α-mangostin content via qNMR. The availability of α-mangostin from the fresh mangosteen pericarps in small scale system is being favored as compared to its production in large-scale system of isolation. Since the former has α-mangostin content in a range of 7 mg/g as the lowest and about 15 mg/g as the highest in EtOAc, while the latter α-mangostin content was about 7.5 g/kg. The fresh pericarps provided a plausible alternative to the production of α-mangostin and in most cases explored is being favored over its dried counterpart.

There is a relative preference for these solvents especially EtOAc which was listed as one of the solvents that showed great potential as environmentally friendly solvents for extracting bioactive compounds relevant to the pharmaceutical industry since they demonstrated high efficiency in extraction, exceptional selectivity, and offered advantages such as time and energy savings as such they were considered economically viable and environmentally sustainable methods (Bundeesomchok et al., 2016).

Repeated column chromatography on ethyl acetate extract of fresh pericarp led to the isolation of alpha-mangostin (33.25%) as the major compound, beta-mangostin (0.225%) and gartanin (7.57%). Their antioxidant scavenging activities were investigated against 2,2-diphenyl-1-picrylhydrazyl DPPH radical and anticancer activities against neuroblastoma SH-SY5Y cancer cell line in MTT assay.

Recommendation

Currently there is overwhelming progress in preclinical studies, but no translation of **α**-mangostin yet into the clinic. According to the drug development pipeline, however, **α**-MG is still at the preclinical stage now, at which point pharmacodynamics, toxicology, and pharmacokinetic studies should be extensively performed before the drug can enter into the clinical testing phase.(Zhang et al., 2017) However, with continuous extensive and profound research, application of **α**-mangostin is hereby recommended to transit from experimental studies to evidence-based, clinically applicable pharmacotherapy.

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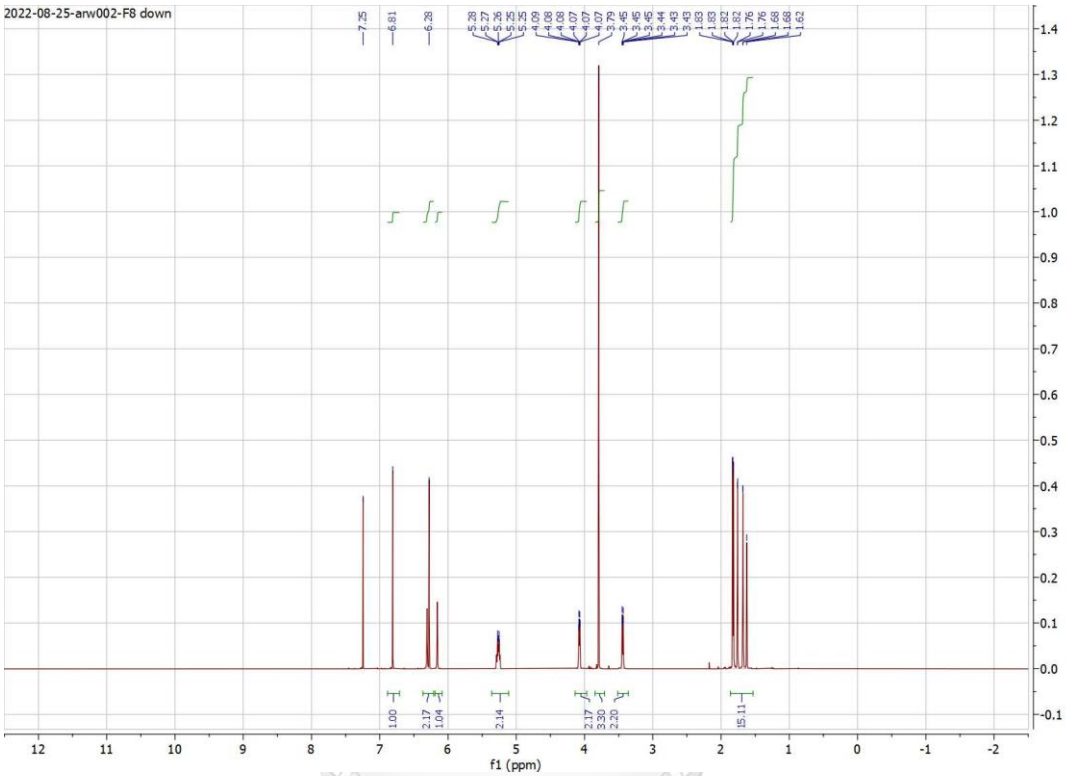
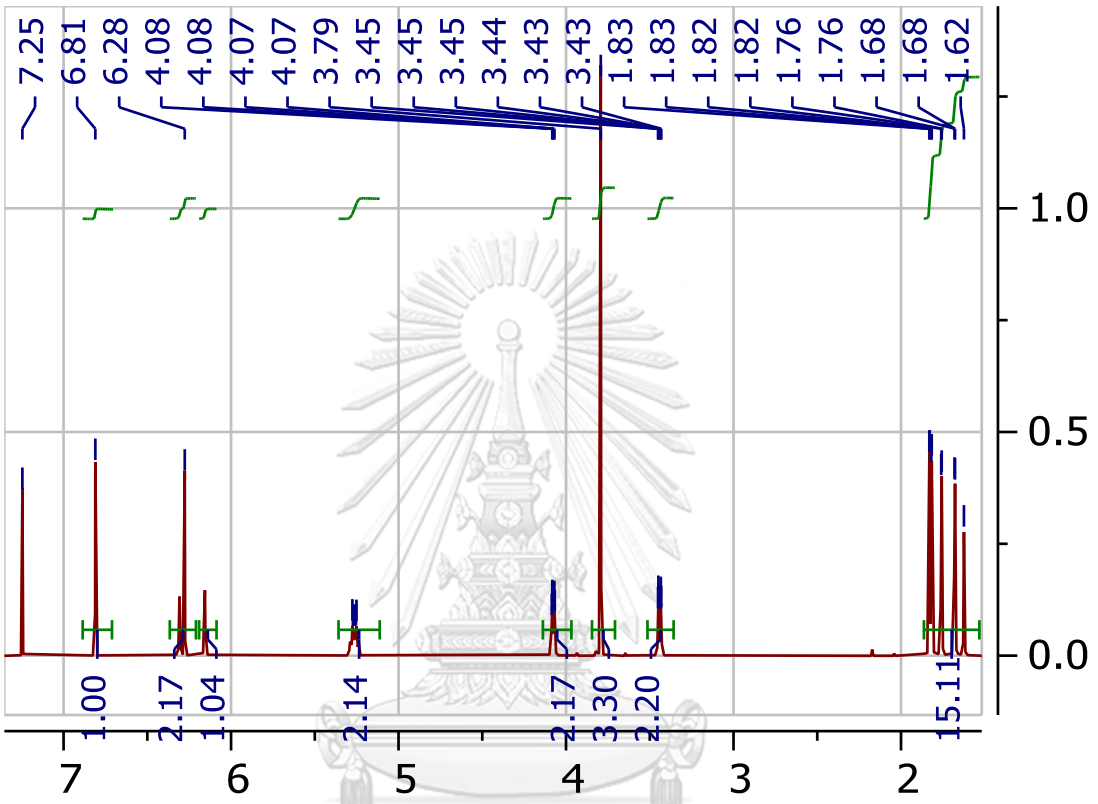
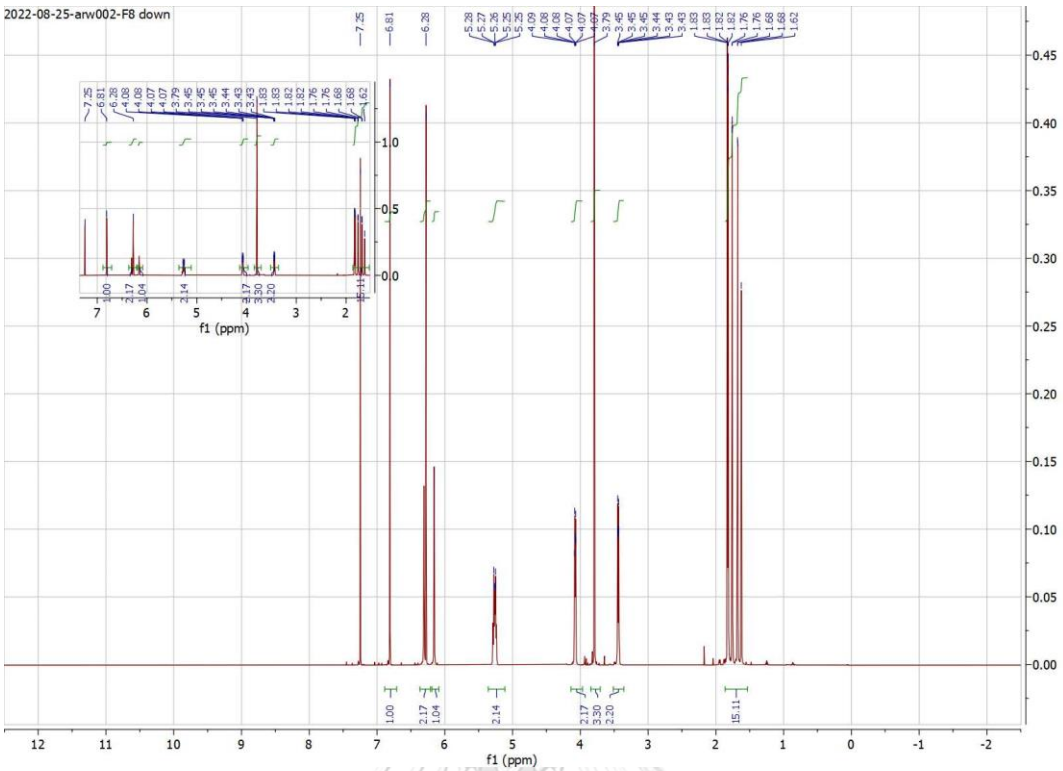
APPENDICES

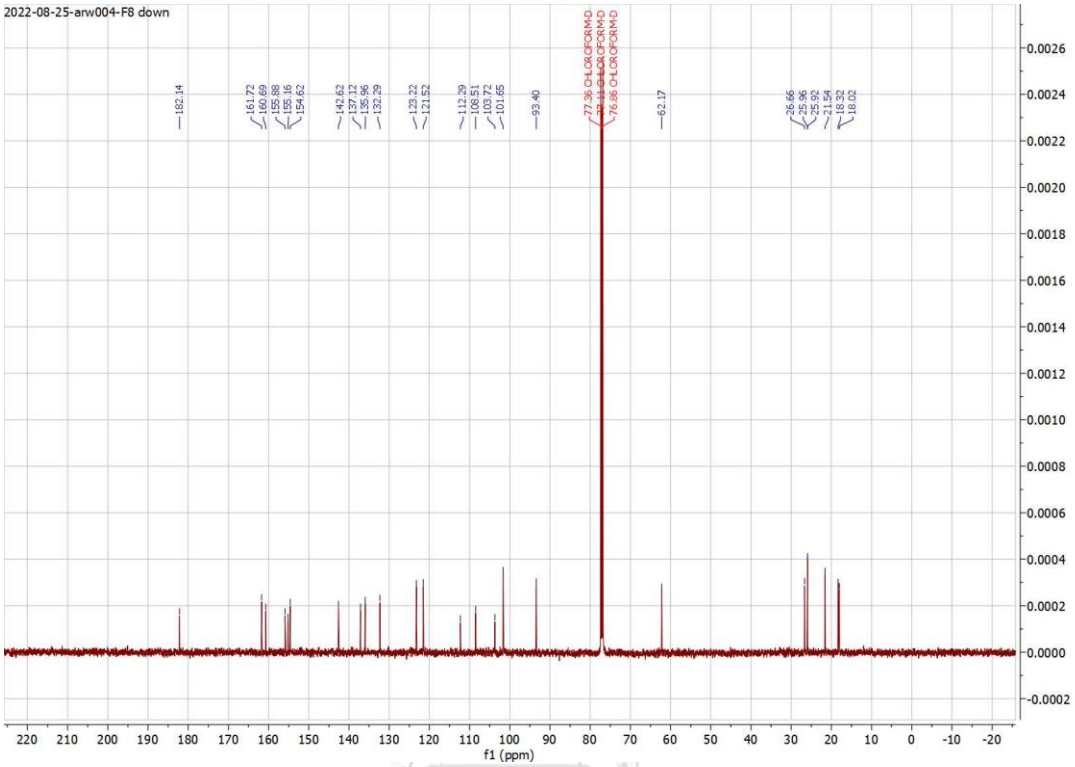
Appendix A. ^{1}HNMR spectrum of compound 1

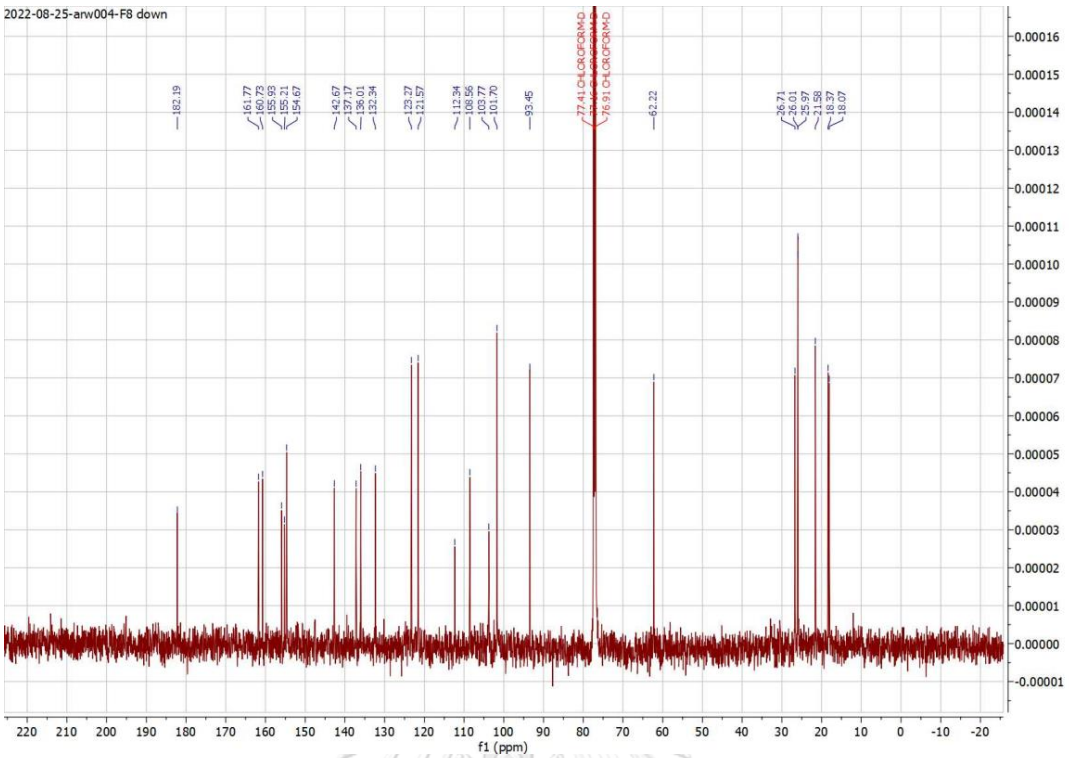


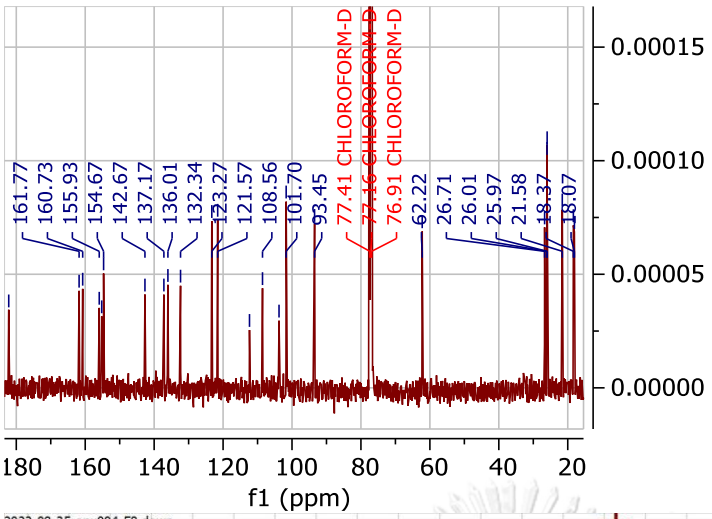


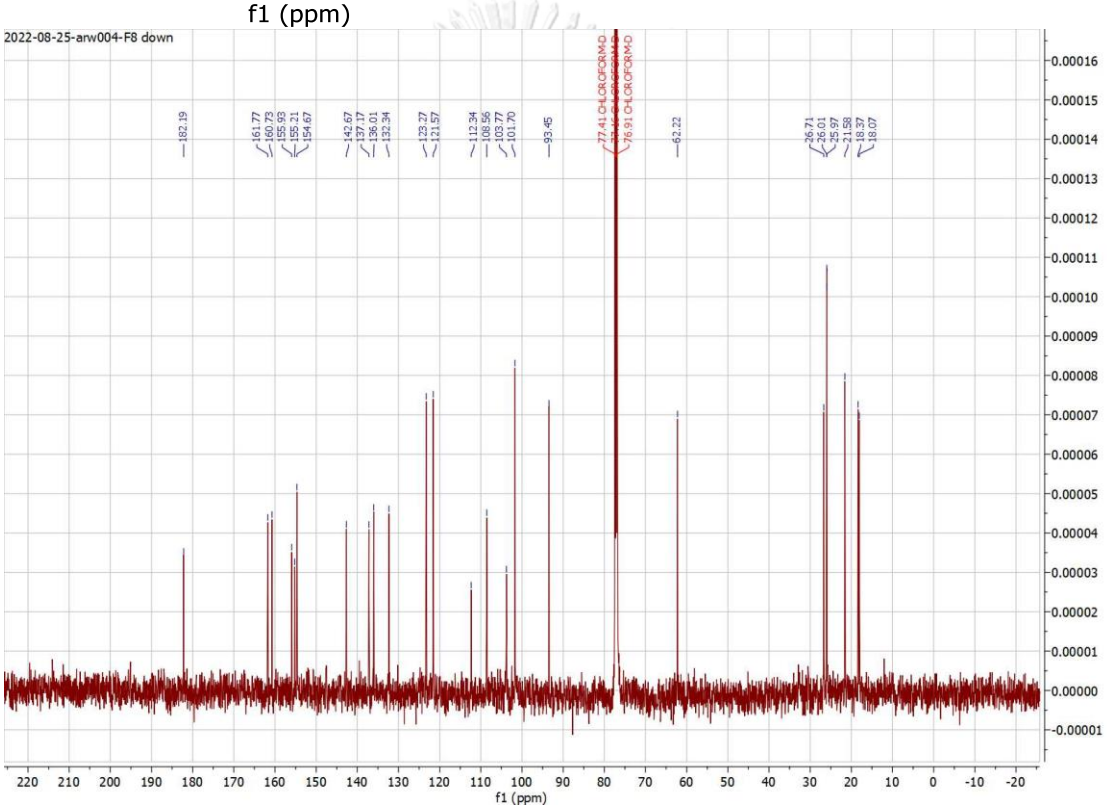


Appendix B. ^{13}C-NMR Spectrum for compound 1






161.77
160.73
155.93
154.67
142.67
137.17
136.01
132.34
123.27
121.57
108.56
101.70
93.45
77.41 CHLOROFORM-D
77.16 CHLOROFORM-D
76.91 CHLOROFORM-D
62.22
26.71
26.01
25.97
21.58
18.37
18.07
0.00015
0.00010
0.00005
0.00000
180 160 140 120 100 80 60 40 20
f1 (ppm)


2022-08-25-arw004-F8 down
182.19
161.77
160.73
155.93
155.21
154.67
142.67
137.17
136.01
132.34
123.27
121.57
112.34
108.56
103.77
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77.41 CHLOROFORM-D
77.16 CHLOROFORM-D
76.91 CHLOROFORM-D
62.22
26.71
26.01
25.97
21.58
18.37
18.07
0.00016
0.00015
0.00014
0.00013
0.00012
0.00011
0.00010
0.00009
0.00008
0.00007
0.00006
0.00005
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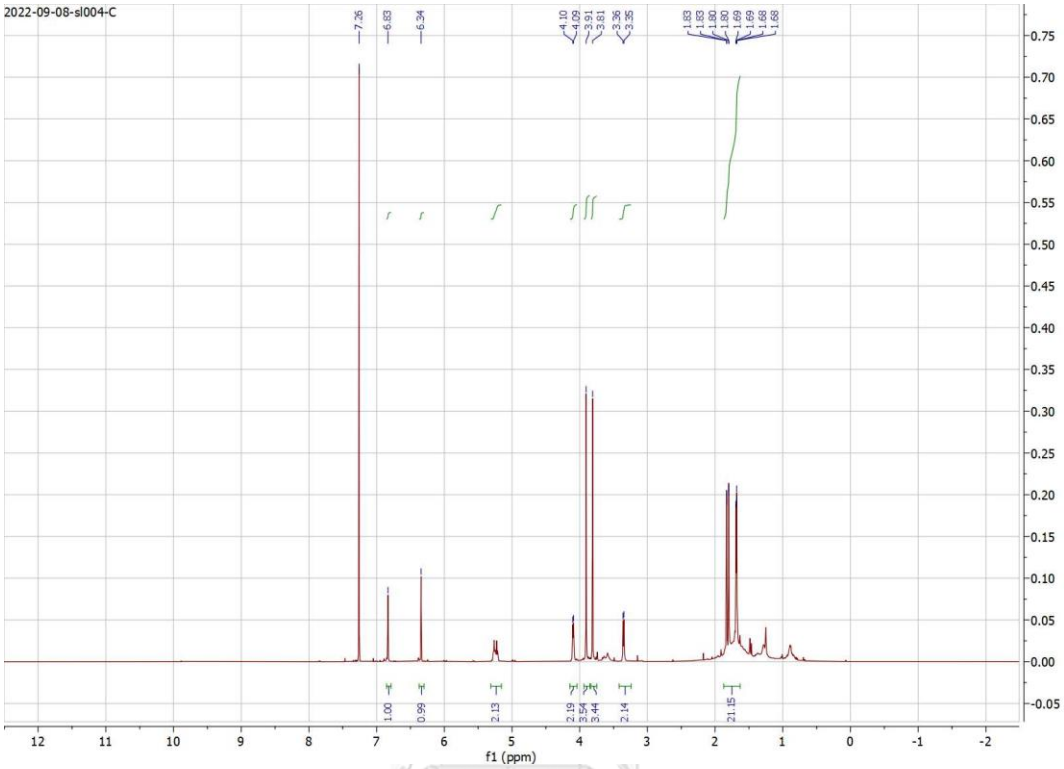
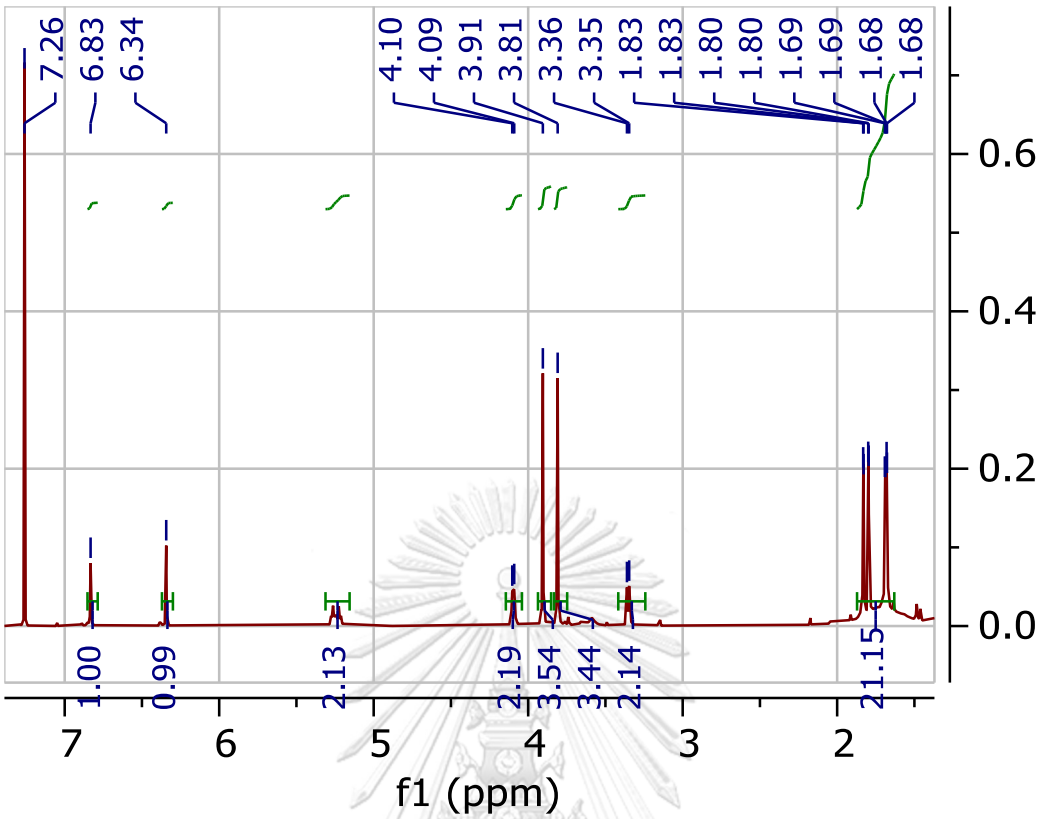
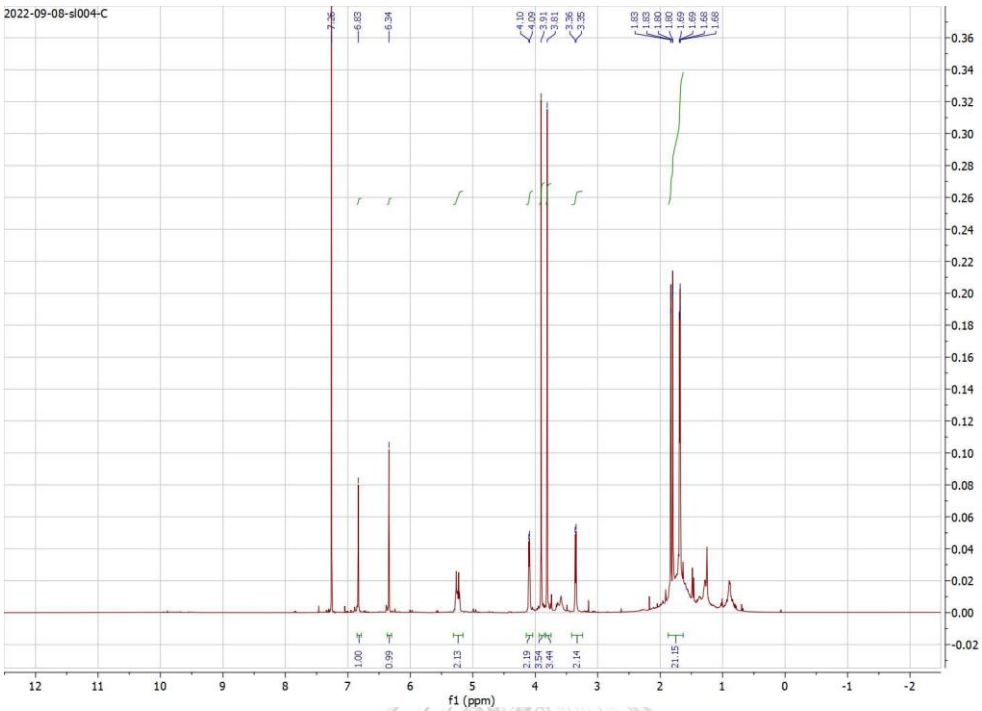
0.00002
0.00001
0.00000
-0.00001
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

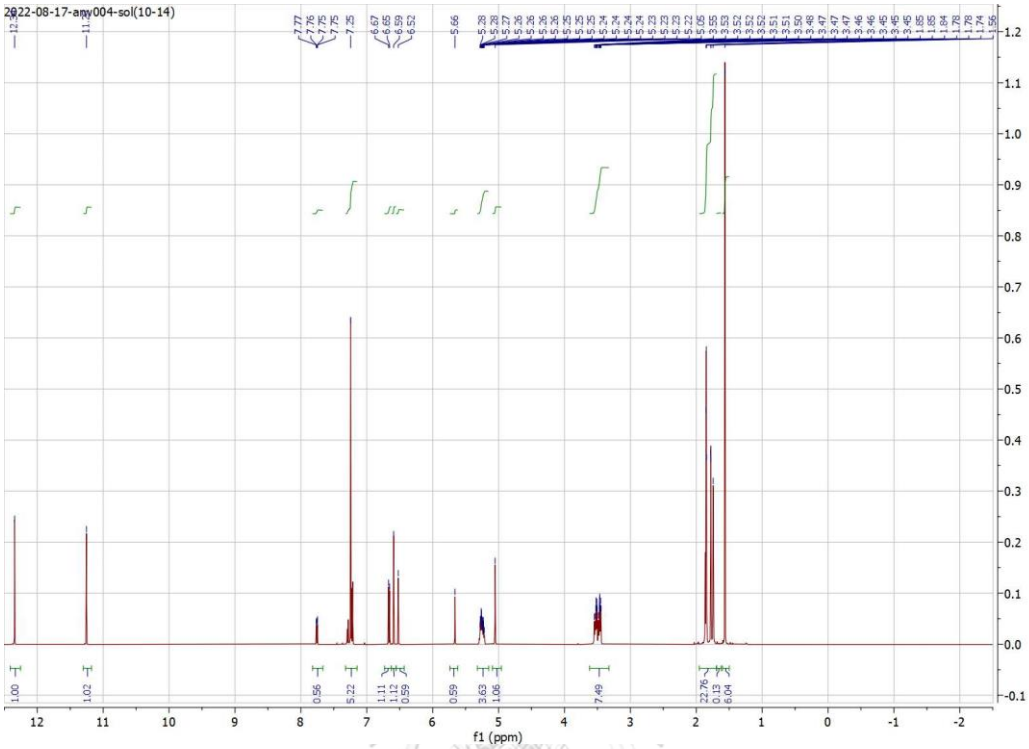
Appendix C. ^{1}H-NMR Spectrum for compound 2

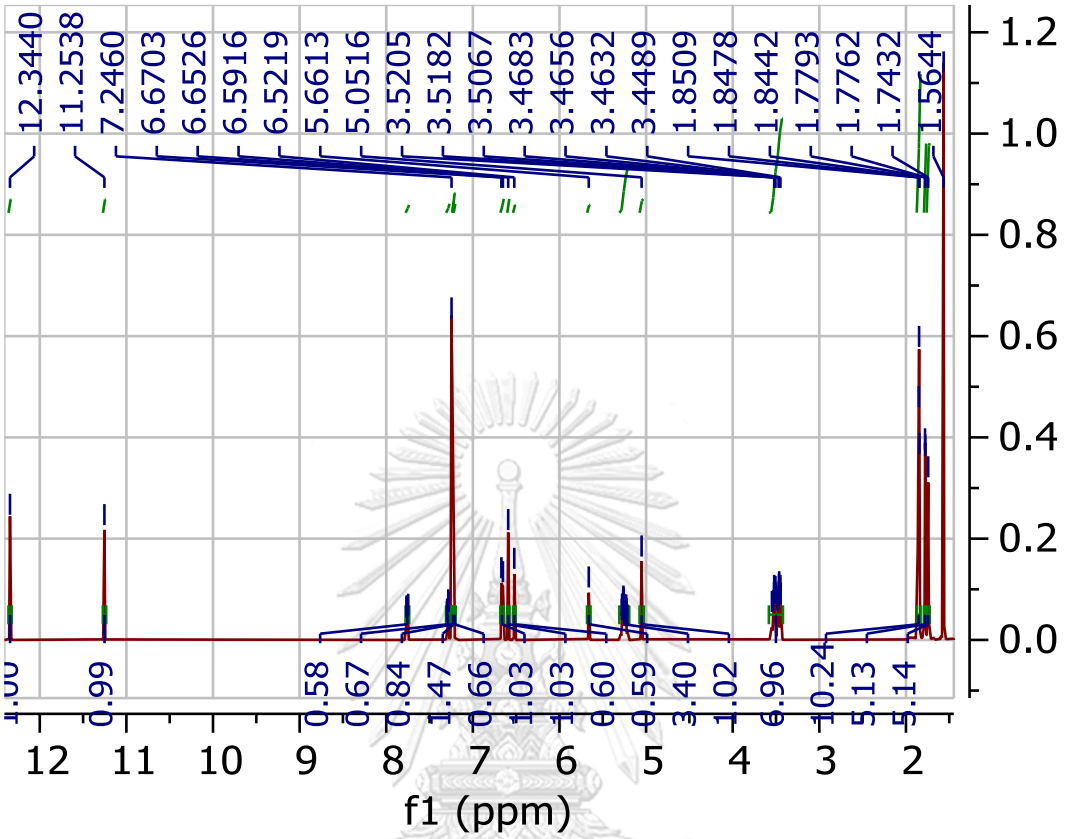


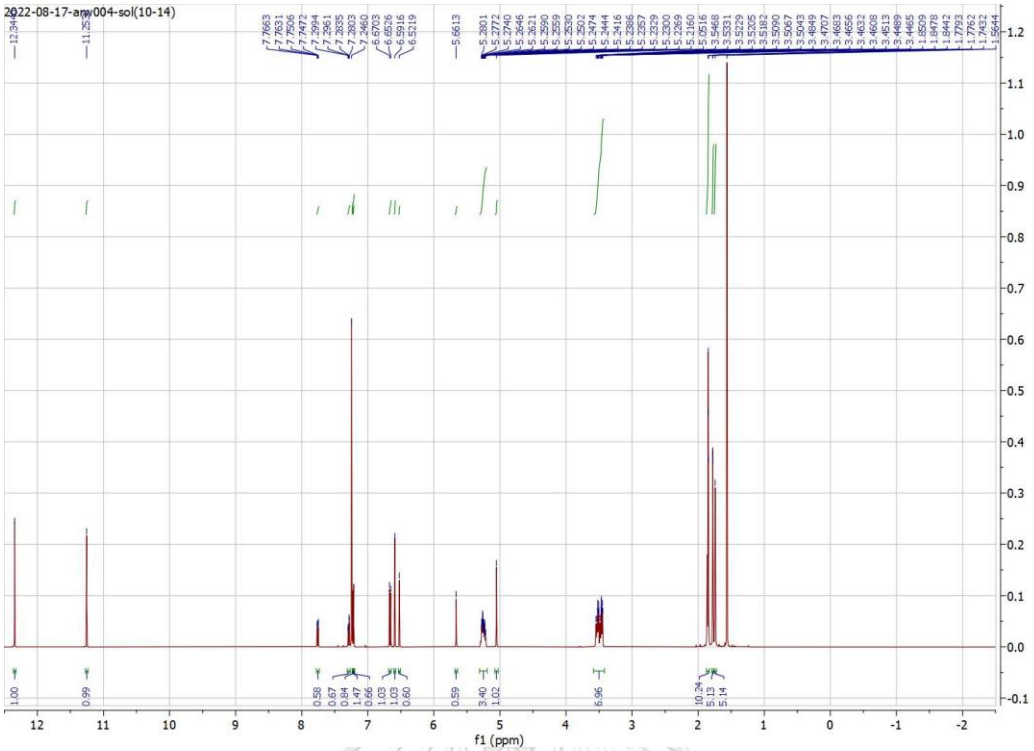




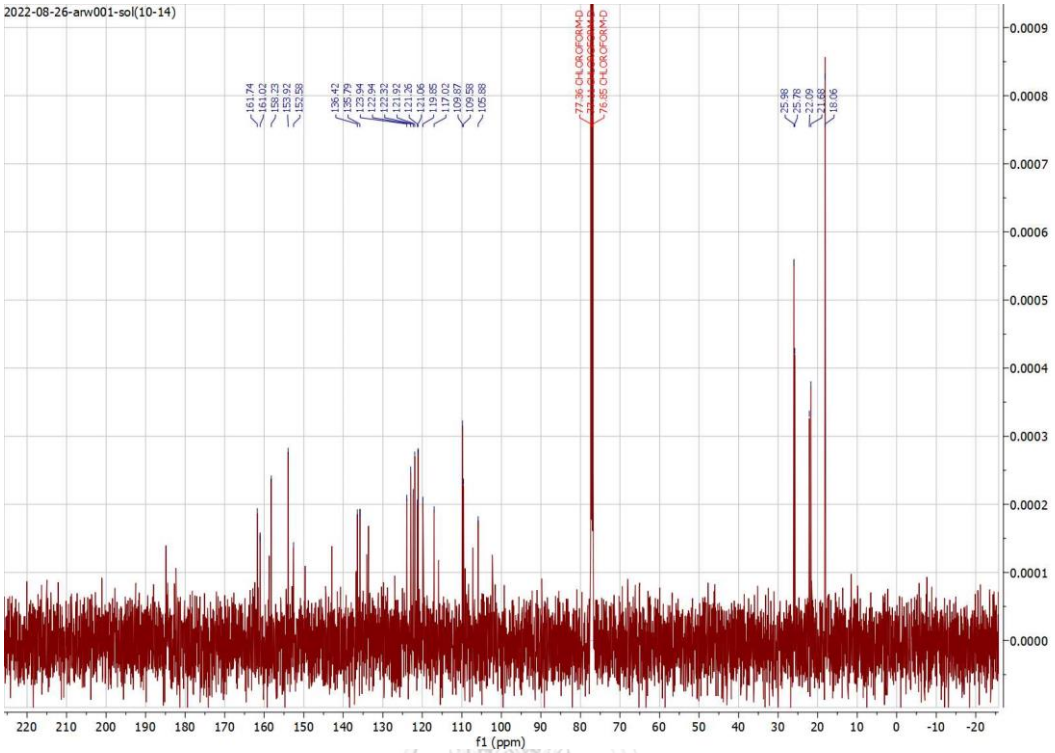
Appendix D. ^{1}H-NMR Spectrum for compound 3

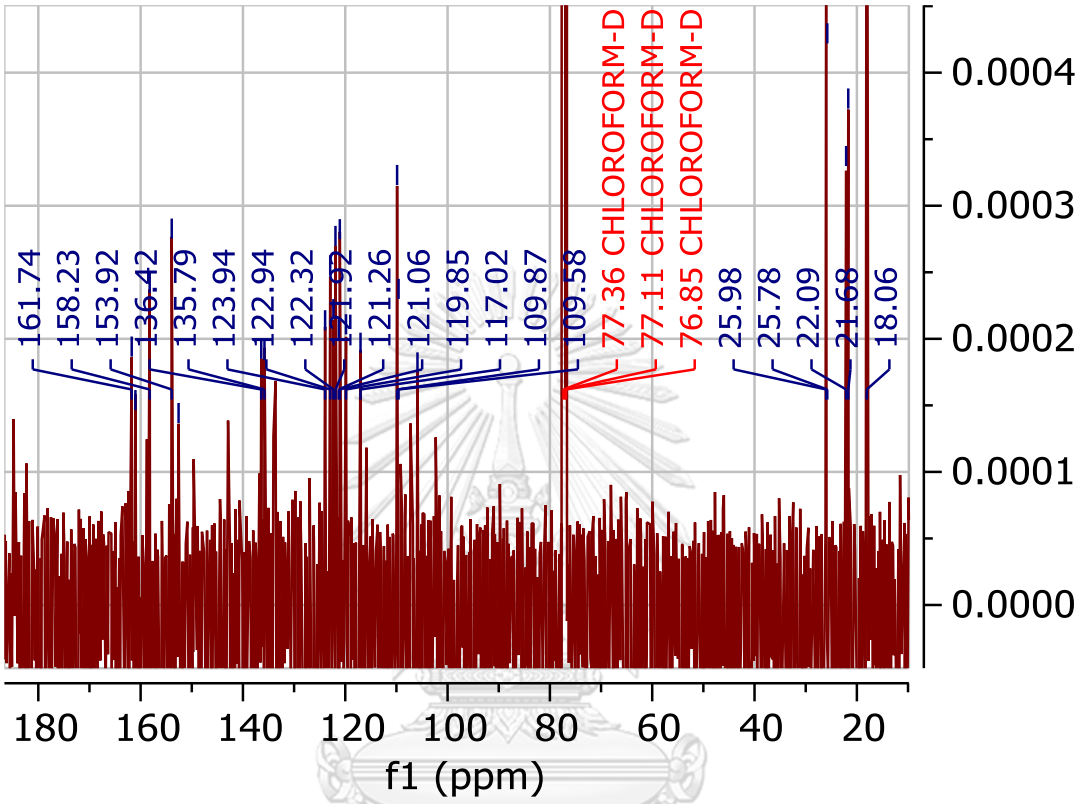


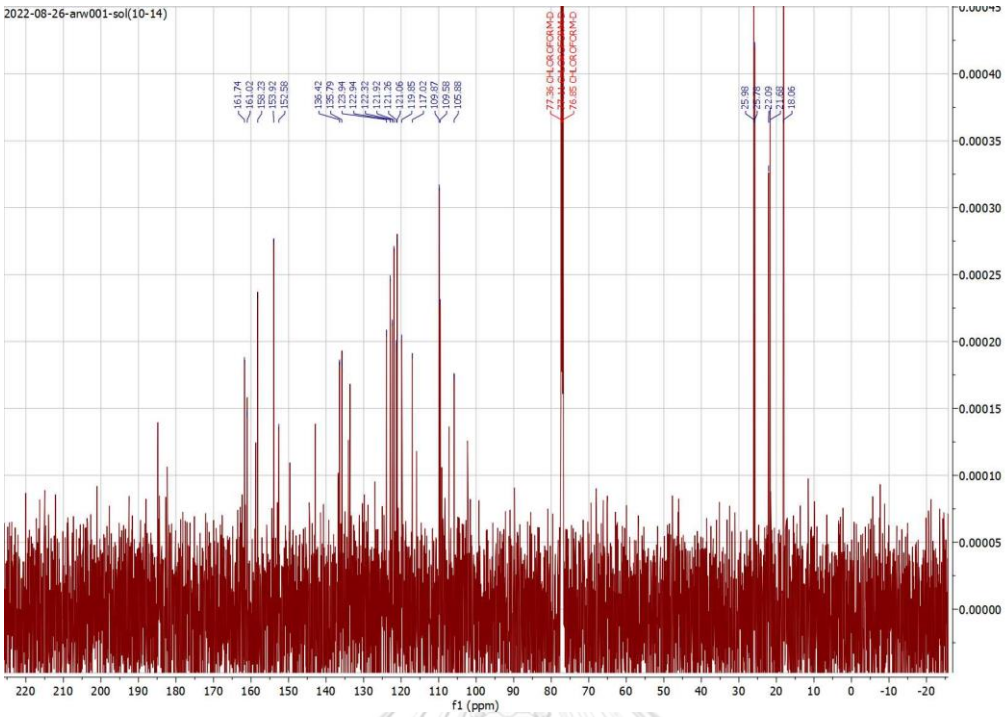




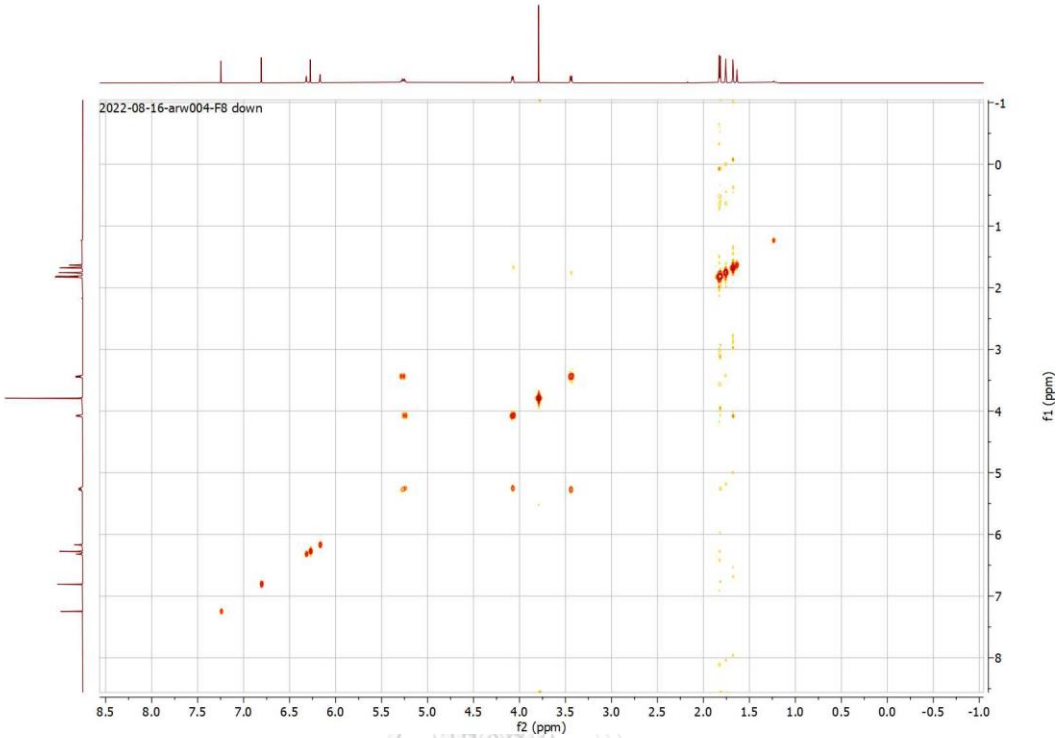
Appendix E. ^{13}C-NMR Spectrum for compound 3

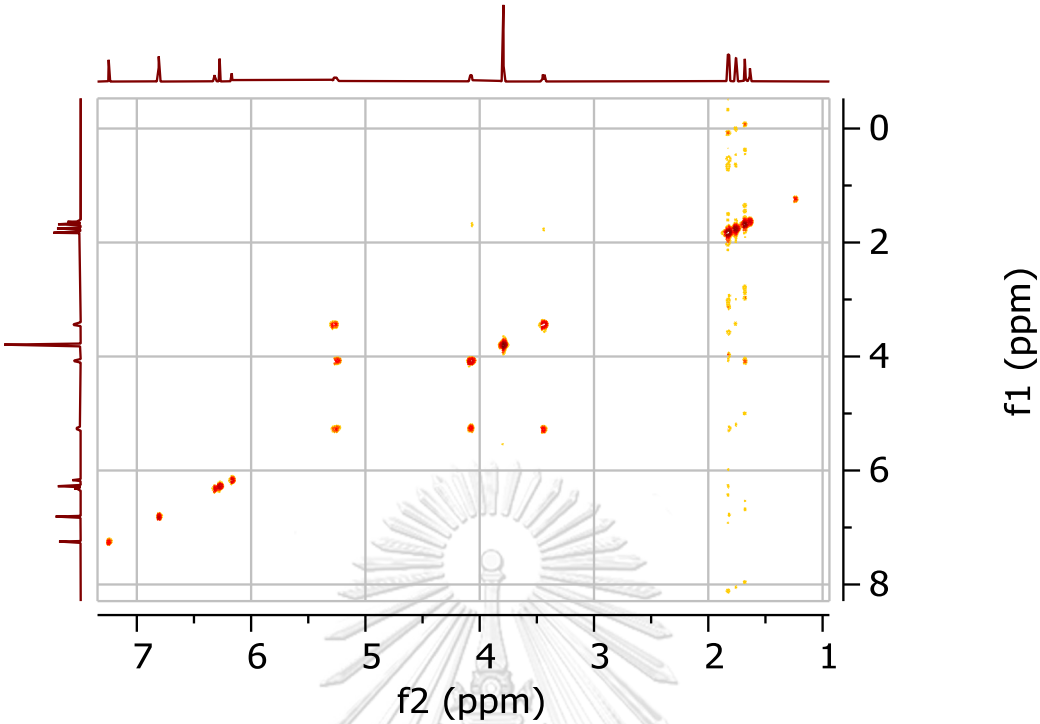




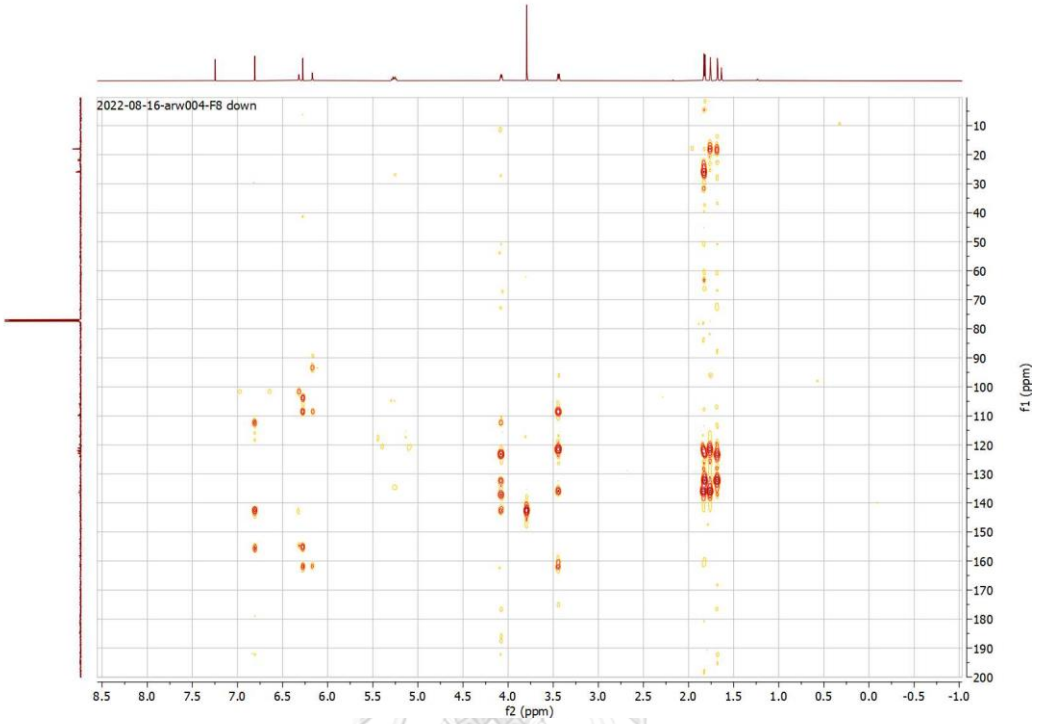


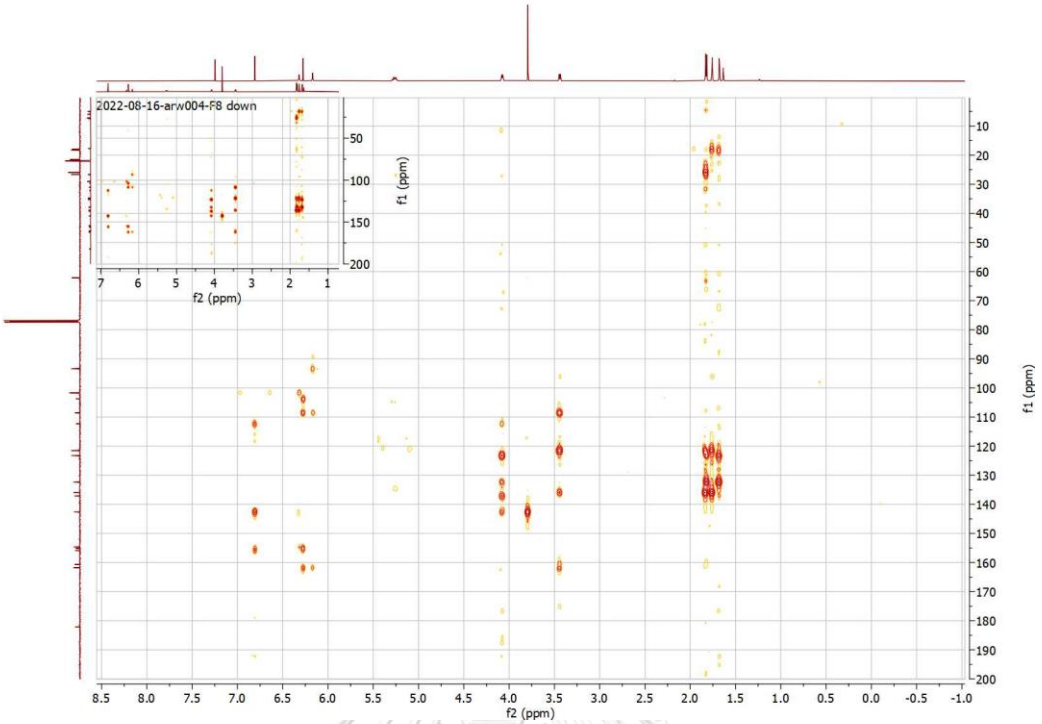
Appendix F. COSY-NMR Spectrum for compound 1

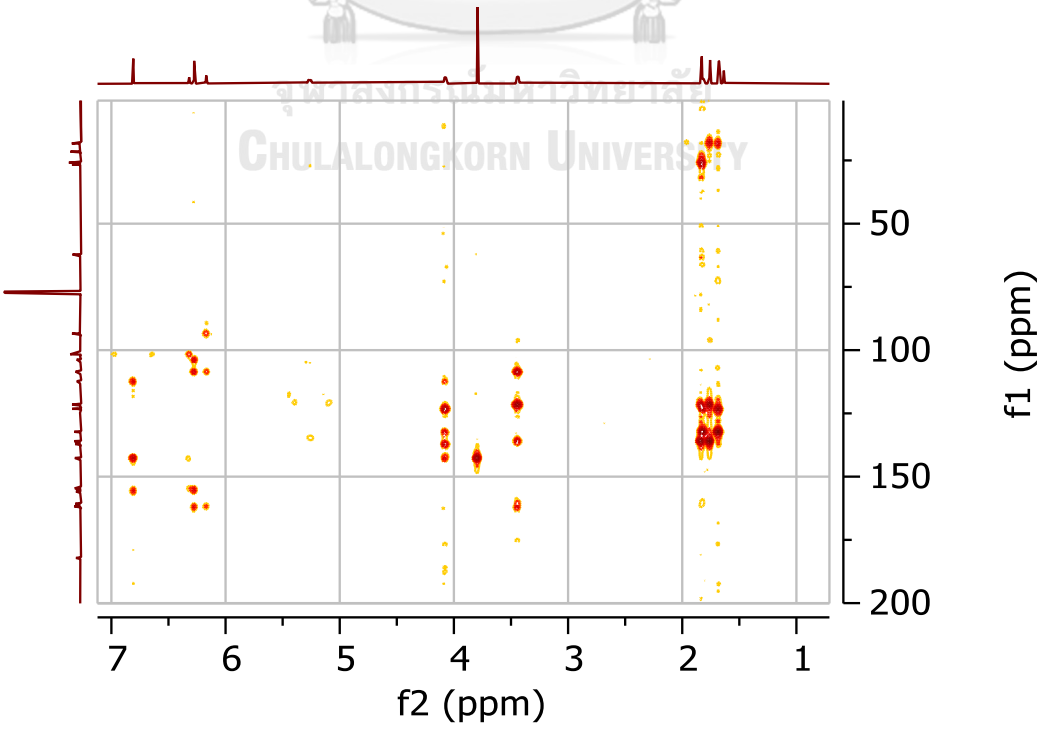




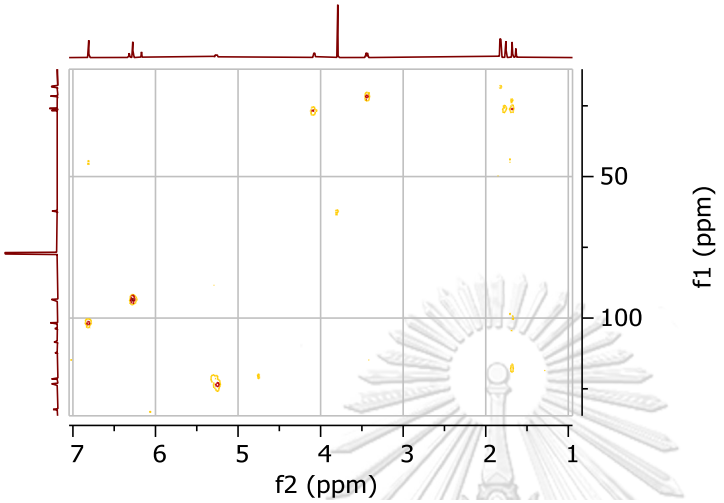
Appendix G. HMBC-NMR Spectrum for compound 1

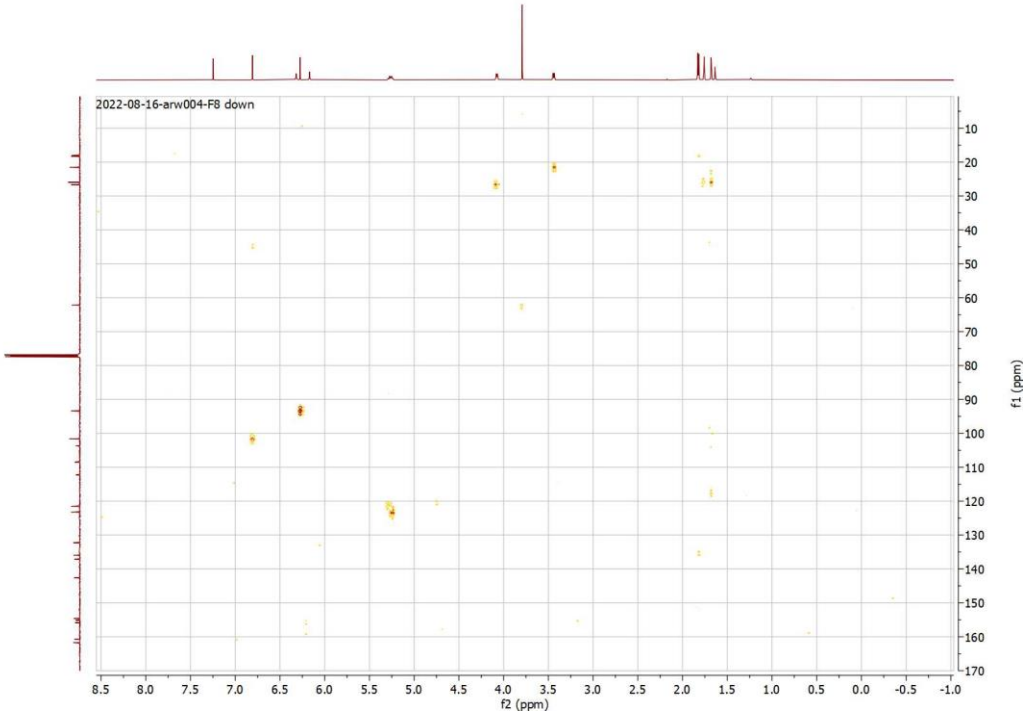




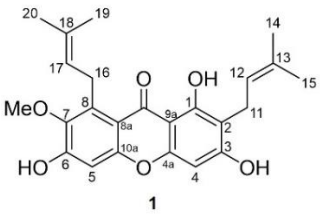


Appendix H. HSQC- NMR spectrum for compound 1

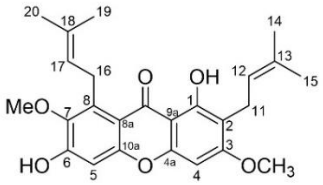




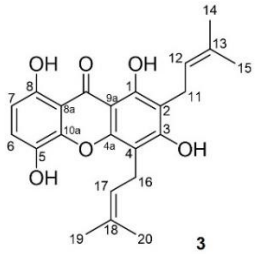
Appendix I. Structure of compounds from the fresh pericarps



1



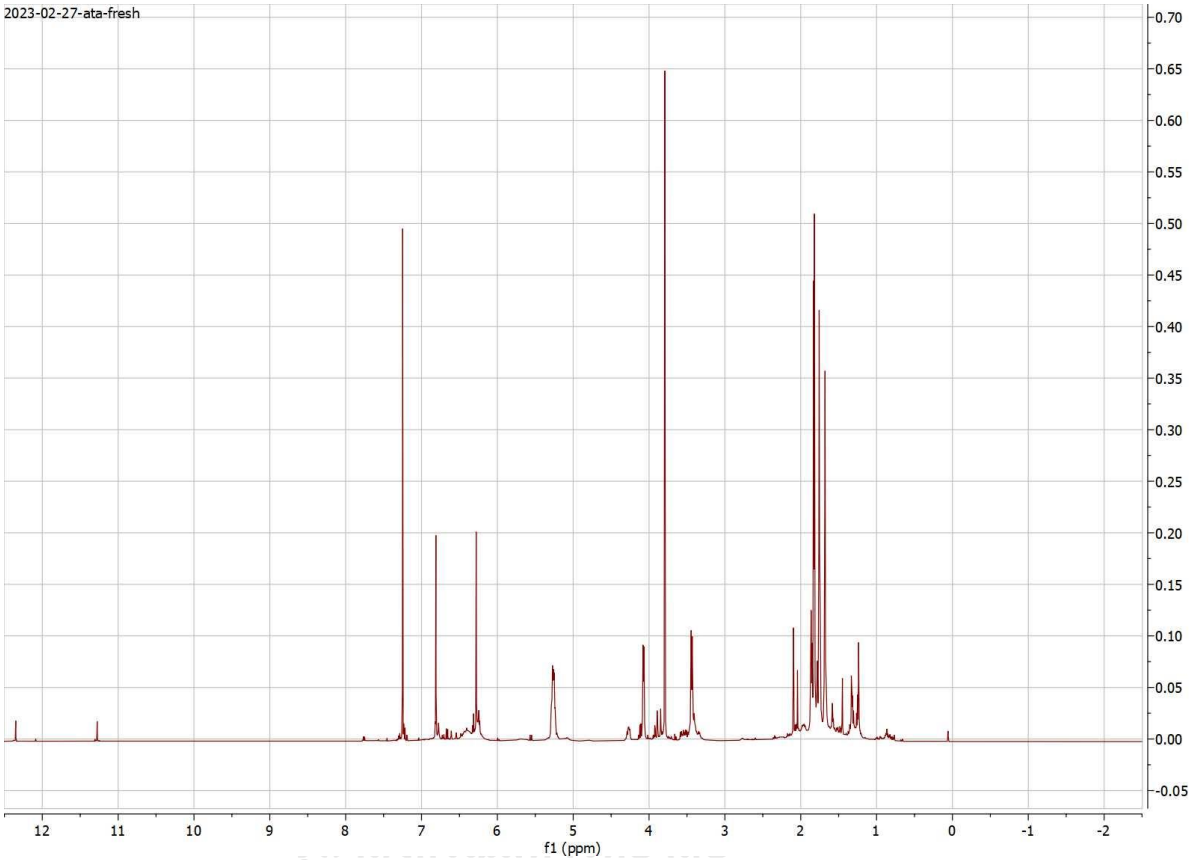
2



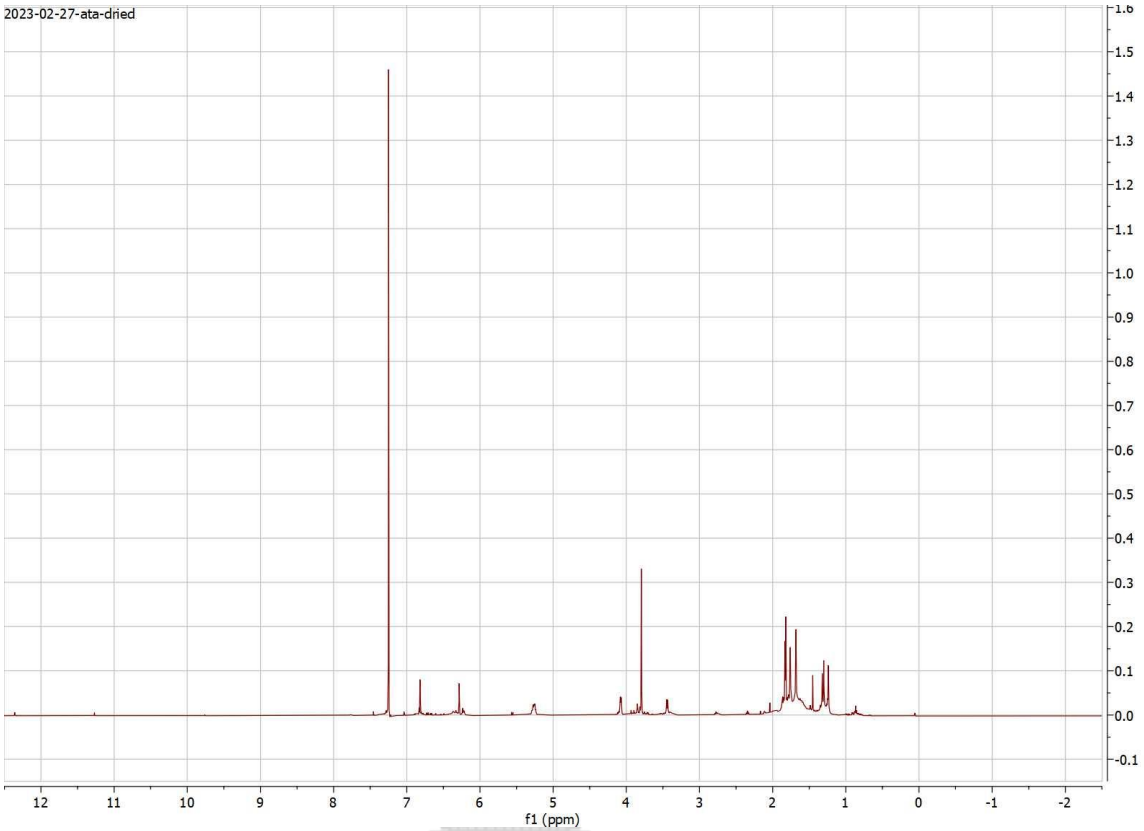
3

Appendix J. Spectrum of crude extract of fresh and dried pericarps.

Fresh



Dried



Appendix K. ^{1}H-NMR, ^{13}C-NMR, IR, MP, and UV data for isolated compounds

α-Mangostin (1): Yellow solid; MP: 180-181°C. IR (KBr) (cm^{-1}): 3418, 3239, 2962, 1639, 1373, 1238, 1169, 1050, 946, 839. UV λ_{max} MeOH nm (log ε): 315 (4.23), 243 (4.41), 204 (4.44)(Anggia et al., 2015). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.81 (s, 1H, H-5), 6.28 (s, 1H, H-4), 5.29 (dddd, *J* = 7 Hz , 7 Hz, 1H, 1H, H-12, H-17), 4.08 (d, *J* = 6.3 Hz, 2H, H-16), 3.79 (s, 3H, H7-OMe), 3.44 (d, *J* = 7.1, 2H, H-11), 1.82 (s, s, *J* = 6.1, 1.4 Hz, 3H, 3H, H-19, H-15), 1.76 (s, *J* = 1.4 Hz, 3H, H-14), 1.68 (s, *J* = 1.5 Hz, 3H, H-20). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.14(C-9), 161.72(C-3), 160.69(C-1), 155.88(C-6), 155.16(C-10a), 154.62(C-4a), 142.62(C-7), 137.12(C-8), 135.96(C-13), 132.29(C-18), 123.22(C-17), 121.52(C-12), 112.29(8a), 108.51(C-2), 103.72(C-9a), 101.65(C-5), 93.40(C-4), 62.17(C7-OMe), 26.66(C-16), 25.96(C-19), 25.92(C-14), 21.54(C-11), 18.32(C-15), 18.02(C-20).

β-mangostin (2): Yellow solid; MP: 160-162°C. IR (KBr) (cm^{-1}): 3384, 2933, 1645, 1600, 1482, 1456, 1381, 1281, 1148, 1169, 939, 882. UV λ_{max} MeOH nm (log ε): 347(4.48), 315(4.87), 258 (4.98), 244(5.05), 203 (5.12)(Anggia et al., 2015). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.83 (s, 1H, H-5), 6.34 (s, 1H, H-4), 5.20 - 5.16 (d, d, 1H, 1H, H-17, H-12), 4.10 (d, *J* = 7.2 Hz, 2H, H-16), 3.91 (s, 3H, H3-OMe), 3.81 (s, 3H, H7-OMe), 3.35 (d, *J* = 7.2 Hz, 2H, H-11), 1.87 – 1.63 (s, s, s, s, 12H, H-14, H-15, H-19, H-20). ^{13}C NMR (126 MHz,

$CDCl_3$) δ 181.9(C-9), 163.5(C-3), 159.69(C-1), 155.6(C-6), 155.2(C-10a), 154.4(C-4a), 142.5(C-7), 137.02(C-8), 132(C-13), 131.7(C-18), 123.21(C-17), 122.3(C-12), 112.29(C-8a), 111.5(C-2), 103.79(C-9a), 101.6(C-5), 88.81(C-4), 62.1(C7-OMe), 55.8(C3-OMe), 31.2(C-16), 26.69(C-19), 25.8(C-14), 21.29(C-11), 18.2(C-15), 17.8(C-20).

Gartanin (3): Yellow needles; MP: 160-162°C. IR (KBr) (cm^{-1}): 2970, 2908, 1626, 1580, 1486, 1381, 1282, 1177, 1073, 966, 829. UV λ_{max} MeOH nm (log ε): 351 (4.14), 319 (4.19), 283 (4.32), 257 (4.47), 243 (4.51), 203 (4.79)(Anggia et al., 2015). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.34 (s, 1H, H1-OH), 11.25 (s, 1H, H8-OH), 7.37 (d, 1H, H-6), 6.74 (d, 1H, H-7), 6.33 (s, 1H, H3-OH), 5.69 (m, *J* =7 Hz, 1H, H-17), 5.39 (m, *J* =7 Hz, 1H, H-12), 5.1 (s, 1H, H5-OH), 3.53 (d, *J* =6.5 Hz, 2H, H-16), 3.45 (d, *J* =7 Hz, 2H, H-11), 1.92 – 1.17 (s, s, s, s, 12H, H-14, H-15, H-19, H-20). ^{13}C NMR (126 MHz, $CDCl_3$) δ 184.6(C-9), 161.74(C-3), 158.23(C-1), 153.92(C-8), 152.58(C-4a), 142.2(C-10a), 135.79(C13), 135.74(C-5), 133.94(C-18), 122.32(C-6), 121.92(C-17), 121.06(C-12), 109.85(C-7), 109.5(C-2), 107.0(C-8a), 105.88(C-4), 102.0(C-9a), 25.98(C-14), 25.96(C-20), 25.78, 22.09(C-16), 21.18(C-11), 18.06(C-15), 18.0(C-19).

Apendix L. [1]HNMR spectra of α-Mangostin, β-mangostin, and gartanin by Vivi Anggia.

Table 1. [1]H-NMR (500 MHz) spectral data of isolated xanthones **1-3** and their references

Position	Compound 1 ($CDCl_3$)	*α*-mangostin [12] ($CDCl_3$)	Compound 2 (Aseton)	*β*-mangostin [13] ($CDCl_3$)	Compound 3 ($CDCl_3$)	Gartanin [12] ($CDCl_3$)
1	13.78, *s* (OH)	13.80, s (OH)	13.65, *s* (OH)	13.42, *s* (OH)	12.34, *s* (OH)	12.34, *s* (OH)
3	6.15, s (OH)	6.12, br (OH)	-	-	6.59, *s* (OH)	6.58, *s* (OH)
4	6.3, *s*	6.27, s	6.52, *s*	6.24, *s*	-	-
4a	-	-			-	-
5	6.83, *s*	6.81, s	6.87, *s*	6.74, *s*	5.1, s (OH)	5.02, br *s* (OH)
6	6.3, s (OH)	6.27, s (OH)	-	-	7.22, *d* (*J*=8.5 Hz)	7.22, *d* (J=7 Hz)
7	-	-	-	-	6.66, *d* (*J*=9Hz)	6.63, *d* (*J*=7 Hz)
8	-	-	-	-	11.26, *s* (OH)	11.25, *s* (OH)
11	3.46, *d* (*J*=7.5 Hz)	3.45, *d* (*J*=7.3 Hz)	3.32, *d* (*J*=7.5 Hz)	3.37, *d* (*J*=7.2 Hz)	3.46, *d* (*J*=7 Hz)	3.46, *d* (*J*=6 Hz)
12	5.29, *t* (*J*=7 Hz)	5.25, *t* (*J*=7.3 Hz)	5.28, *d* (*J*=6.5 Hz)	5.17, *d* (*J*=7.2 Hz)	5.26, *m* (*J*=7 Hz)	5.23
14	1.77, *s*	1.75, *s*	1.83, *s*	1.75, *s*	1.86, *s*	1.8, br *s*
15	1.83, *s*	1.81, *s*	1.65, *s*	1.62, *s*	1.76, *s*	1.86, br *s*
16	4.09, *d* (*J*=6.5 Hz)	4.07, *d* (*J*=7.0 Hz)	4.13, *d* (*J*=6.5 Hz)	4.09, *d* (*J*=7.2 Hz)	3.52, *d* (*J*=6.5)	3.51, *d* (*J*=6 Hz)
17	5.29, *t* (*J*=7 Hz)	5.28, *t* (*J*=7.3 Hz)	5.21, *d* (*J*=7.5 Hz)	5.18, *t* (*J*=7.2 Hz)	5.26, *m* (*J*=7 Hz)	5.23, *t* (*J*=6 Hz)
19	1.84, *s*	1.82, *s*	1.64, *s*	1.61, *s*	1.79, *s*	1.86, br *s*
20	1.69, *s*	1.67, *s*	1.77, *s*	1.72, *s*	1.86, *s*	1.8, br *s*
3-OMe	-	-	3.8, *s*	3.82, *s*	-	-
7-OMe	3.8, *s*	3.79, *s*	3.97, *s*	3.80, *s*	-	-

Appendix M ^{13}CNMR spectra of α-Mangostin, β-mangostin, and gartanin by Vivi Anggia.

Table 2. ^{13}C-NMR (125 MHz) spectral data of isolated xanthones **1-3.**

Position	Compound 1 ($CDCl_3$)	α-mangostin [12] ($CDCl_3$)	Compound 2 (Aseton)	β-mangostin [13] ($CDCl_3$)	Compound 3 ($CDCl_3$)	Gartanin [12] ($CDCl_3$)
1	160.6	160.6	160.5	159.7	158.1	158
2	108.4	108.4	111.1	111.5	109.5	109.5
3	161.6	161.6	164.6	163.5	161.6	161.6
4	93.5	93.3	89.9	88.8	105.8	105.8
4a	154.5	155.1	156.2	154.4	152.5	152.7
5	101.6	101.5	102.7	101.5	135.7	135.7
6	155.1	154.5	156.3	155.6	122.8	122.8
7	142.6	142.5	144.6	142.5	109.8	109.8
8	137	137	138.1	137	153.8	153.9
8a	112.2	112.2	112.1	112.3	107.1	107
9	182	182	182.9	181.9	184.7	184.7
9a	103.6	103.6	104.2	103.8	102.2	102.0
10a	155.8	155.8	157.6	155.2	142.8	142.2
11	21.5	21.4	21.9	21.3	21.9	21.1
12	121.4	121.4	124.7	122.3	121.8	121.0
13	132.2	135.9	131.5	132	133.9	136.5
14	25.8	25.9	25.9	25.8	25.7	25.9
15	18.2	18.2	18.3	18.2	17.9	18.0
16	26.6	26.6	26.9	31.2	21.6	22.1
17	123.1	123.5	123.3	123.2	120.9	121.8
18	135.8	132.2	131.5	131.7	136.3	133.9
19	17.9	17.9	17.9	17.8	17.9	18.0
20	25.9	25.8	25.9	26.7	25.9	25.9
3-OMe	-	-	56.5	55.8	-	-
7-OMe	62.1	62.1	61.4	62	-	-

REFERENCES

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