สมบัติของฟิล์มแบคทีเรียเซลลูโลสจากการสังเคราะห์โดย *ACETOBACTER XYLINUM* ที่มีสารสกัดจากเปลือกมังคุด

นายณัฐวุฒิ นุ่นแก้ว

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CHARACTERISTICS OF BACTERIAL CELLULOSE FILM SYNTHESIZED BY *ACETOBACTER XYLINUM* CONTAINING *GARCINIA MANGOSTANA* EXTRACT

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering Program in Chemical Engineering
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Thesis Title	CHARACTERISTICS OF BACTERIAL CELLULOSE FILM SYNTHESIZED BY *ACETOBACTER XYLINUM* CONTAINING *GARCINIA MANGOSTANA* EXTRACT
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ณัฐวุฒิ นุ่นแก้ว : สมบัติของฟิล์มแบคทีเรียเซลลูโลสจากการสังเคราะห์โดย *ACETOBACTER XYLINUM* ที่มีสารสกัดจากเปลือกมังคุด. (CHARACTERISTICS OF BACTERIAL CELLULOSE FILM SYNTHESIZED BY *ACETOBACTER XYLINUM* CONTAINING *GARCINIA MANGOSTANA* EXTRACT) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. เหมือนเดือน พิศาลพงศ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ดร. ผ่องพรรณ ศิริพงษ์, 126 หน้า.

ปัจจุบันวัสดุพอลิเมอร์หลายชนิดได้ถูกนำมาประยุกต์ใช้ในงานวิจัยด้านการแพทย์ แบคทีเรียเซลลูโลสเป็นวัสดุชีวภาพชนิดหนึ่งที่ได้รับความสนใจในการประยุกต์ใช้ทางด้านการแพทย์เพราะสมบัติอันเป็นเอกลักษณ์ของวัสดุ ด้วยวัตถุประสงค์ที่จะปรับปรุงสมบัติทางชีวภาพของฟิล์มแบคทีเรียเซลลูโลสจึงได้ทำการศึกษาการดัดแปลงสมบัติโดยการเติมสารสกัดจากเปลือกมังคุดเข้าไปในแผ่นฟิล์มด้วยสารสกัดด้วยน้ำที่ความเข้มข้นร้อยละ 2.5-10.0 (น้ำหนักโดยปริมาตร) และด้วยสารสกัดด้วยเอทานอลที่ความเข้มข้นร้อยละ 0.25-1.00 (ปริมาตรโดยปริมาตร) จากการผลของอินฟราเรดทางโครงสร้างโมเลกุล ชี้ให้เห็นถึงการเกิดปฏิสัมพันธ์ระหว่างหมู่ฟังก์ชันของแบคทีเรียเซลลูโลสและสารสกัดเปลือกมังคุดที่ได้ถูกดูดซับเข้าไปในแผ่นฟิล์ม ค่าความเค้นแรงดึง (Tensile Strength) และค่าการยืดสูงสุด (elongation at break) ของแผ่นฟิล์มที่มีสารสกัดด้วยน้ำมีค่าลดลงเมื่อความเข้มข้นของสารสกัดมีค่าเพิ่มขึ้น ในขณะที่ค่าสมบัติเชิงกลของแผ่นฟิล์มที่มีสารสกัดด้วยเอทานอลมีค่าเพิ่มขึ้น พบว่าเมื่อทำการเติมสารสกัดด้วยน้ำลงในแผ่นฟิล์ม ความสามารถในการดูดซับน้ำและอัตราการซึมผ่านของไอน้ำจะมีค่าเพิ่มขึ้นเนื่องจากคุณสมบัติความชอบน้ำของแผ่นฟิล์มที่เพิ่มขึ้น ทั้งแผ่นฟิล์มแบคทีเรียเซลลูโลสที่มีสารสกัดด้วยน้ำและเอทานอลแสดงผลในการยับยั้งการเจริญเติบโตของเชื้อ *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes* และ *Aspergillus niger* ยกเว้น *Escherichia coli* ที่ถูกยับยั้งด้วยฟิล์มแบคทีเรียเซลลูโลสที่มีสารสกัดด้วยน้ำเท่านั้น นอกจากนี้ฟิล์มแบคทีเรียเซลลูโลสที่มีสารสกัดด้วยน้ำและเอทานอลของมังคุดยังแสดงผลในการยับยั้งการเจริญเติบโตของเซลล์มะเร็งผิวหนัง (B16 melanoma) อีกด้วย ดังนั้นแผ่นฟิล์มแบคทีเรียเซลลูโลสที่ถูกดัดแปลงด้วยการเติมสารสกัดจากเปลือกมังคุดจึงมีสมบัติที่เป็นประโยชน์ที่อาจนำไปประยุกต์ใช้ในทางการแพทย์ที่หลากหลาย

ภาควิชา	วิศวกรรมเคมี	ลายมือชื่อนิสิต	ณัฐวุฒิ นุ่นแก้ว
สาขาวิชา	วิศวกรรมเคมี	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก	
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NATTHAWUT NUNKAEW: CHARACTERISTICS OF BACTERIAL CELLULOSE FILM SYNTHESIZED BY *ACETOBACTER XYLINUM* CONTAINING *GARCINIA MANGOSTANA* EXTRACT. ADVISOR: ASSOC. PROF. MUENDUEN PHISALAPHONG, Ph.D., CO-ADVISOR: PONGPUN SIRIPONG, Ph.D., 126 pp.

Many kinds of polymeric materials recently have been applied in biomedical researches. Bacterial cellulose (BC) is the one of biomaterials, which has been interested for medical application because of its unique properties. In order to improve their biological properties, the modifications by means of impregnation of the extract of *Garcinia mangostana* fruit rind into the BC film by using 2.5-10.0%w/v aqueous extract and 0.25-1.00%v/v ethanolic extract were investigated. From FT-IR analysis, the interaction between BC functional groups and mangosteen extract compounds, which absorbed into the BC film, was indicated. The tensile strength and elongation at break of the BC films with the aqueous extract (BCWM) were decreased by increasing extract concentration, whereas those of the BC films with the ethanolic extract (BCEM) were increased. The water absorption capacity and water vapor transmission rate of BCWM were increased due to the more hydrophilic property after adding *G. mangostana* aqueous extract. Both BCWM and BCEM films exhibited antibacterial and antifungal effects on the growths of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Aspergillus niger*, whereas only BCWM films exhibited antibacterial effect against *Escherichia coli.* In addition, the BCWM and BCEM films exhibited cytotoxic effects on B16 melanoma cells. Therefore, the modified BC films containing *G. mangostana* extracts exhibit advantageous properties for many possible medical applications.

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Finally, I would like to dedicate this thesis to my parents and my families for their always support and encouragement as well as my friends in the Department of Chemical Engineering, Chulalongkorn University.

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CHAPTER I

INTRODUCTION

Biomedical materials have been substantially developed in recent years. Many scientists were interested in developing novel biomaterials from synthetic polymers which be used in a variety of biomedical applications, including *in vitro* and *in vivo* tissue engineering, drug delivery, wound dressings, and medical implants. However, synthetic polymers often do not provide the appropriate mechanical properties and are usually not biocompatible. (Czaja *et al.*, 2007)

Recently, cellulose, the most abundant biopolymer from wood has been isolated, purified and used as biomaterial for many medical applications (Hoenich, 2006). Natural cellulose fiber from plants is unpurified and usually found some kinds of natural fibers, namely, lignin, hemicellulose, pectin and other biogenetic products. Moreover, the production of plant cellulose products is facing some environmental problems (Saied *et al.*, 2004). According to some weak points of plant cellulose in environment issues, the new sources of cellulose generated from biosynthesis has been studied. Bacterial cellulose or BC can be produced by some microbial cells; however, only cellulose synthesized from secretion of Acetobacter strain is enough for the commercial interest and has the high quality of microfibrils. By comparing to planted cellulose, the major advantage of bacterial cellulose is an absence of other bio-products in its structure of microfibril (Jonas and Farah, 1998; Jung *et al.*, 2005). Despite their identical chemical composition, the structure and mechanical properties of microfibrils synthesized by microbial cells differ from that isolated from plants. BC microfibrils have high mechanical properties including tensile strength and modulus,

high water holding capacity, moldability, crystallinity, and biocompatibility. (Retegi *et al.*, 2010)

Because of its excellent propeties, BC has been used in several biomedical applications, for instance, artificial skin for humans with extensive burns (Fontana *et al.*, 1990), artificial blood vessels for microsurgery (Klemm *et al.*, 2001), scaffold for tissue engineering of cartilage (Svensson *et al.*, 2005) and wound-dressing (Czaja *et al.*, 2006). Due to many advantages of BC on wound healing such as wound exudates control, ability to retain moisture, biocompatibility, and so on, it is one of biomaterials which has been interested for using as a wound dressing. Previously, in order to provide antimicrobial ability of this biocompatible and nontoxic material, the addition of antimicrobial chemical agents into the films including inorganic salts, organometallics, iodophors, phenols and thiophenols, onium salts, heterocyclics with anionic groups, nitro compounds, formaldehyde derivatives, and some amines, has been applied. However, many of these agents were often toxic to human body and its degradation in the environment was not easy (Maneerung *et al.*, 2008; Hou *et al.*, 2009)

Mangosteen (*Garcinia mangostana* Linn.), a tropical fruit tree belonging to the family Guttiferae, has been planted in South and Southeast Asian countries where climate is suitable for mangosteen growing. Its fruit is very famous and has been known as the “Queen of fruits” in Thailand. The main areas which have appropriate conditions for cultivating mangosteen in Thailand, are located in the eastern and southern parts of the country (Pothitirat and Gritsanapan, 2008). The traditional medicinal treatment has been used the fruit hull of *G. mangostana* for relieving and curing illness such as abdominal pain, diarrhoea, dysentery, leucorrhoea, gonorrhoea, skin infection, wounds, etc (Yu *et al.*, 2007).

From many reports, mangosteen extract from all parts of mangosteen, especially the fruit rind of *G. mangostana* contains many complex phenolic compounds such as xanthones, flavonoids, tannins, and other bioactive substances (Phothitirat *et al.*, 2009). Several studies indicated that xanthones particularly α-mangostin which is a major xanthone, exhibits antioxidant, antitumoral, antiinflammatory, anti-allergic, antibacterial and antifungal activities. Owing to its pharmacological activities, it is popularly applied as an ingredient in herbal cosmetics and pharmaceutical products (Pothitirat and Gritsanapan, 2009). In the United States, products of mangosteen are now extensively available and are highly popular because of their obvious role in enhancing human health (Chin *et al.*, 2008).

In this present study, the bacterial cellulose film was modified by supplementation of crude extract from fruit rind of *G. mangostana*. Mangosteen extract was impregnated into the bacterial cellulose film by means of immersion of film samples in mangosteen extract solution. The changes in physical and biological properties such as surface morphology, mechanical strengths, water holding capacity, antibacterial activity and antifungal activity against pathologic microorganisms as well as antiproliferation activity against melanoma cells of the bacterial cellulose film containing *G. mangostana* extract were investigated. Furthermore, the absorption and release characteristics of *G. mangostana* extract on the modified bacterial cellulose films are demonstrated.

Objectives

1. To develop BC films containing aqueous (BCWM) and ethanolic (BCEM) extracts of *G. mangostana*.
2. To investigate the effect of the impregnation of aqueous and ethanolic extracts of *G. mangostana* into BC films on the physical and biological characteristics of the films.

Research scopes

1. Prepare BC films from biosynthesis under static conditions by *Acetobacter xylinum*.
2. Examine the absorption and release characteristics of bioactive compounds from the BC films containing aqueous (BCWM) and ethanolic (BCEM) extracts of *G. mangostana*.
3. Characterize the physical properties of modified BCWM and BCEM films by
    a. Scanning electron micrographs (SEM) for preliminarily investigating morphology.
    b. Fourier Transform Infrared (FT-IR) spectrometer for identifying the chemical structure.
    c. Universal testing machine for determining the mechanical properties of the films.
    d. Oxygen permeation tester for measuring oxygen transmission rate (OTR)
    e. Water vapor permeation tester for measuring water vapor transmission rate (WVTR)
    f. Water absorption capacity (WAC)

4. Characterize the biological properties of modified BCWM and BCEM film by
    a. Antibacterial and antifungal activities
    b. Inhibitory effects on mouse melanoma cells

CHAPTER II

THEORY

2.1 Cellulose

Cellulose, the basic structure of the plant cell walls, many fungi and some algae, is the most biopolymer existing on Earth. Moreover, several studies reported that some microbial cells such as *Acetobacter*, *Agrobacterium*, *Rhizobium*, and *Sarcina* strains could produce cellulose (Jonas and Farah, 1998). The composition of cellulose is an assembly of β-1,4-linked D-glucose (glucan) and its degree of polymerization varies from 100-15,000 glucose units with the crystallized long linear chains of microfibrils (Koyama *et al.*, 1997). Due to cellulose's unique structure, which is quite different from the common synthetic polymers and patterned by linkage of repeated glucose building blocks, the highly functionalized, the linear stiff-chain homopolymer is characterized by its biodegradability, hydrophilicity, chirality and broad chemical-modifying capacity (Klemm *et al.*, 2006). Cellulose is insoluble in water and most organic solvents. However, concentrated acids treatment at high temperature could result in chemical breakdown of its structure into glucose units.

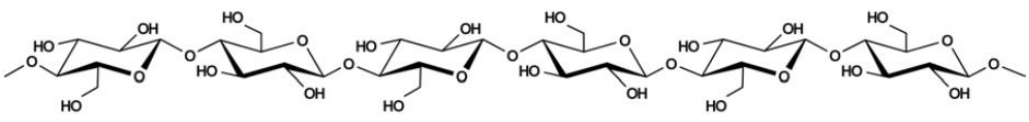


Figure 2.1 Structure of Cellulose (Gardner and Blackwell., 1974)

The cellulose's structure which indicated by its difference in polarity of the cellulose chains, has at least two major types. They are cellulose I and cellulose II. The backbone conformations of both celluloses are essentially identical (Gardner and Blackwell., 1974). Cellulose I is exclusively parallel chains and found synthesized in nature. The appearance of parallel conformation has been proved by many approaches, for example, electron diffraction, enzymatic degradation, and silver labeling of the reducing ends (Koyama *et al.*, 1997). The cellulose I allomorph is the thermodynamically metastable structure of cellulose (Ranby, 1952). Similar to cellulose I, cellulose II can be produced by natural organisms or under specific conditions; however, is generally generated by a chemical treatment or irreversible process of cellulose I. From the change of its form arrangement, the structural matrix of cellulose II is more thermodynamically stable than cellulose I and the glucan chains of this allomorph are arranged in an antiparallel manner (Saxena and Brown, 2008).

Recent research and development fields are focused on cellulose based polymers. The interests of these polymers are more functional groups, combinations of different functional groups, new functionalities as well as substituents with special properties. The growing tendency of cellulose based polymers development following to the fact that cellulose, as the main component of plant cell wall, is renewable.

In the past, cellulose can be produced from sources such as several kinds of wood or the highly pure sources such as cotton (see in Table 2.1). Because of the by-products containing in cellulose, the possibility of application problems and difficulties in chemical modifications are occurred. However, recent isolation and purification processes of cellulose yield materials of high purity and variability (Heinze and Liebert, 2001).

Table 2.1 Chemical composition of some cellulose source (Heinze and Liebert, 2001)

Source	Composition (%)			
	Cellulose	Hemicellulose	Lignin	Extract
Wheat straw	30	50	15	5
Bagasse	40	30	20	10
Softwood	40-44	25-29	25-31	1-5
Hardwood	43-47	25-35	16-24	2-8
Flax (retted)	71.2	20.6	2.2	6.0
Jute	71.5	13.6	13.1	1.8
Henequen	77.6	4-8	13.1	3.6
Ramie	76.2	16.7	0.7	6.4
Cotton	95	2	0.9	0.4

2.2 Bacterial Cellulose (BC)

Unless cellulose can generated by isolation or purification from plants, cellulose can also be synthesized by some microbial cells such as *Acetobacter*, *Agrobacterium*, *Rhizobium*, and *Sarcina* strains. However, only *Acetobacter* strains produce interesting amount of cellulose. *Acetobacter xylinum* was given a special attention first by Brown in 1886 (Jonas and Farah, 1998). *A. xylinum* which has an ability to synthesize a large amount of excellent quality of cellulose formed as twisting ribbons of microfibrillar bundles, is a simple Gram-negative bacterium (Czaja *et al.*, 2006).

The process of bacterial cellulose biosynthesis is shown in Figure 2.2. Various carbon compounds such as glucose, other simple carbohydrates, alcohols or polyalcohols, are used as the nutrition medium to produce uridine diphosphate-glucose (UDPGlc), the cellulose precursor, and then it is polymerized into linear β-1,4-glucan chains and finally secreted outside the cells. The nascent chain association of the β-1,4-glucan chains organizes outside of the cell. First, subfibrils locate on their outer membrane, then a large amount of nascent chains become microfibrils, and finally bundles of microfibrils consisting of individual cellulose chains about thousands (Ross *et al*., 1991). According to the danger in environment, bacteria build BC and confine themselves in it to protect themselves from enemies and heavy-metal ions while nutrients can be supplied by diffusion (Sangrungraungroj, 2003).

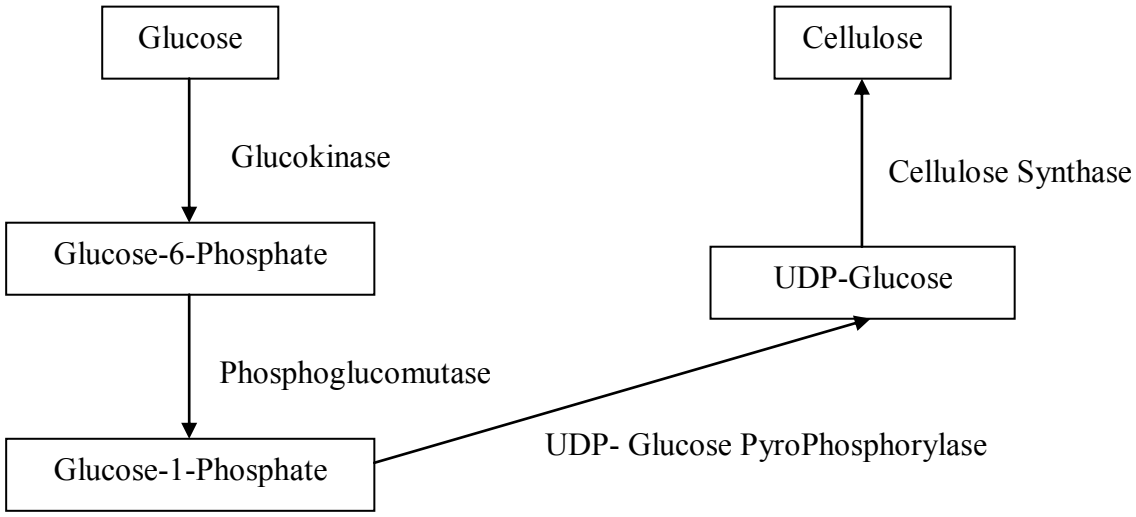


Figure 2.2 Proposed biochemical pathway for cellulose synthesis in *A. xylinum* (Ross *et al*., 1991)

BC is traditionally synthesized in form of white gelatinous pellicle which floates on the surface of the liquid medium in a static culture. Cellulose nanofibrils produced by bacteria are 3-8 nm in diameters (see in Table 2.2). Futhermore, the mesh of these fibrils forms a gelatinous membrane. The size of bacterial fibrils is smaller than that of plant cellulose about 100 times. Both of them are shown in Figure 2.3 (Czaja *et al*., 2006). From Figure 2.4, the swollen bacterial cellulose expresses that there is random association of microfibrils of less than 100 °A diameter in bacterial cellulose's structure. The structure of microfibril could be as simple as drawn in Figure 2.5 compared to the "fringed micelle" of vegetable fibres which were visualized in some old day studies. In fact, bacterial cellulose microfibril is the finest natural structure with long chain molecules extended in idealized parallel form; that is to say, several researches have been interested in the efforts of developing super-strong fibres and growing extended chain single-crystals of BC in the last few decades (Iguchi et al., 2000).

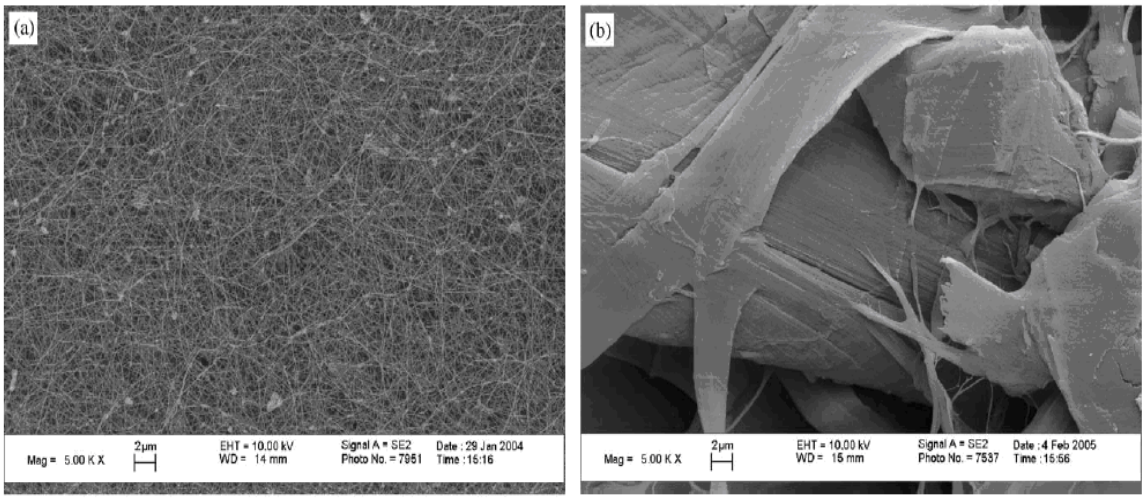


Figure 2.3 A comparison of microfibrillar organization between bacterial cellulose (a) and Wood pulp (b) at the same magnification (Czaja *et al*., 2006)

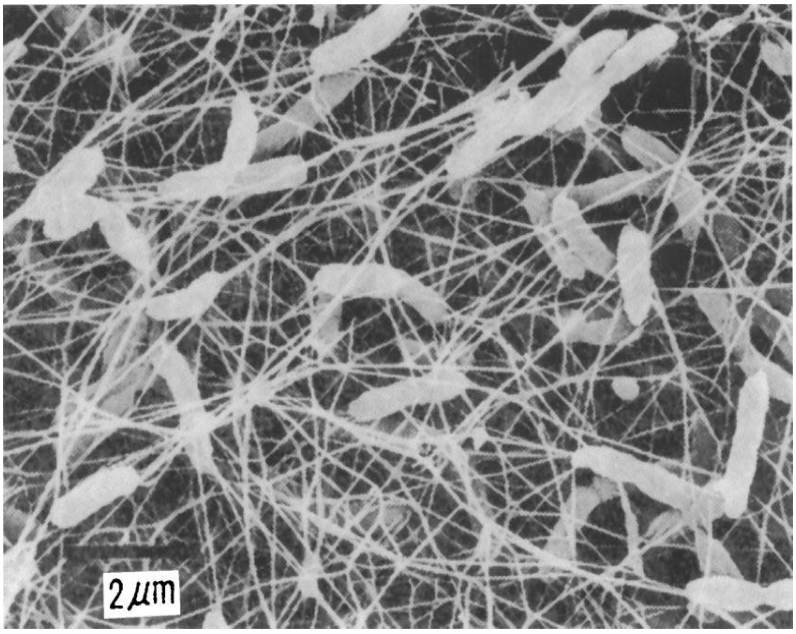


Figure 2.4 A scanning electron micrograph of freeze-dried surface of bacterial cellulose gel (Iguchi et al., 2000)

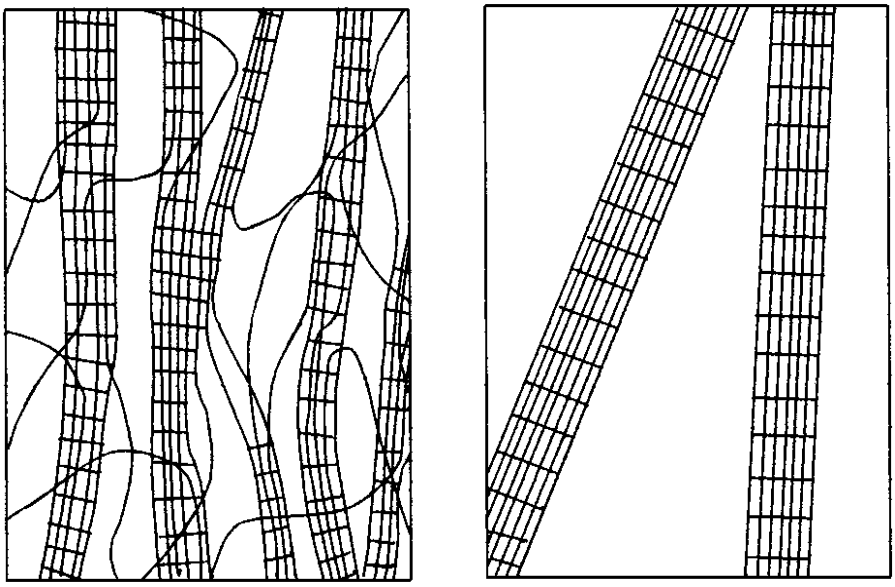


Figure 2.5 Schematic models of BC microfibrils (right) drawn in comparison with the fringed micelles (Iguchi et al., 2000)

Microbial strains which produce many structures of cellulose, is presented in Table 2.2. The BC structure depends on the kind of organism. *Acetobacter xylinum* strain was reported that most representative and high quality bacterial cellulose producer.

Table 2.2 Effect of microorganisms on cellulose producers (Jonas and Farah, 1998)

Organisms(genus)	Cellulose produced	Biological role
Acetobacter	Extracellular pellicle Cellulose ribbons	To keep in aerobic environment
Achromobacter	Cellulose fibrils	Flocculation in wastewater
Aerobacter	Cellulose fibrils	Flocculation in wastewater
Agrobacterium	Short fibrils	Attach of plant tissues
Alcaligenes	Cellulose fibrils	Flocculation in wastewater
Pseudomonas	No distinct fibrils	Flocculation in wastewater
Rhizobium	Short fibrils	Attach of plant tissues
Sarcina	Amorphous cellulose	Unknown
Zoogloea	Not well defined	Flocculation in wastewater

Cellulose from plants is usually a mixture with hemicellulose, lignin, pectin and biogenetic products. On the other hand, BC is free of other bio-products. The unique nano-structure of BC could result in a larger surface area. Consequently, the major advantages of BC, which differ from planted cellulose, are its excellent mechanical strength, high crystallinity, fine fibre network structure and especially remarkable water holding capacity. It is extremely hydrophilic absorbing 60 to 700

times its weight in water. From above reasons, BC has been used for several applications such as food supplement, modified paper pulp, membranes, high performance speaker diaphragms, paint thickeners and biomedical applications like artificial skin, artificial bone, tissue engineering and others (Jung *et al.*, 2005; Keshk and Sameshima, 2006; Suwanmajo, 2006).

2.3 Mangosteen

Mangosteen (*Garcinia mangostana* Linn.) is a tropical fruit tree in plant family "Guttiferae". It is planted widely in area of Asia such as India, Srilanka, Myanmar, Malaysia, Indonesia, Philippines and especially Thailand. The fruit rind of *G. mangostana* has been used as traditional medicines of these countries, for treatments for abdominal pain, diarrhoea, dysentery, leucorrhoea, gonorrhoea, skin infection, wounds, etc. Besides, the extract from fruit rind of *G. mangostana* has been documented for inflammatory inhibition, antitumor ability, antioxidant activity, and antibacterial activity (Yu *et al.*, 2007).

2.3.1 Characteristics of Mangosteen

Mangosteen, a fruit tree widely planted in tropical climate, is suitable to be grown in warm condition. In general, the mature plant of mangosteen will be killed by cold weather (temperature below 4 ^{o}C). Full grown tree produces an average yield about 500 fruits. When the fruit tree is 30 years old, the harvest of mangosteen steadily increases up to 1,000-2,000 fruits. Mangosteen trees are cultivated in the eastern region of Thailand. For example, Rayong, Chanthabuti and Trat, that is fruitful from April to June. In addition, the southern region is also proper for

cultivated area such as Chumphon, Surat Thani and Nakhon Si Thammarat, which produce fruits from June to September (Kosem, 2008).

The fruit appearance of mangosteen is similar to tangerine for size and shape and has 5-8 fruit segments. The fruit peel is hard, dark purple or reddish when ripe and thickness of mangosteen pericarp is about 6-10 mm. When the fruit can be eaten and seeds have finished developing, the color and softening of mangosteen rind will change which are the natural processes of ripening. The edible pulp is white-colored, soft and juicy. It has a slightly acid and sweet flavor. The fruit of mangosteen is usually composed of 1-3 seeds. The fruit tree is 6-25 m in height, pyramidal crown or conical shape, and slows to grow. Mangosteen has simple ovate-elliptic-oblong leaf. Its width and length are 6-11 cm and 15-25 cm, respectively. The leaf is leathery and glabrous, dark green above and dull pale green beneath. Flower is a unisexual-dioecious or polygamous type. Before the mature plant produce fruits, yellowish green with red edges or almost entirely red flower will bloom in uppermost leaf-axil (Satyavati *et al.*, 1976; Farnsworth and Bunyapraphatsara, 1992; Jung *et al.*, 2006).

2.3.2 Phytochemical Composition

Recenly, many researches reported that the extract from rind of *Garcinia mangostana* has many complex phenolic compounds such as xanthones, flavonoids, tannins, and other bioactive substances (Phothitirat *et al.*, 2009). Owing to the existense of botanical substances, there are several studies which interested in various medical benefits of mangosteen.

2.3.2.1 Phenolic compounds

Phenolic compounds, the most important substance of secondary metabolites which are produced by mechanism of all plants to prevent them from disease, consist of at least one aromatic ring (C6) binding with one or more hydroxyl groups. They can be divided into several different groups which depend on the number of constitutive carbon atoms in association with the structure of the basic phenolic backbone (Michalak, 2006). The variety of phenolic compounds content in fruits is a result of the maturity of fruits at harvest, fruits' genetic differences (cultivar), preharvest environmental conditions, post-harvest storage conditions and processing (Zadernowski, 2009). Phenolic compounds are generally founded in plants, vegetables and fruits and may be distributed into 3 major groups:

Table 2.3 The kind of flavonoids and their typical sources (Luapattarakasem, 2005)

Class	Typical sources	Representative (aglycone)
Flavonol	Tea, onions, red wine, fruit	Quercetin
Flavones	Vegetables, citrus fruits	Apigenin
Flavonones	Citrus fruit	Hesperitin
Anthocyanidins	Berries, colored fruit	Cyanidin
Catechins	Tea, wine	Epigallocatechin
Isoflavonoids	Legumes	Genistein

1) Simple phenols or phenolic acid and derivatives are such as gallic acid, ellagic acid, tannic acid, vanillin, catechol, resorcinol and salicylic acid, etc. They are available in many kinds of fruit, for example, raspberry and blackberry.

2) Phenylpropanoids, phenolic compounds which aromatic ring in their structures binding with three-carbon side chain, are such as hydroxycinnamic acids (ferulic acid, caffeic acid or coumaric acid), coumarins (umbelliferone, scopoletin, aesculetin or psoralen), lignans (pinoresinol, eugenol or myristicin). They can be founded in apple, pear, coffee, etc.

3) Flavonoids are the major part of phenolic compounds which have the structure of C6-C3-C6. The flavonoids' types compose of catechin, proanthocyanins, anthocyanidins, flavones, flavonols, flavonones and isoflavones. Researchers reported that flavonoids are antioxidant and chemoprevention which have a lot of benefits to our body and the mechanism of disease prevention (Luapattarakasem, 2005).

Due to the antioxidant activity of phenolic compounds, they are implied to be very important in the part of disease prevention, since they can inhibit or postpone the formation of oxidizing substrate chain reaction (Jung *et al.*, 2006).

2.3.2.2 Xanthones

Xanthones is the natural essential composition consisting considerably in mangosteen. The whole fruit, trunk, branches and leaves of mangosteen have xanthones entirely; although, the content of xanthones in the rind of fruit is higher than other parts (Chaverri *et al.*, 2008). From chemical examination of mangosteen, many chemical compounds in xanthones group are found in its hull such as α-mangostin, β-mangostin, γ-mangostin, mangostatin and gartanin, etc.

The chemical structure of xanthones consists of two benzene rings connected by carbonyl group (C=O) and oxygen. Each ring is conjugated in a fused formation not allowing free rotation about the carbon-carbon bonds. The xanthones' backbone is attached to distinct functional groups at benzene ring in various positions which the difference of functional groups and positions affect to specific functionalities or properties of xanthones (Jantaravinid, 2009).

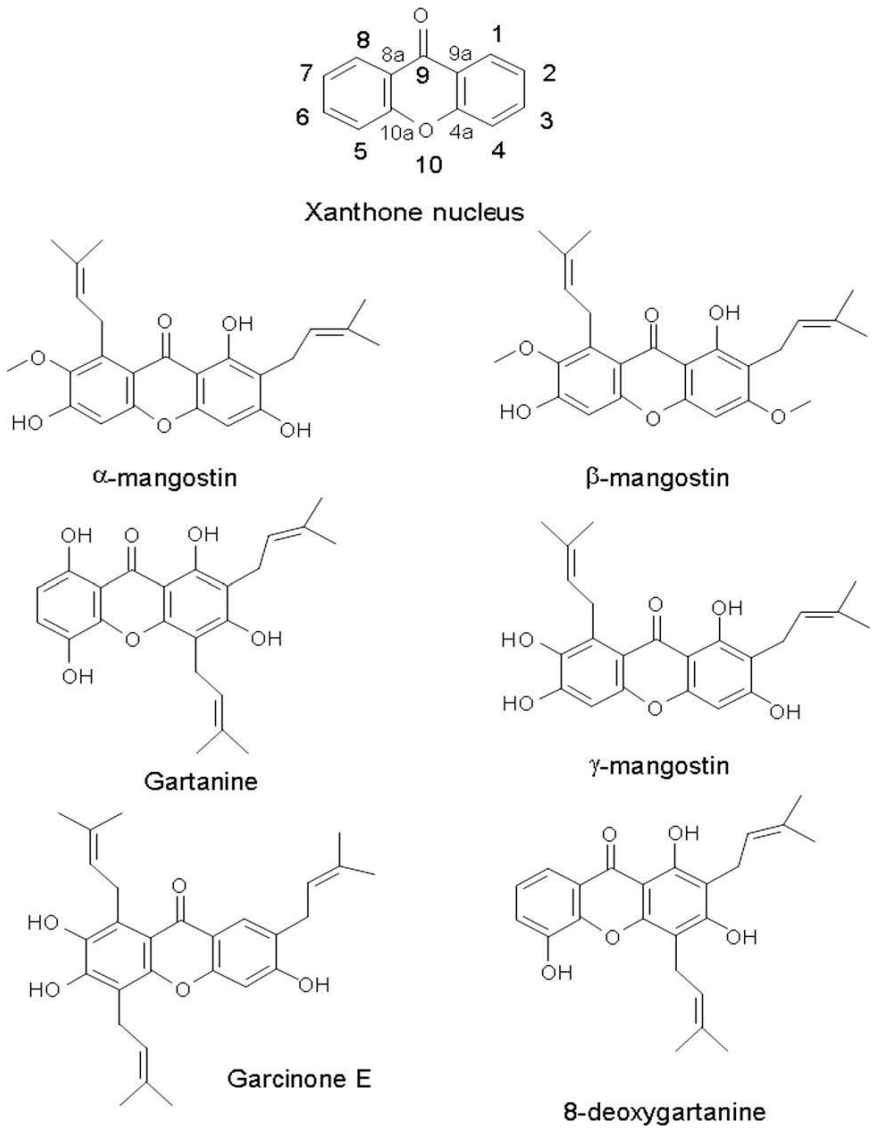


Figure 2.6 Xanthone nucleus (backbone) with IUPAC numbers of carbons and chemical structure of the most studied xanthones (Chaverri *et al.*, 2008)

2.3.2.3 Alpha-Mangostin (α- Mangostin)

Among a lot of bioactive compounds consisting in mangosteen, α-mangostin has been mainly focused on its biological properties. α-mangostin, yellow-colored, is the one of compounds in xanthones. The chemical IUPAC name of α-mangostin is 1,3,6-Trihydroxy-7-methoxy-2,8-diprenylxanthone having molecular weight 410.47 g/mole. Its boiling point is about 180-181 ºC. The solubility of α-mangostin in water is so poor, whereas it solutes clearly in organic solvent, for example, ethanol, chloroform and methanol, etc (Phadungkarn *et al.*, 2009).

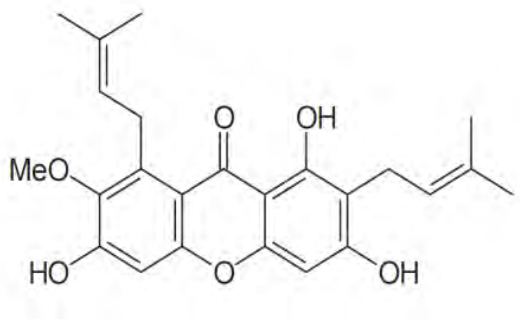


Figure 2.7 Chemical structure of α-Mangostin (Phadungkarn *et al.*, 2009)

According to many researches on biological properties of α-mangostin, it was found to that have anti-inflammatory activity, antibacterial activity (such as *S. aureus*, *P. aeruginosa*, *S. thypimurium*, *B. Subillis*), antifungal activity (such as *E. floccosum*, *A. solani*, *Mucor* sp., *Rhizopus* sp., *C. echinulata*), acne-inducing bacteria inhibitory property (*P. acnes* and *S. epidermidis*), activity against vancomycin resistant *Enterococci* (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA), antioxidant activity (such as The copper-induced LDL oxidation inhibitory property), human leukemia cell line HL60 inhibitory property and so on (Sundaram *et al.*, 1983;

Mahabusarakam *et al.*, 2000; Matsumoto *et al.*, 2003; Chomnawang *et al.*, 2005; Sakagami *et al.*, 2005; Chen *et al.*, 2008).

In 2009, Yodhnu *et al.* studied in the stability of α-mangostin from dichloromethanic extraction. It was found that α-mangostin was stable in these condition: storage at 80 ºC for 3 h, storage under UV-light at 254 or 366 nm of wavelength for 6 h and under 3 N NaOH solution supplementation followed by heating at 80 ºC for 3 h.

Table 2.4 Xanthones isolated from *G. mangostana* pericarp (Chaverri *et al.*, 2008)

Xanthone	**References**
α-Mangostin	Schmid (1855), Yates and Stout (1958) and Stout and Krahn (1968)
β-Mangostin	Dragendorff (1930), Yates and Bhat (1968) and Mahabusarakam *et al.* (1987)
γ-Mangostin	Jefferson *et al.* (1970), Mahabusarakam *et al.* (1987) and Jinsart *et al.* (1992)
Gartanin	Govindachari *et al.* (1971), Mahabusarakam *et al.* (1987) and Asai *et al.* (1995)
8-Deoxygartanin	Gopalakrishnan *et al.* (1997), Govindachari *et al.* (1971) and Huang *et al.* (2001)
Garcinone E	Dutta *et al.* (1987), Sakai *et al.* (1993) and Asai *et al.* (1995)

CHAPTER III

LITERATURE REVIEW

3.1 Medical Application of BC

Biomaterials, natural or synthesized materials which are applied in medical fields, can be produced by using many materials such as biopolymer, synthesized polymer, metallic components, ceramics and others through chemical and/or physical processes. Recently, the major applications of biomaterials are artificial tissue or skin, joint and bone replacement, blood vessels, heart valves, breast implants and contact lenses, etc. The required properties for utilization in biomedical applications are the important consideration under the environments or conditions where biomaterials replace in or on our body. Biomaterials' mechanical properties must be suitable for the body replacement; besides, they must be also compatible with our body called "biocompatibility".

In the present, many kinds of polymeric materials have been investigated for wound dressing application. However, adequate materials which have same properties and functionalities as human skin for the skin, are still be researched. According to the biocompatibility of BC, it can be applied as artificial skin in treating extensive burns (Czaja *et al*., 2006).

Owing to several advantages of BC comparing to plant cellulose, it highly has been focus on its proposing properties especially for further studies in biomedical applications. BC provides many excellent properties, for example, crystalline structure, high water absorption capacity and high mechanical strength in wet state

(Czaja *et al*., 2007). Moreover, it also offers the purity and uniformity (Mayall *et al*., 1990), substantial permeability for liquids and gases, low irritation of skin (Saied *et al*., 2004), low toxicity, chemical stability (Kurosumi *et al*., 2009) and transparency which is the one of required properties for the temporary covering of wounds, allows clinical observer to perceive the continuous healing progress (Saied *et al*., 2004). The main biomedical applications of BC are the following.

The first study about the application of BC was reported by Fontana *et al*. (1990) as temporary skin substitutes. Biofills, the biocellulose wound dressing in commercial grade, provide non-woven, shaped objects in medicine such as artificial arteries, vessels, skin, and etc. Afterwards, Mayall *et al*. (1990) used a Biofill skin substitute in the treatment of trophic ulcerations of the limbs and expressed that this biocellulose material had excellent potentials such as the cicatrisation time shortening, the contamination reducing, and the cost of treatment saving. Furthermore, it has been still utilized for the treatment of several skin injuries, for instance, basal cell carcinoma/skin graft, severe body burns, facial peeling, sutures, dermabrasions, skin lesions, chronic ulcers, and both donor and receptor sites in skin grafts (Czaja *et al*., 2006).

The results from the treatment by artificial skin's BC utilization covering on burn and skin injuries illustrated dramatic clinical results such as immediate pain relief, diminished post-surgery discomfort, faster healing, reduced infection rate and reduced treatment time and cost (Fontana *et al*., 1990). Similarly, Czaja *et al*. (2006) reported that never-dried BC are fully biocompatible and also successfully protected burn wounds from excessive external fluid loss resulting in accelerating the entire process of healing.

Further studies by Kucharzewski group in 2003 reported that BC wound dressing was more effect than Unna's boot in the chronic venous leg ulcers treatment. Alvarez *et al*. (2004) reported that the use of BC in the hydrated form for the treatment of chronic venous ulcers generate more potential than a standard protocol (non-adherent cellulose acetate gauze) in the process of autolytic debridement.

Considering to the lack of antimicrobial activity of BC, many researches have interested in how to improve the ability against microbial strains for medical application. Chitosan was chosen to blend with BC due to its structure is similar to cellulose's structure and its antibacterial activity. Wu *et al.* (2004) prepared membranes of chitosan and cellulose blends by using trifluoroacetic acid as a cosolvent and reported that the modified chitosan/cellulose blend membranes demonstrated antibacterial activity against *E. coli* and *S. aureus*.

Another application of BC is artificial vessels prepared by special technologies. Owing to its mouldability *in situ*, hollow fibers [bacterial synthesized cellulose (BASYC)-tubes] is possible to create as artificial blood vessels and ureters. Artificial vessels are used to replace arteries or veins damaged as a result of tumors or accidents. Because of high mechanical strength in wet state, enormous water retention contents, low roughness of the inner vessel surface, and a vitalization of BASYC, it could interpose microvessel completely in rat experiments. These results demonstrated that BASYC has high potential to use as an artificial blood vessel in microsurgery (Klemm *et al.*, 2001; Saied *et al*., 2004).

In addition, Shi *et al*. (2009) developed nanocomposites (CaDHCAp/BC) consisting of calcium-deficient carbonate-containing hydroxyapatite (CaDHCAp) in the three-dimensional (3D) network of BC nanofibers. The experimental results expressed that the attained nanocomposites has remarkable osteoconductivity and

bioactivity. Similarly, Zimmermann *et al.* (2009) explored BC/hydroxyapatite nanocomposites for bone healing applications prepared by using BC with various surface morphologies (pellicles and tubes) adsorbed carboxymethyl cellulose (CMC) to initiate nucleation of calcium deficient hydroxyapatite (cdHAp). The results demonstrated that the presence of cdHAp crystals on BC surfaces resulted in the increased cell attachment and the cdHAp crystal size increased with increasing nanocellulose fibril density.

3.2 Medical Application of Mangosteen Extract

A lot of tropical plants which have interesting biological activities and abilities to use as therapeutic applications, have been studied on their appearances of antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial, antifungal and antiviral properties. Because mangosteen was used as a traditional medicine for the treatment of abdominal pain, diarrhea, dysentery, infected wound, suppuration, and chronic ulcer in some South and Southeast Asia countries, so many researchers have been interested in analyzing the composition of bioactive substances in various parts of mangosteen such as edible pulp, peel, branches, leaves and so on. It was then investigated for biological activities. Mangosteen is the rich sources of xanthones, a class of phenolic compounds, which have potentials in the medicinal treatment. The most studied xanthones are α-, β-, and γ-mangostins, garcinone E, 8-deoxygartanin, and gartanin (Chaverri *et al.*, 2008).

3.2.1 Anti-Inflammatory Activities of Mangosteen Extract

Currently, there are many interests in the cares of skin. The major issue of teenagers is acne, the cause of skin inflammation, with occurred on various parts of their skin; thus, commercial products and scientific researches have focused on this problem for a while. *P. acnes* has been reported to be the important origin in the chronic inflammatory disease of the pilosebaceous follicle or acne vulgaris by being the stimulator of the production of pro-inflammatory cytokines. Owing to the ability of *P. acnes* to cause cytokinesis, the release of reactive oxygen species which is highly harmful to the cells, is generated. The destructive phenomenon leading to scarring will be perceived when these inflammatory mediators are excessive in the cells environment. Chomnawang *et al.* (2007) studied the anti-inflammatory activity of Thai medicinal plants, *G. mangostana*, *H. cordata*, *S. siamea*, *E. odoratum*, and S. *alata*, in terms of free radical scavenging and cytokine reducing activity. The results indicated that *G. mangostana* exists the most striking antioxidant activity at IC_{50} of 6.13 μg/ml. Similar to the DPPH radical scavenging test, the reducing of ROS production by using *G. mangostana* was remarkably highest with inhibitory ratio at 77.80±1.28% in comparison to *H. cordata*, *S. siamea*, *E. odoratum*, and *S. alata* which showed moderate antioxidant and inhibitory activity. Moreover, *G. mangostana* extract could significantly reduce the TNF-α production produced from PBMC by stimulating with *P. acnes*.

According to the study of Chen *et al.* (2008), it suggested that α- and γ-mangostins could significantly decrease PGE_2 levels through inhibition of COX-2 activity and NO production. The two mangostins were isolated from the fruit rinds of *G. mangostana*, and their anti-inflammatory activities were examined. The results showed that the inhibitory abilities of α-mangostin were weaker than γ-mangostin. α-

and γ-mangostins could inhibit NO and PGE_2 production and iNOS expression by LPS-stimulated RAW 264.7 cells; however, iNOS activity and COX-2 expression were not inhibited by α-mangostin or γ-mangostin.

3.2.2 Antibacterial Activities of Mangosteen Extract

Chomnawang *et al.* (2005) conducted the experiments about antimicrobial activities of Thai medicinal plants against agents of acne vulgaris (*P. acnes* and *S. epidermidis*). The antimicrobial activities of crude extracts of 19 medicinal plants were investigated by disc diffusion and broth dilution methods. From the experimental results, *Senna alata*, *Eupatorium odoratum*, *Garcinia mangostana*, and *Barleria lupulina* had strong inhibitory effects; however. *G. mangostana* showed the greatest antimicrobial effect. The minimal inhibitory concentration (MIC) values against both organisms were equal (0.039 mg/ml) and the minimal bactericidal concentration (MBC) values were 0.039 and 0.156 mg/ml against *P. acnes* and *S. epidermidis*, respectively.

Enterococci and *S. aureus* are the leading causes of nosocomial infections in long-term healthcare facilities. Vancomycin resistant *Enterococci* (VRE) and methicillin resistant *S. aureus* (MRSA) infections in hospitals have been increasing worldwide in recent years. The results from Sakagami and co-worker in 2005 expressed that α-mangostin is more effective than β-mangostin for both of anti-VRE and anti-MRSA activities with MIC values of 6.25 and 6.25 to 12.5 mg/ml, respectively. The synergism between α-mangostin which was extracted from the stem bark of *G. mangostana* and commercially available antibiotics were studied. It showed that α-mangostin alone or combination with gentamicin (GM) against VRE

and vancomycin hydrochloride (VCM) against MRSA might be profitable in controlling VRE and MRSA infections.

Under the study of 17 medicinal plants effecting to MRSA strain by disc diffusion method, *G. mangostana* was identified as the strongest MRSA inhibitor with MIC and MBC values of 1.95 and 3.91 μg/ml as a result of the existence of α-mangostin, the prenylated xanthone, in *G. mangostana* extract (Chomnawang *et al.*, 2009).

Tadtong *et al.* (2009) determined the tyrosinase (enzyme for melanin synthesis) inhibitory ability and antibacterial activities against the pathogenic bacteria in the oral cavity of the extract from mangosteen pericarp. The results expressed that the mangosteen pericarp extract inhibited the tyrosinase enzyme at IC_{50} = 67 ng/ml. In addition, the mangosteen pericarp extract also provided the antibacterial activities against the pathogenic bacteria in the oral cavity, *S. mutans* DMST18777, P. *gingivalis* DMST2136, and *S. pyogenes* DMST17020 at minimal inhibitory concentration (MIC) of 0.01 mg/ml, and *S. aureus* ATCC25923 at MIC of 0.1 mg/ml by agar dilution method.

3.2.3 Antioxidant Activities of Mangosteen Extract

In 1994, mangostin isolated from *G. mangostana* was investigated the possible antioxidant effects on metal ion dependent (Cu^{2+}) and independent (aqueous peroxyl radicals) oxidation of human LDL. Mangostin prolonged the lagtime to both metal ion dependent and independent oxidation of LDL in a dose dependent manner over 5-50 μm as monitored by the formation of conjugated dienes at 234 nm ($P<0.001$). There was no significant effect of mangostin on the rate at which conjugated dienes were formed in the uninhibited phase of oxidation. Levels of thiobarbituric reactive

substances (TBARS) generated in LDL were measured 4 and 24 h after oxidation with 5 μM Cu^{2+} in the presence or absence of 50 μM or 100 μM mangostin. From study observation, an inhibition of TBARS formation with 100 μM mangostin at 4 h (P=0.027) but not at 24 h (P=0.163). Similar results were observed in the presence of 50 μM mangostin. Mangostin, at 100 μM, retarded the relative electrophoretic mobility of LDL at both 4 and 24 h after Cu^{2+} induced oxidation. Mangostin (100 μM) significantly inhibited the consumption of α-tocopherol in the LDL during Cu^{2+} initiated oxidation over a 75 minute period (P<0.001) (Williams *et al.*, 1994).

Many benefits and inhibitory mechanisms of *G. mangostana* have been still unknown because few scientific reports are available in the literature. Kosem *et al.* (2007) investigated the methanolic extract from the hulls of *G. mangostana* (GME) for its antioxidant and cytoprotective activities. The results showed that GME composed of phenolic compounds and possessed reducing power as well as Fe^{2+} chelating activity. In this study, nitric oxide and lipid radicals in dose-dependent manners (scavenging DPPH) were used to determine the antioxidant properties of GME. The powerful scavenging activities against hydroxyl and superoxide radicals were especially obtained. Furthermore, GME also increased the cell survival by decreasing the oxidative damage in ECV304 endothelial cells after H_2O_2 exposure.

Owing to antioxidant properties of α-Mangostin isolated from mangosteen fruit, Chaverri *et al.* (2009) studied the reactive oxygen species (ROS) scavenging capacity and the potential protective effect of α-mangostin against the mitochondrial toxin 3-nitropropionicacid (3-NP) in primary cultures of cerebellar granule neurons (CGNs). The results showed that α-mangostin has the scavenging capacity in a concentration-dependent way with singlet oxygen, super oxide anion and peroxynitrite anion. On the other hand, hydroxyl radicals and hydrogen peroxide were

not inhibited by α-mangostin. Moreover, α-mangostin was able to ameliorate in a concentration-dependent way, the neuronal death induced by 3-NP. This protective effect was associated with an amelioration of 3-NP-induced reactive oxygen species formation.

3.2.4 Anticancer Activities of Mangosteen Extract

Matsumoto *et al.* (2003) examined the effects of six xanthones from the pericarps of mangosteen on the cell growth inhibition of human leukemia cell line HL 60 by vitro cytotoxic test at 72 h after the exposure of HL60 cells in extract concentration from 5-40 μM. All xanthones displayed significant growth inhibitory effects. α-, β- and γ-mangostin were particularly effective even at the low dose of 10 μM. Among six xanthones, α-mangostin showed complete inhibition at 10 μM through the induction of apoptosis, so it was further examined for the cell growth inhibitory activity against other leukemia cell lines, namely, K562, NB4 and U937. α-Mangostin showed inhibitory effects markedly all cell lines at 10 μM, especially HL60, NB4 and U937, but K562 cells seemed to be most resistant to it.

On account of antioxidant activity of mangosteen extract, Sun *et al.* (2009) demonstrated the antioxidant activity of mangostin or MAG. In addition, the influence of MAG on K562 cells in 5-aminolevulinic acid (ALA)-based PDT was studied. The results indicated that hydroxyl radical, superoxide anion, and hydrogen peroxide could be scavenged by MAG and MAG inhibited the formation of malondialdehyde (MDA), but increased the amounts of singlet oxygen in cell-free systems. Besides, MAG was inhibitory to K562 cell proliferation and elevated cell apoptosis, lipid peroxidation, and DNA damage in ALA-PDT on K562 cells.

CHAPTER IV

EXPERIMENTAL

4.1 Materials

4.1.1 Microbial Strains

The *A. xylinum* strain AGR60 was isolated from Nata de coco. The stock culture was kindly supplied by Pramote Thammarad, the Institute of Research and Development of Food Product, Kasetsart University, Bangkok, Thailand.

4.1.2 Chemicals

The details of chemicals used in this experiment are shown in Table 4.1

Table 4.1 The chemicals used in this experiment

Chemical	Supplier (country)
Sucrose	Ajax Finechem (Australia)
Ammonium sulfate	Ajax Finechem (Australia)
Sodium hydroxide	Rankem (India)
Acetic acid	QRec (New Zealand)
Mangosteen aqueous extract	Thiptipa (Thailand)
Mangosteen ethanolic extract	Thai-China Flavours and Fragrances Industry (Thailand)
Folin-Ciocalteu reagent	Sigma (Switzerland)
Sodium bicarbonate	Sigma-Aldrich (Germany)

Table 4.1(continue) The chemicals used in this experiment

Chemical	Supplier (country)
Gallic acid	Sigma-Aldrich (China)
α-Mangostin	ChromaDex (USA)
Methanol	QRec (New Zealand)
Ethanol	Mallinckrodt (Malaysia)
Acetone	QRec (New Zealand)
Sodium acetate	Loba Chemie (India)
Tween 80	Rankem (India)
N,N-Dimethylacetamide	Loba Chemie (India)

4.1.3 Equipments

- Scanning electron microscopy, SEM (JOEL JSM-5410LV, Tokyo, Japan)
- Fourier Transform Infrared (FT-IR) spectrometer (Perkin Elmer Spectrum One Massachusetts, USA)
- Universal Testing Machine (Hounsfield H 10 KM, Redhill, England)
- UV-vis spectrophotometer (Shimadzu UV-2550, Tokyo, Japan)
- Oxygen permeation analyzer (Illinois Instruments, Model 8000, Johnsburg, IL)
- Autoclave (Model Tomy Autoclave SS-325, Nerima-ku, Tokyo, Japan)
- Microplate reader (Bio-Rad Benchmark 550, California, USA)

4.2 Culture Media and Method

Cell cultivation was performed in a simple and inexpensive way modified from the procedure developed by Pramote Thammarad (The institute of Food Research and Product Development, Kasetsart University). The medium for the inoculums was coconut-water with 5 g of sucrose, 0.5 g of ammonium sulfate and 1 mL of 30.0%v/v acetic acid. The medium was sterilized at 110 °C for 5 min. The 5% v/v precultures were prepared by transferring 15 mL of a stock culture to 300 mL medium and incubated statically at 30 ^{o}C for 7 days. After that, the 5%v/v preculture broth was added to the main culture medium. The activated main medium (75 mL) was inoculated in a in a 14.5 cm diameter Preti-dish and kept at 30 °C for 7 days.

All sample films were first purified by washing with distillated water for 1 hand then was treated with 1% w/v NaOH at room temperature for 24 h to remove bacterial cells, neutralized by 1%v/v acetic acid for 1 h and then rinsed with DI water until pH became neutral. Then, air-dried at room temperature (30 ^{o}C) for 3-4 days and stored in plastic film at room temperature.

4.3 Preparation of Bacterial Cellulose Films containing *G. Mangostana* Aqueous and Ethanolic Extracts

The purified BC films in wet state were immersed in a solution of aqueous mangosteen extract (purchased from Thiptipa Co., Ltd., Pathumthani, Thailand) with concentrations of 2.5, 5.0, and 10.0%w/v or in an ethanolic extract solution of mangosteen (kindly supported by Thai-China Flavours and Fragrances Industry Co., Ltd., Phra Nakhon Si Ayutthaya, Thailand) with solution concentrations of 0.25, 0.50,

and 1.00%v/v at room temperature for 2 days. The excess content of the extract compounds on BC film surface was removed by rinsing with 20%v/v ethanol solution and then the films were air dried at room temperature for 3-5 days and stored in plastic film.

In this study, BCWM, BCEM and BC film refers to BC film with the mangosteen aqueous and ethanolic extract impregnation, and without, respectively.

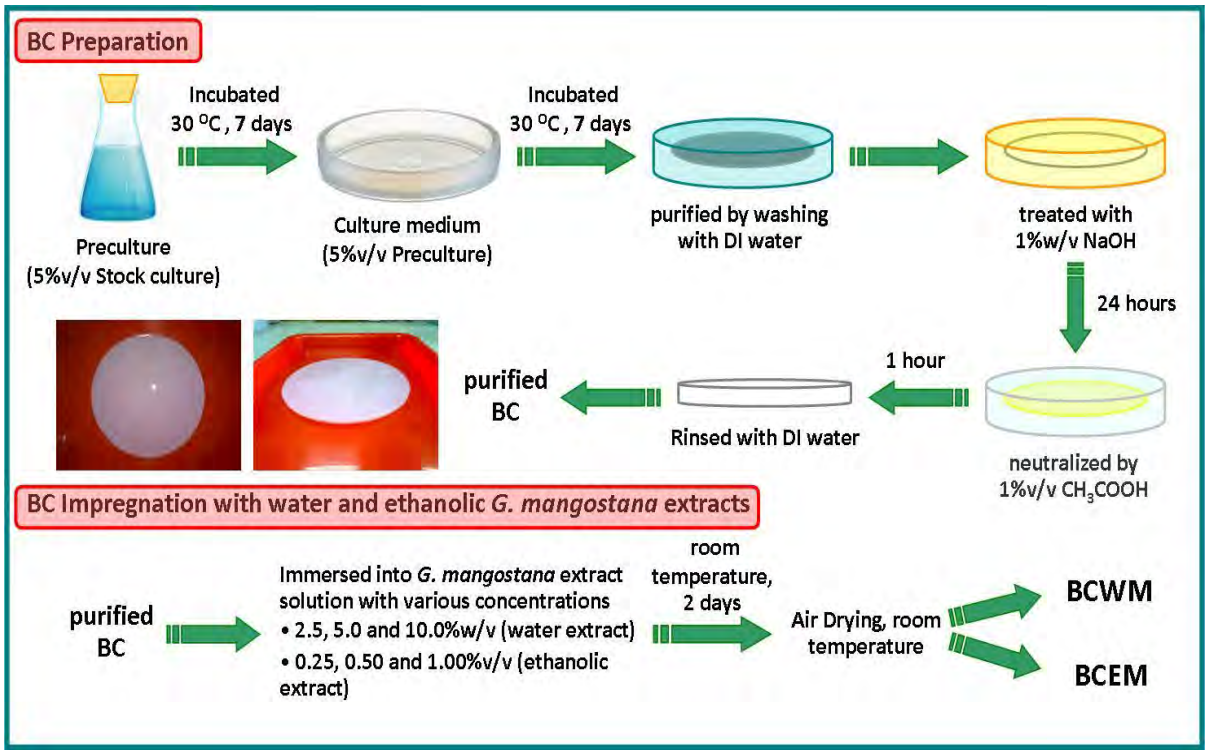


Figure 4.1 Schematic diagram of BC, BCWM and BCEM films preparation

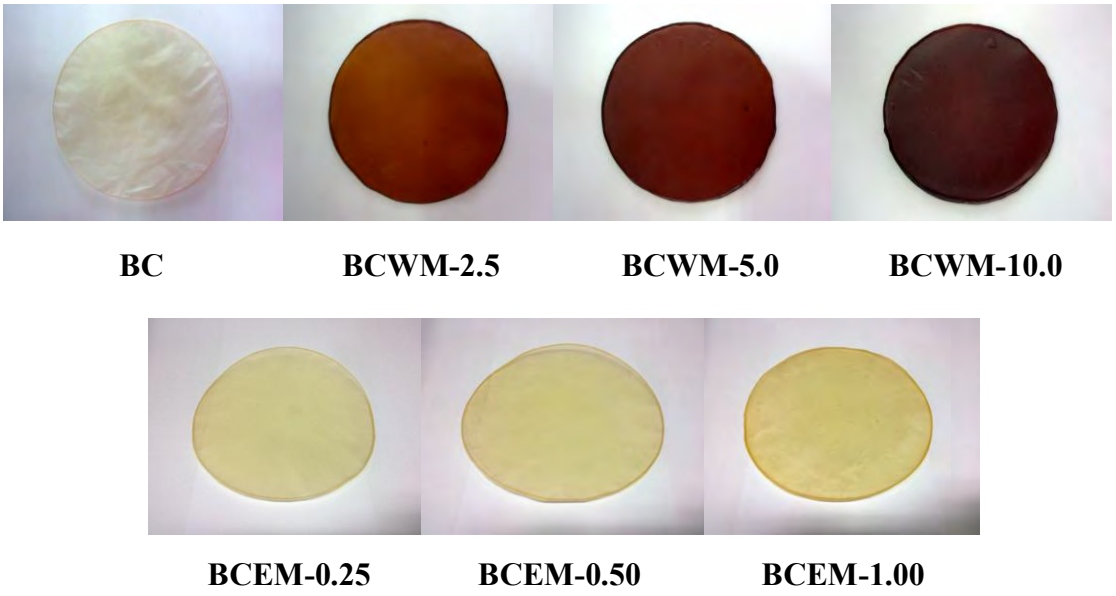


Figure 4.2 Dried BC, BCWM and BCEM films

4.4 Quantitative Determination of Bioactive Compounds Content in The Extract of *G. Mangostana*

4.4.1 Phenolic Compounds Analysis Method

The total phenolic compounds content was evaluated using Folin-Ciocalteu method (Pothitirat *et al.*, 2009). Mangosteen extract sample, 0.4 mL was mixed with 1.6 mL of sodium bicarbonate solution (7.5% w/v) and then was added with 1.0 mL of the Folin-Ciocalteu reagent (previously diluted at 1:10 with deionized water). The mixture was shaken for well-mixing and incubated at room temperature for 30 minutes. The content of total phenolic compounds was calculated as mean (n=3) and expressed as grams of gallic acid equivalents (GAE)/100g of the extract by using a UV–visible spectrophotometer (Shimadzu UV-2550, Tokyo, Japan) measured the absorbance of the mixture at 765 nm.

4.4.2 Mangostins Content Analysis Method

The analytical condition of mangostins content was developed by Pothitirat and Gritsanapan (2008). A stock solution of α-mangostin reference standard was prepared by dissolving 10 mg of α-mangostin in 100 mL of methanol in a volumetric flask. Afterwards, various concentrations of the standard solution were prepared to provide final concentrations at 4, 6, 8, 10 and 12 μg/mL. The absorbances of all samples and the standard solution were measured at 320 nm by UV–visible spectrophotometer (Shimadzu UV-2550, Tokyo, Japan). The mangostins content was calculated as mean (n=3) and expressed as grams of α-mangostin equivalents (AME)/100g of the extract.

4.5 Characterizations of BC, BCWM and BCEM Films

4.5.1 Scanning Electron Microscope (SEM)

The examination of the surface properties was performed by JOEL JSM-5410LV scanning electron microscopy (SEM) (Tokyo, Japan) at Scientific and Technological Research Equipment Centre, Chulalongkorn University. The BC, BCWM and BCEM films in dried form were sputtered with gold in a Balzers-SCD 040 sputter coater (Balzers, Liechtenstein). The accelerating voltage was adjusted to 15 kV. The specimens were examined at magnification 10,000X for surface morphology and both 10,000X and 3500X for cross sectional morphology.

4.5.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is used primarily to identify the chemical structure of the samples (BC film, BCWM and BCEM films with different concentrations of

mangosteen aqueous and ethanilic extract content, *G. mangostana* aqueous extract (powder), and *G. mangostana* ethanolic extract (solution)). All samples were ground (only solid state) into small pieces, mixed with Potassium bromide (KBr) in the ratio 1:100 by weight (sample 1 mg/KBr 100mg) and then compressed into pellet. FTIR spectra of the developed film were recorded with Perkin Elmer (Spectrum One, Massachusetts, USA) in the region of 4000–450 cm^{-1} and the resolution equal to 4.0 cm^{-1} at Scientific and Technological Research Equipment Centre, Chulalongkorn University.

4.5.3 Mechanical Testing

The tensile strength and elongation at break of BC, BCWM and BCEM dried films were measured by Universal Testing Machine (Hounsfield H 10 KM, Redhill, England) at Scientific and Technological Research Equipment Centre, Chulalongkorn University. The load cell capacity was 1 kN. The film samples were cut into strip-shaped specimens 10 mm width and 10 cm length. The test conditions followed ASTM D882 as a standard test method for tensile elastic properties. Two ends of the specimens were placed between the upper and lower jaws of the instrument, leaving a length of 6 mm of film in between the two jaws. Extension speed of the instrument was 10 mm/min. The tensile strength and break strain were the average value determined from at least five specimens.

4.5.4 Water Absorption Capacity (WAC)

Water absorption capacity (WAC) was determined by immersing the preweighted of dried BC, BCWM and BCEM films in distilled water at room temperature until equilibration. The film was then removed from the water. After

excess water at the surface of the film was blotted out with Kim wipes paper, the weight of the swollen film was measured and the procedure was repeated until there was no further weight change. Water content was calculated using the following formula:

$$WAC(\%) = \frac{W_h - W_d}{W_d} x100$$

Where W_h and W_d denoted the weight of hydrate and dry membrane, respectively.

4.5.5 The Oxygen Permeability Measurement

Oxygen transmission rate (OTR) of the dried BC, BCWM and BCEM films was determined with a oxygen permeation analyzer: Illinois Instruments (Johnsburg, IL) Model 8000 at Thai packaging centre, Thailand Institute of Scientific and Technological Research. The test condition followed ASTM D3985. The determination of OTR was done at 23 ^{o}C and 0% relative humidity. The film was held in such a manner that it separated two side of test chamber. One side was exposed to a nitrogen atmosphere. Testing was completed when the concentration of oxygen in the nitrogen side was constant.

4.5.6 The Water Vapor Permeability Measurement

Water vapor transmission rate (WVTR) of the BC, BCWM and BCEM films with area of 50.00 cm^2, was measured at Thai packaging centre, Thailand Institute of Scientific and Technological Research. The test conditions followed ASTM E-96 with desiccant method. The determination of WVTR was done at 38 ^{o}C and 98% relative humidity. The test specimen was sealed to the open mount of test dish containing a

desiccant, and the assembly placed in a controlled atmosphere. Periodic weighting was performed to determine the rate of water vapor movement through the specimen into the desiccant.

4.5.7 Antibacterial and Antifungal Test

The antimicrobial properties of BC, BCWM, and BCEM films were examined against 6 pathogenic bacterial and fungal strains: *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Aspergillus niger (A. niger*), *Staphylococcus epidermidis* (*S. epidermidis*), *Propionibacterium acnes* (*P. acnes*), and *Candida albicans* (*C. albicans*). The film samples were cut into round-shaped sample of 38 mm diameter according to the method described by AATCC TM 39-1989. The incubation was 24 h at 37 °C under aerobic conditions for *E. coli*, *S. aureus*, *S. epidermidis*, *and C. albicans* while the inoculated plates of *P. acnes* was incubated anaerobically at 37 °C for 72 h and the test of *A. niger* was performed in the AGAR plate for a week of inoculation at 30 °C. Before the antibacterial and antifungal assay, all BC, BCWM, and BCEM films were sterilized by UV irradiation for 20 min in each side.

4.6 Release of Bioactive Compounds from BCWM and BCEM Films

4.6.1 Preparation of Acetate Buffer

Acetate buffer was used to simulate the human skin pH condition of 5.5. For 1000 ml acetate buffer solution preparation, 150 g of sodium acetate was dissolved in 250 ml of distilled water and then exactly 15 ml of glacial acetic acid was added very

slowly into the sodium acetate aqueous solution. Finally, distilled water was added into the solution to fill the volume.

4.6.2 Actual Bioactive Compounds Content

Actual amount of bioactive compounds content in the BC films containing aqueous (BCWM) and ethanolic (BCEM) extracts of *G. mangostana* were determined. Each specimen (square plate; 2.5 x 2.5 cm^2) was immersed 2:1 v/v acetone/dimethylacetamide (DMAc) solution at room temperature (30°C). After that, the solution was collected and the actual amount of bioactive compounds content were measured by Shimadzu UV-2550 UV-vis spectrophotometer at the wavelength of 765 nm for phenolic compounds and 320 nm for mangostion following analytical methods which were previously described in part 4.4.

4.6.3 Bioactive Compounds Release Assay

The release characteristics of bioactive compounds from the BC films containing aqueous and ethanolic extracts of G. mangostana were investigated by total immersion (Suwantong *et al.*, 2007). The B/T/M releasing medium (96.5%v/v acetate buffer with 0.5%v/v Tween 80 and 3%v/v methanol) was used. Each specimen (square plate; 2.5 x 2.5 cm^2) was immersed in 30 ml of the medium at the temperature of 37 ^oC. At a specified immersion period ranging from 0 to 48 h (2880 min), either 1 ml of a sample solution was withdrawn and an equal amount of the fresh medium was refilled. The amounts of bioactive compounds in the sample solutions were determined using the UV-vis spectrophotometer at the wavelength of 765 nm for phenolic compounds and 320 nm for mangostion.

4.7 Cytotoxicity Study of BC, BCWM and BCEM Films

In this study, cytotoxicity study of the BC, BCWM and BCEM films against B16 mouse melanoma cells, dermal cancer cells, were kindly evaluated by Dr.Pongpun Siripong, Jantana Yahuafai and Kitiya Rassamee at Natural Products Research Section, Research Division, National Cancer Institute of Thailand.

4.7.1 Cell Proliferation Assay

The air-dried BC, BCWM and BCEM films were punched into round-shaped samples of 14 mm diameter. The samples were sterilized by autoclaving at 121 °C for 15 min and transferred aseptically to 24-well culture plates. The experiments were conducted in triplicate. One milliliter of DMEM culture medium with 10% fetal calf serum was added to each well to equilibrate the samples for 30 min before cell seeding. Briefly, B16 mouse melanoma cells were seeded into 24-well culture plates (Costar, Corning, NY) at an initial density of 3×10^4 cells per well on the BC, BCWM and BCEM films, and the control without BC membrane. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h. Then, the culture medium was removed and replaced for another 3 h by melanoma B16 complete growth medium and serum-free DMEM for the cultures of B16 mouse melanoma cells. The number of living cells was determined using the MTT assay.

4.7.2 MTT Assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was used to determine the amount of purple formazan crystals formed which could indicate the number of viable cells. First, the culture medium was aspirated, washed

the PBS and replace with 20 µL MTT solution [(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide), 5 mg/mL in PBS]. After that, the plate was incubated at 37^{o}C for 3 h. After centrifugation at 2,200 rpm for 5 minutes at 4 ^{o}C, the medium was aspirated and the formazan product was dissolved in 100 µL DMSO in each well. The absorbance was measured using a Microplate reader (Benchmark 550, Bio-Rad, USA) at 550 nm wavelength.

CHAPTER V

RESULTS AND DISCUSSIONS

The bacterial cellulose (BC) was generally synthesized by *Acetobacter xylinum* in form of a pellicle which floated on culture medium in static condition. Owing to its excellent mechanical properties, BC has been used for various applications especially biomedical application. Generally, BC has no antimicrobial activity in itself. Previously, there have been many attempts to generate antimicrobial activity of this biocompatible and nontoxic material by the supplementation of antimicrobial agents, but these agents is not easy to decompose in the environment and have toxicity to human. In this present work, in order to enhance antimicrobial activity of the BC film, the crude aqueous and ethanolic extracts from fruit rind of *Garcinia mangostana* was impregnated into the BC film at various concentrations. The BC films modified by supplementation of the aqueous extract (BCWM) and modified by supplementation of the ethanolic extract (BCEM) were then characterized for the changes of physical and biological properties, for example, surface and cross sectional morphologies, chemical structure and interactions, tensile properties, water absorption capacity, water vapor permeability, oxygen permeability, antibacterial activities and antifungal activities, comparing with the unmodified BC film (without *G. mangostana* extract). In addition, the cytotoxic effects of BCWM and BCEM films on B16 mouse melanoma cells (dermal cancer cells) were also studied.

5.1 Bioactive Compounds Analysis in *G. Mangostana* Aqueous and Ethanolic Extracts

Before the modification of BC film by loading *G. Mangostana* aqueous and ethanolic extracts with various concentrations, both *G. Mangostana* aqueous and ethanolic extracts were analyzed by UV-Vis spectroscopic technique for determining the contents of bioactive compounds consisting in those extracts. Two major bioactive compounds were measured for quantitative analysis in this present work: phenolic compounds which were expressed by gallic acid equivalents (GAE) and mangostins, principal compounds in xanthones, which were revealed by alpha-mangostin equivalents (AME). The appearance of *G. Mangostana* aqueous extract was a dried powder. Meanwhile, *G. Mangostana* ethanolic extract which kindly provided by Thai-China Flavours and Fragrances Industry Co., Ltd., was a liquid solution. Therefore, the contents of bioactive compounds in *G. Mangostana* aqueous and ethanolic extracts were shown in the weight of bioactive compounds per 1 g of aqueous extract and 1 mL of ethanolic extract, respectively.

Table 5.1 The contents of bioactive compounds in *G. Mangostana* aqueous and ethanolic extracts

Extracts	Phenolic compounds	Mangostins
Water	198.88±10.81 (mg GAE/ g extract)	20.98±1.69 (mg AME/ g extract)
Ethanol	34.11±0.65 (mg GAE/ mL extract)	31.98±0.49 (mg AME/ mL extract)

When *G. Mangostana* aqueous extract was analyzed by UV-Vis spectroscopy, the result expressed that *G. Mangostana* aqueous extract composed of mangostins 20.98±1.69 mg AME/ g extract. In fact, mangostins were non-polar phenolic compounds and the solubility of mangostins in water is so poor or not soluble (Phadungkarn *et al.*, 2009; Nguyen and Marquis, 2011), so the content of mangostins could not be detected in aqueous extract. From the study of Ngawhirunpat *et al.* (2010) on different solvent extracts from the fruit hull of mangosteen, there was no mangostins in water fraction, but it could be found in methanol and hexane extracts. Similarly, the HPLC chromatogram of *G. Mangostana* aqueous extract did not displayed a main peak of alpha-mangostin at approximately 21 min of the retention time in this study (see the HPLC chromatograms of standard and *G. Mangostana* extracts in Appendix (Figures3-6). Thus, the content of mangostins in aqueous extract obtained from UV-Vis spectrophotoscopic measurement might be some phenolic compouds which their structures were close to mangostins or have the same functional groups as mangostins, but not alpha-mangostin.

Tannin, polyphenolic compounds found in the plant kingdom, can be divided into two groups by their structures: hydrolysable and condensed tannins. Gallic acid is a one of hydrolysable tannins and as part of tannic acid molecule as shown in Figure 5.1 (Bajpai and Patil, 2008; Sariözlü and Kivanç, 2009; Moosophin *et al.*, 2010). Moreover, tannins could be highly soluble in water and they were also the major compounds in *G. Mangostana* aqueous extract (Ngawhirunpat *et al.*, 2010). In consequence, the content of phenolic compounds in *G. Mangostana* aqueous extract which was shown as gallic acid consisting in aqueous extract equal to 19.89±1.08 %w/w extract, should be imply to the content of tannins or other simple phenolic compounds in aqueous extract.

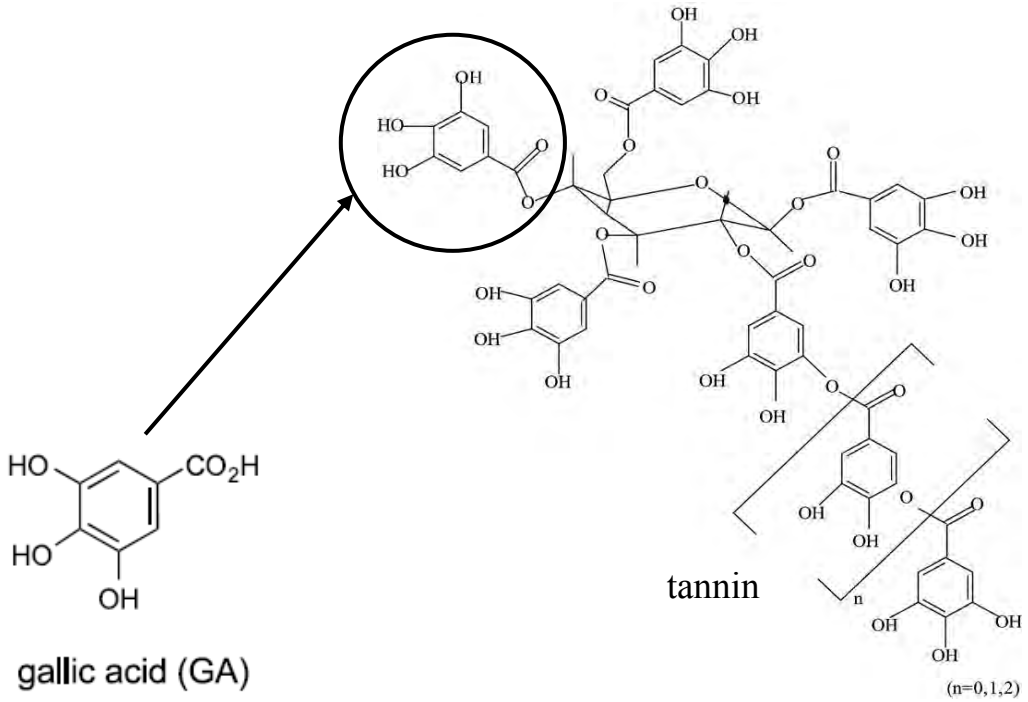


Figure 5.1 Chemical structures of gallic acid and tannin (Lü *et al*, 2004; Agarwal *et al.*, 2006)

Mangostins are belong to phenolic compounds in *G. Mangostana* ethanolic extract. Owing to the high content of mangostins comparing with the phenolic compounds (31.98±0.49 mg AME/ mL extract and 34.11±0.79 mg GAE/ mL extract, respectively), *G. Mangostana* ethanolic extract could be indicated that composing of mangostins principally. Similar to the HPLC analysis, the content of mangostins in ethanolic extract was 31.95±0.77 mg AME/ mL extract, so this ethanolic extract was rich of mangostins. Due to the non-polarity of mangostins which were the major compounds in *G. Mangostana* ethanolic extract, mangosteen ethanolic extract might be defined as hydrophobic component (Nguyen and Marquis, 2011).

5.2 Characterization of BC Films Modified by Supplementation of *G. Mangostana* Aqueous Extract (BCWM)

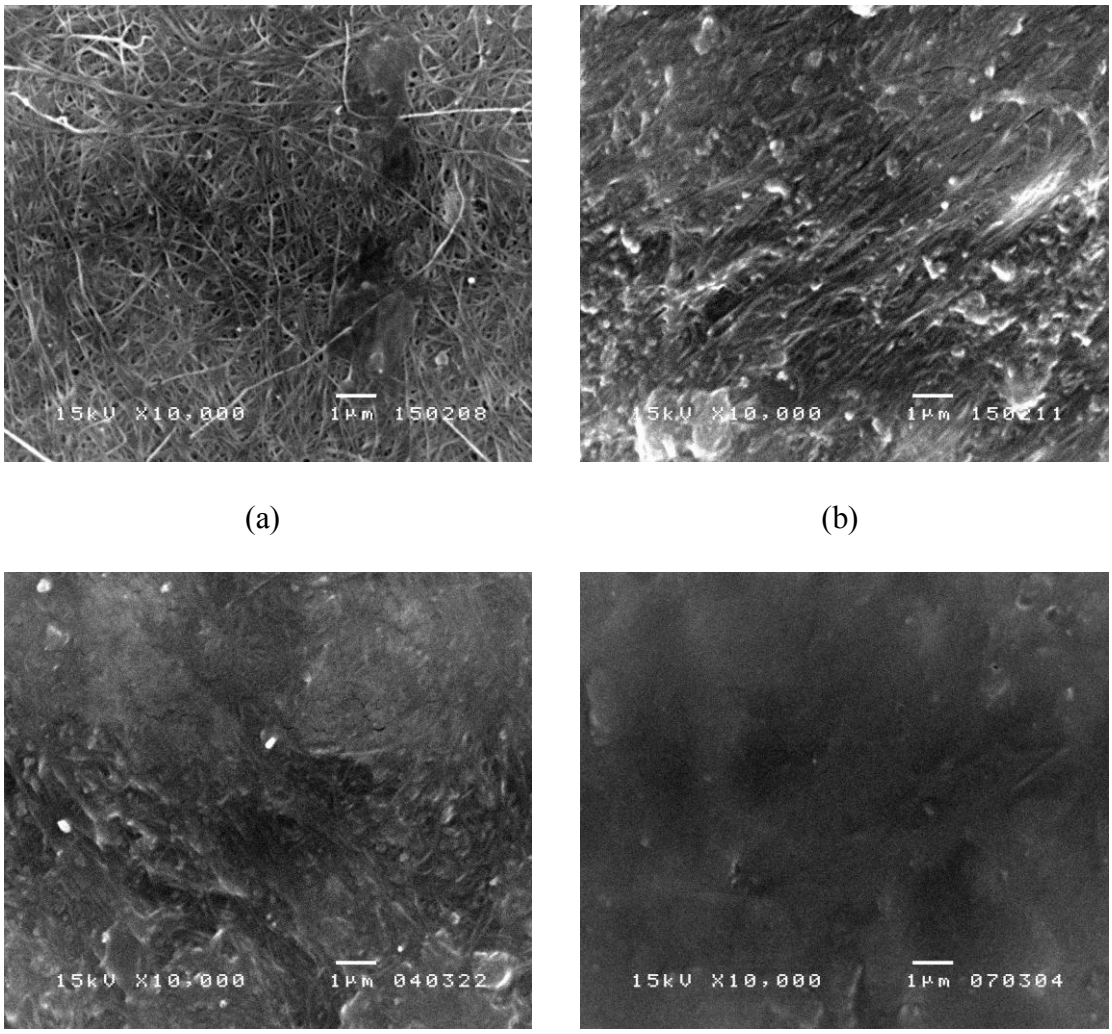
The characteristics of modified BC films prepared by aqueous *G. mangostana* extract impregnation were investigated for physical and biological properties in this part. All of modified BC films prepared by means of immersion of film samples in mangosteen aqueous extract solution were referred as BCWM-n, where n is the concentration of *G. mangostana* aqueous extract solution (%w/v) used for the BC films immersion.

5.2.1 Morphology

Scanning electron microscope (SEM) technique widely used for revealing the fine biological structure was applied to study the morphologies of BC and Modified BC films prepared by *G. mangostana* extract impregnation in this study. Figure 5.2 shows the surface morphologies of BC and BCWM films at 10,000 magnifications. As the results on SEM images, ultrafine fiber network structure and ribbon-shaped fibrils could be observed on the surface of BC film with approximately 0.06-0.08 μm of BC fibrils diameter (Figure 5.2(a)). With *G. mangostana* aqueous extract impregnated into BC film, the BC film exhibited denser surface morphologies related to the extract contents. From Figure 5.1(b) and (c), the modification by 2.5 and 5.0%w/v *G. mangostana* aqueous extract immersion causing BC film coated with dark layer of amorphous compounds of mangosteen extract, but still in the fribillar structure of nano-cellulose fibers. However, the fibrillar structure could not be observed on the surface of BCWM-10.0 (Figure 5.2(d)) because the aqueous extract of *G. mangostana* was thoroughly covered on BC fibrils and formed a thin amorphous

layer on the BC film surface resulting in the reduction of BC film's transparency as shown in Figure 5.2(d).

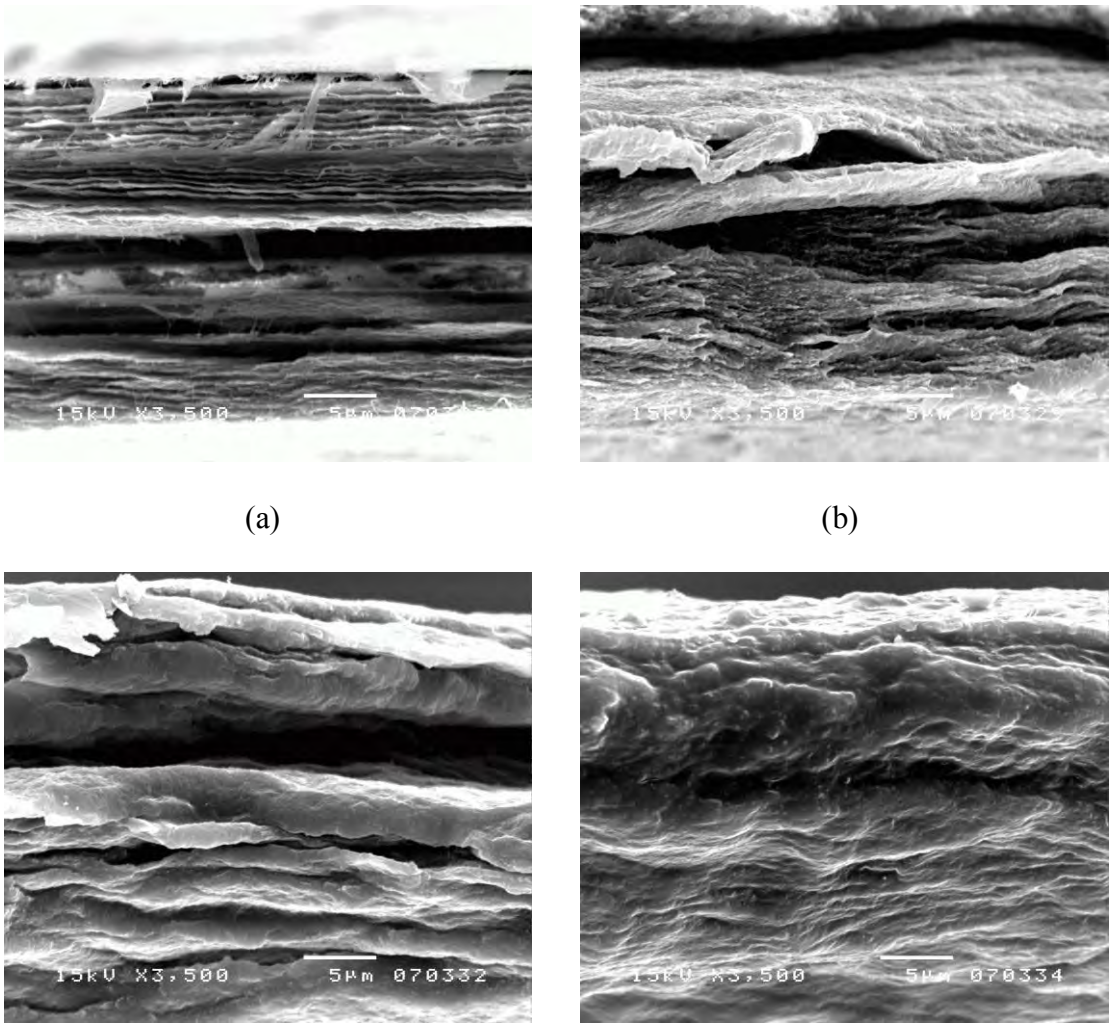
Figures 5.3 and 5.4 express the cross sectional morphologies of BC and BCWM films at 3,500 and 10,000 magnifications, respectively. As seen in Figures 5.3(a) and 5.4(a), BC film was composed of cellulose fiber flat sheets. Because of an assembly of nano-fibrils between BC layers, each of cellulose sheets was bound together causing of porosity in the BC film structure. By adding the aqueous extract of *G. mangostana* into the BC film, amorphous compounds of mangosteen aqueous extract penetrated into the spaces between cellulose sheet layers and into pores of BC sheet. BC is extremely hydrophilic with high water holding capacity (Suwanmajo, 2006; Kanjanamosit *et al.*, 2009), so *G. mangostana* aqueous extract could be absorbed into the structure of BC film due to its hydrophilic property.The BCWM film structure became denser than the BC film structure as shown in Figures 5.3(b-d) and 5.4(b-d). On the observation of the BCWM-2.5 and 5.0 films, there were still a few empty spaces between the BC layers. However, dense structure with no voids or pores was observed in the BCWM-10.0 film as a result of high content of the extract compounds in the film.



(a) (b)

(c) (d)

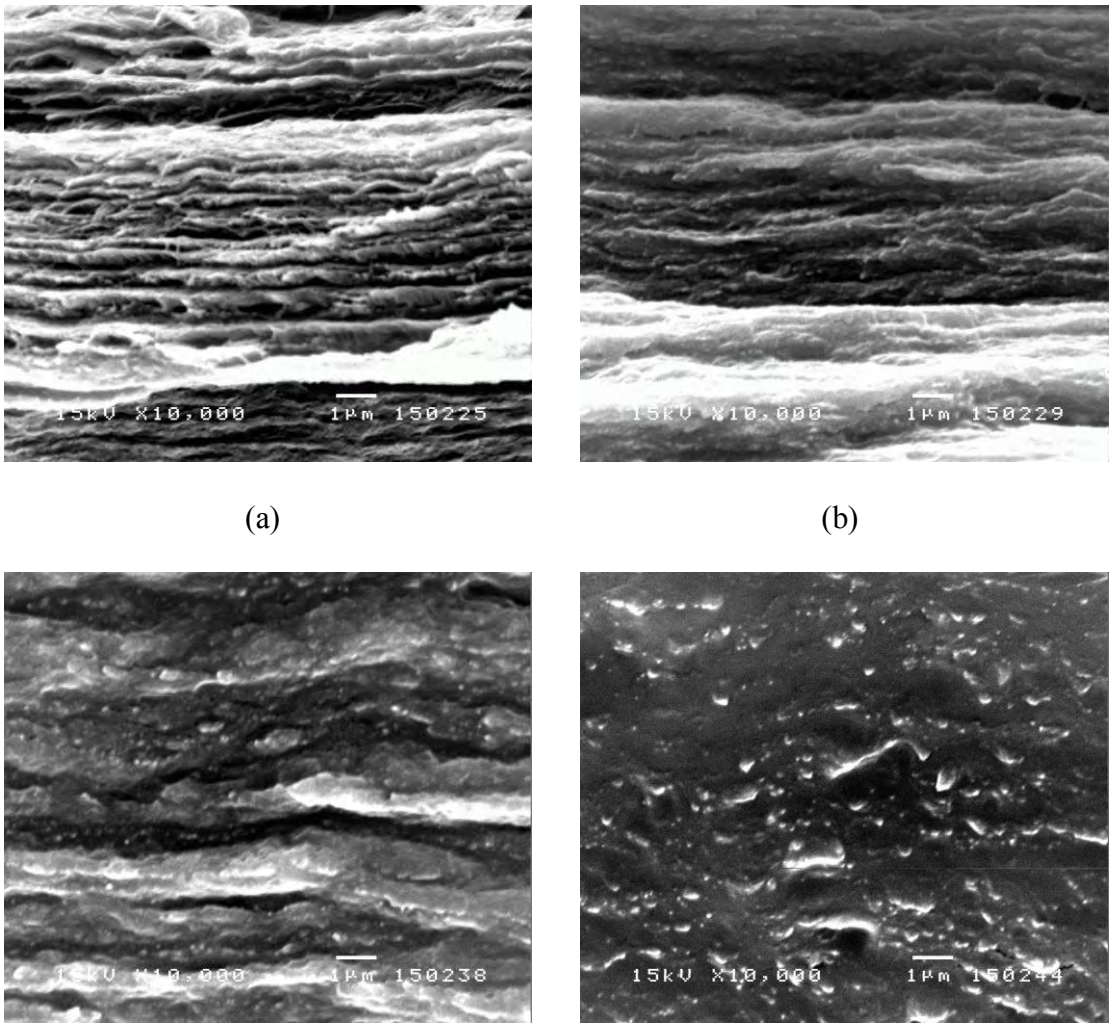
Figure 5.2 SEM images of surface morphology at magnification 10,000X: BC (a), BCWM-2.5 (b), BCWM-5.0 (c) and BCWM-10.0 (d)



(a) (b)

(c) (d)

Figure 5.3 SEM images of cross sectional morphology at magnification 3,500X: BC (a), BCWM-2.5 (b), BCWM-5.0 (c) and BCWM-10.0 (d)



(a) (b)

(c) (d)

Figure 5.4 SEM images of cross sectional morphology at magnification 10,000X: BC (a), BCWM-2.5 (b), BCWM-5.0 (c) and BCWM-10.0 (d)

5.2.2 FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy has often been applied as a useful technique to detect functional groups or chemical bonds that exist in a material. All samples of BC and BCWM films were analyzed by detecting with FTIR spectra at the wavenumbers ranging from 4000 to 450 cm^{-1} as demonstrated in Figure 5.5. The BC film showed a band at 3412 cm^{-1} and 1643 cm^{-1} which were attributed to hydroxyl group (-OH) and glucose carbonyl group (C=O) of cellulose (Phisalapong and Jatupaiboon, 2008; Kanjanamosit *et al.*, 2009). At the band of 1643 cm^{-1}, it also can be assigned to the H–O–H bending vibration of absorbed water molecules consisting in the structure of BC (Proniewicz *et al.*, 2002; Lojewska *et al.*, 2005; Adel *et al.*, 2011). Meanwhile, the characteristic absorptions of mangosteen aqueous extract were the bands at 3399 and 1611 cm^{-1}, which were represented to phenolic hydroxyl group (-OH) and double bond (C=C) in aromatic ring of phenolic compounds, respectively (Ee *et al.*, 2008; Han *et al.*, 2009; Kumar *et al.*, 2010; Veerasamy *et al*, 2011). The FTIR results demonstrate that the peaks attributed to hydroxyl (O-H) and glucose carbonyl (C=O) stretching vibrations of BC film impregnated with various concentrations (2.5, 5.0 and 10.0%w/v) of *G. mangostana* aqueous extract in solution, were slightly shifted from a wave number at 3412 cm^{-1} to a wave number at 3419, 3420 and 3412 cm^{-1}, respectively as showed in Figure 5.5(b-d). Moreover, the new peak expressed on the modified BC films at 1615 cm^{-1}, owing to the presence of aromatic ring consisting in the structure of phenolic compounds (Han *et al.*, 2009; Veerasamy *et al*, 2011). These changes indicated interactions between the functional groups of BC film and mangosteen aqueous extract compounds.

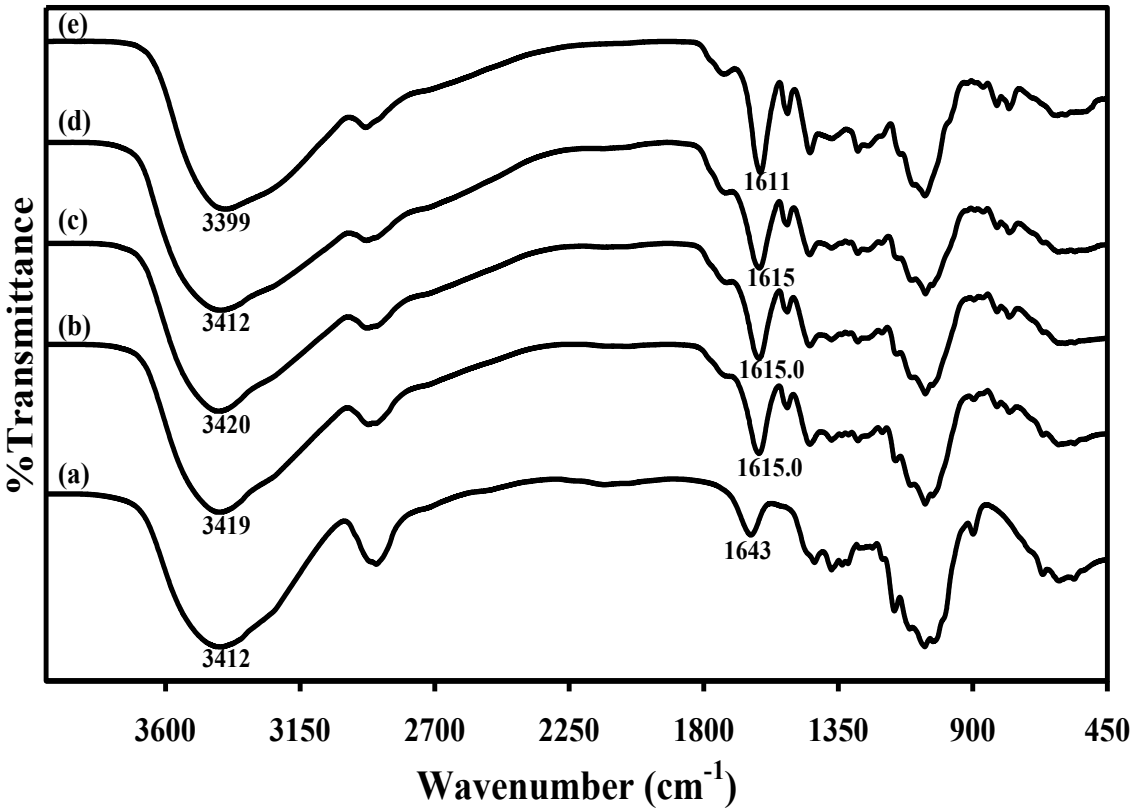


Figure 5.5 The FTIR spectra in wavenumber ranging from 4000 to 450 $cm^{-1}$of (a) BC, (b–d) BCWM films and (e) the aqueous extract of *G. mangostana*. The supplementation of mangosteen extract (%w/v) in BCWM were: (b) 2.5%, (c) 5.0% and (d) 10.0%

5.2.3 Mechanical Property

In general, biomaterials used as wound dressing have to be applied onto wound surfaces. The mechanical properties are often one of the most important properties because virtually all service condition and the majority of end-use applications involve some degree of mechanical loading. Therefore, in this study, the mechanical properties of BC and BCWM films were examined in terms of the tensile strength, elongation at break and Young's modulus.

Figure 5.6 shows the effects of *G. mangostana* aqueous extract addition on tensile strength of the films. The tensile strength of the BC film without the extract supplementation was 107.89 MPa.

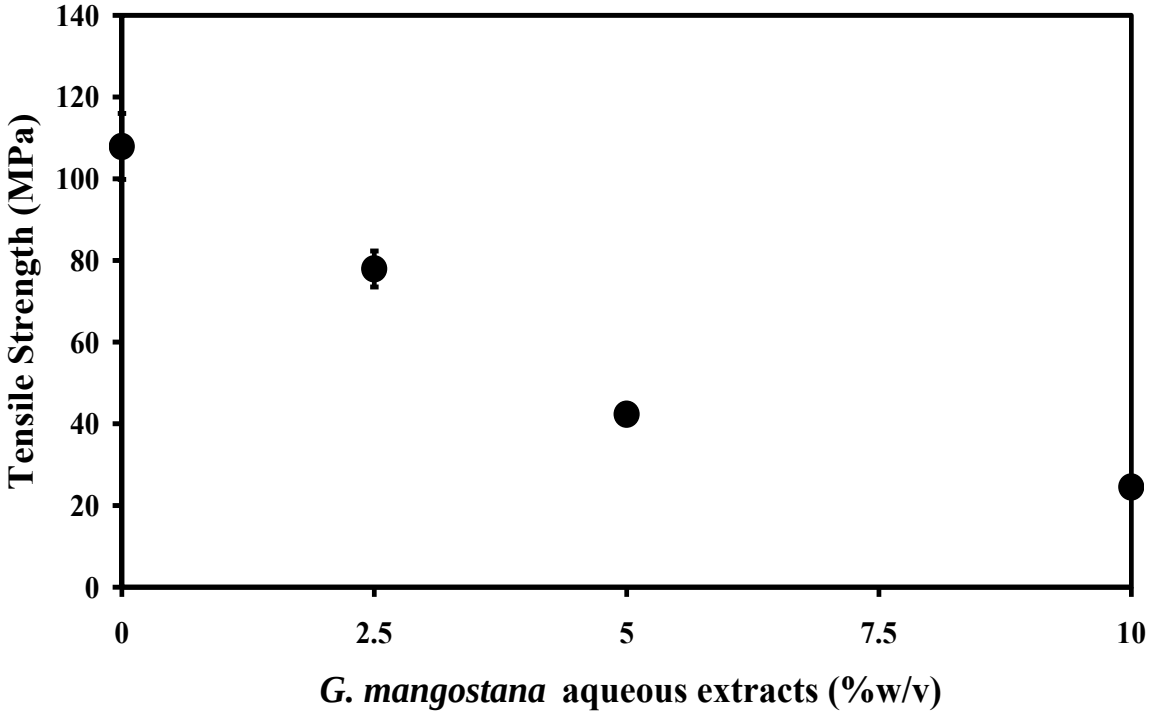


Figure 5.6 Tensile strength of BC and BCWM films as a function of *G. mangostana* aqueous extract supplementation (%w/v)

After the BC films were immersed into *G. mangostana* aqueous extract solution at concentrations of 2.5, 5.0 and 10.0%w/v, the tensile strengths of the BCWM-2.5, 5.0 and 10.0 were decreased to 77.90, 42.33 and 24.52 MPa, respectively. Due to the presence of mangosteen aqueous extract compounds inserting BC fibrils, the films became more amorphous. Amorphous films are usually less rigid, weaker and more easily deformed. Due to the reduced mechanical strength, the BCWM films became less durable than the unmodified one**.**

Figure 5.7 demonstrates the effects of *G. mangostana* extract on percentage of elongation at break of the films. The elongation at break of BC and BCWM-2.5, 5.0 and 10.0 films were 23.97, 20.1, 17.77 and 16.02%, respectively.

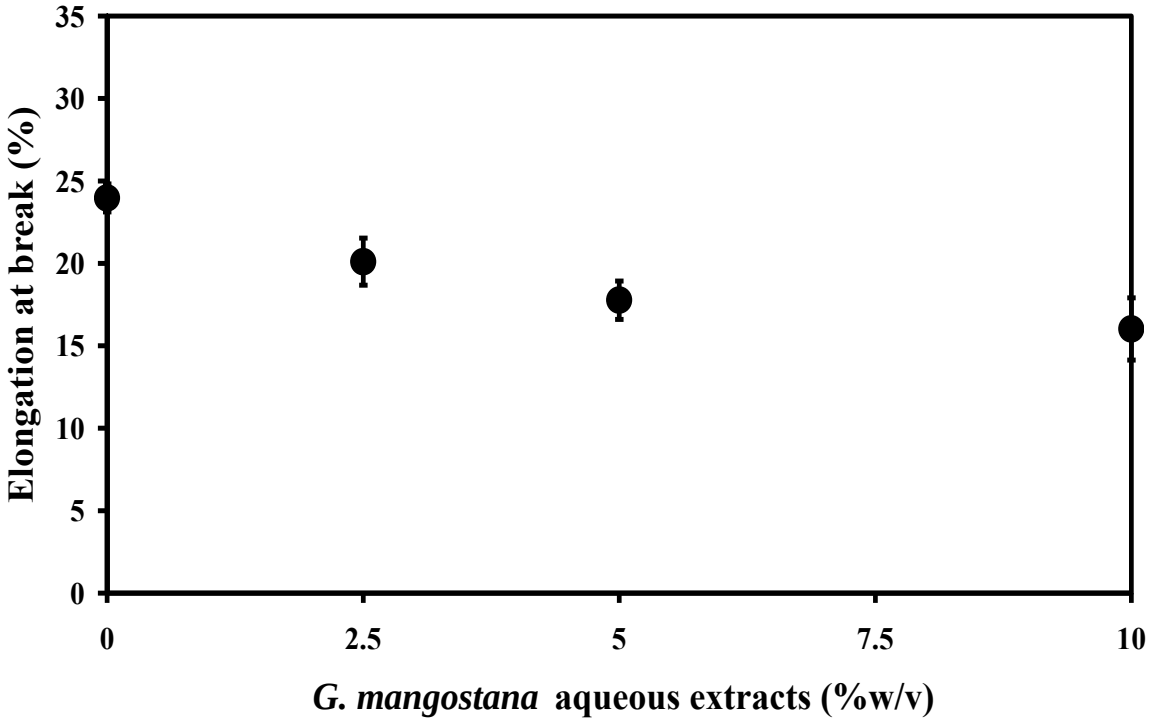


Figure 5.7 Elongation at break of BC and BCWM films as a function of *G. mangostana* aqueous extract supplementation (%w/v)

A slightly decreased tendency of the percentage of elongation at break of the BCWM films with the increased content of *G. mangostana* aqueous extract impregnated into the BC film was observed. When the aqueous extract contents were transferred into the film, the amorphous phase fraction increased. This could weaken affinity of binding of the films.

Young's modulus of BCWM films as a function of *G. mangostana* aqueous extract supplementation is presented in Figure 5.8. By addition of *G. mangostana* aqueous extract with concentrations of 2.5, 5.0, and 10.0%w/v, the modulus of elasticity of the BCM films were 557.21, 251.78 and 140.76 MPa, respectively.

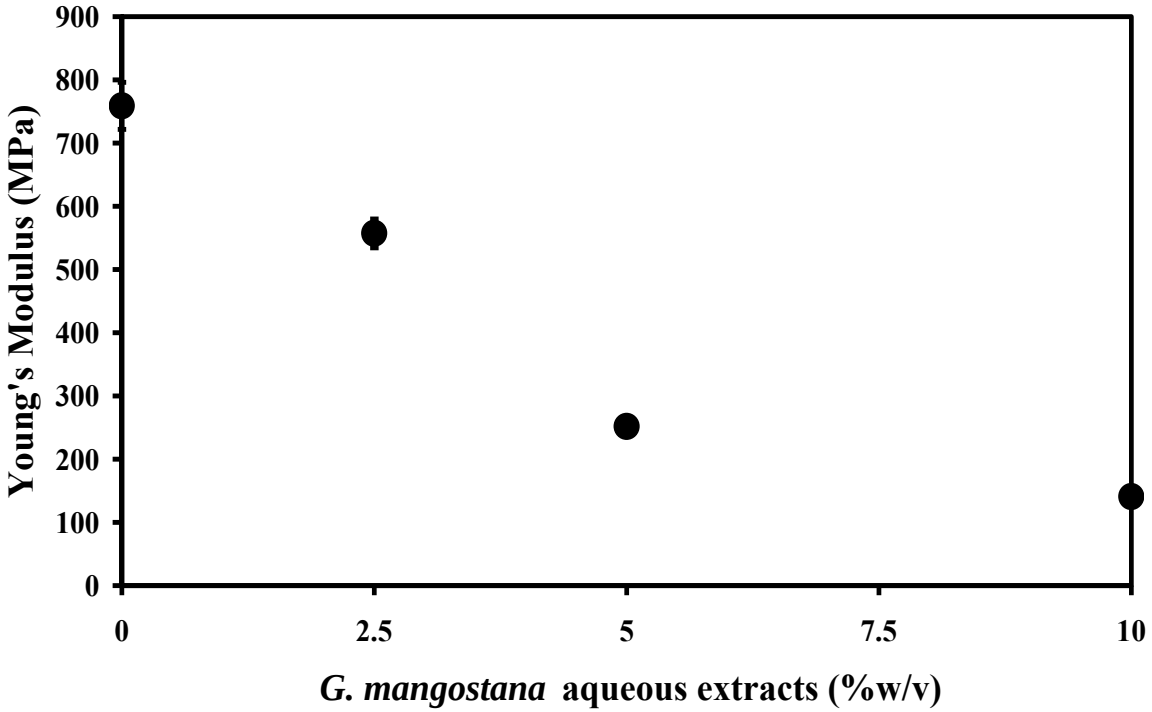


Figure 5.8 Young's modulus of BC and BCWM films as a function of *G. mangostana* aqueous extract supplementation (%w/v)

Compared with Young's modulus of the BC film (758.84 MPa), the reduction of elastic modulus should be arised from the increase of amorphous structure in the BCWM films. The flexibility and endurance of the BCWM films were less than the BC film, which could be related to the less organized fiber-network structure of the BCWM films.

From the previous literature published by Martins *et al.* (2009), thermoplastic starch (TPS) prepared by the disruption of the molecular chain interactions under specific conditions (presence of a plasticizer), was incorporated with vegetable cellulose (VC) and BC fibers as reinforced composite materials in order to obtain new bio-composite with higher mechanical properties. After 1% and 5%w/w of BC supplementation into TPS matrix, the tensile strength and Young's modulus of TPS/BC composites were increased with BC contents in the materials. It could be explained by the inherent morphology of well-organized network structure of BC. The mechanical properties of TPS/BC were higher than TPS/VC composites due to the greater fine network structure of BC comparing with VC fibrils. These results could imply that well-organized-fiber network structure provided the superior mechanical properties. In this work, the fiber network of BCWM became less organized resulting in the inferior mechanical properties of the BCWM films.

5.2.4 Water Absorption Capacity (WAC)

Wound exudates control and ability to retain moisture are essential properties for wound healing process. Therefore, the WAC analysis of BC and BCWM films was examined by immersing the dried films in distilled water at room temperature until equilibration.

Figure 5.9 showes that the WAC of the BC film was 328.77%. After the BC films were immersed into water extract solution at concentrations varied from 2.5, 5.0 and 10.0%w/v, the WACs of BCWM films were increased related to the amount of the supplementation to 577.09, 651.14 and 756.88%, respectively, owing to the more hydrophilic property of the BCWM films.

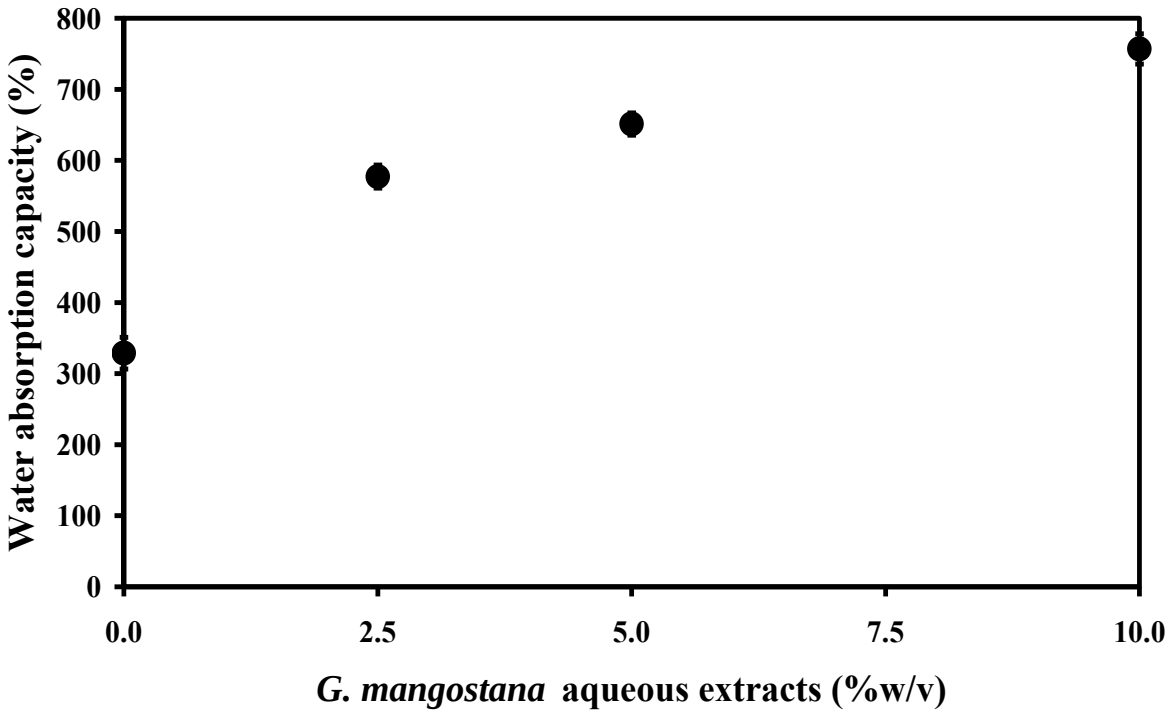


Figure 5.9 The water absorption capacity of BCWM films as a function of *G. mangostana* aqueous extract supplementation (%w/v)

On the previous study, Kanjanamosit *et al.* (2009) developed novel BC composite membrane by adding of sodium alginate into BC matrix. The WAC of the BC and 1.0%w/v alginate in BC (BCA) membranes were 542% and 706%, respectively. It was demonstrated that the WAC of the film was increased with increasing retention of hydrophilic compounds.

5.2.5 Oxygen Permeability Test

In wound healing, oxygen plays a key role by supporting tissue regeneration and repair, by inhibiting anaerobic bacteria and supporting the body's natural defence mechanisms. In this study, all films were analyzed for oxygen transmission rate by following the ASTM D3985.

Table 5.2 shows the results of the oxygen transmission rate (OTR) of the BC and BCWM film. The OTRs of the BCWM-5.0 and BCWM-10.0 was less than that of the BC film because of the reduction of pore volume in these films. After the BC films were impregnated with the aqueous extract of *G. mangostana*, its extract would fill the pores as previously shown in the high-magnified SEM images (Figures 5.2-5.4). As the above result, the OTRs were reduced.

As shown in Table 5.2, the BC and BCWM films had significant less oxygen transmission when compared with commercial wound dressings, for example, Bioprocess® (Cardona et al., 1996) and gauze (Lowe, 2008).

Table 5.2 The oxygen transmission rate of BC and BCWM films (Mean value from duplicate testing) and commercial wound dressings (Cardona *et al.*, 1996; Lowe, 2008)

Material	OTR±S.D. (cc/m^2/day)
BC	2.53±0.00
BCWM-5.0	1.71±0.01
BCWM-10.0	1.45±0.00
Bioprocess®	277.34±54.45
Gauze	14275.00

5.2.6 Water Vapor Permeability Test

The good wound dressings are not only absorb wound exudates to keep the wound dry, but also have to control evaporative fluid loss from wounded skin, which was a necessary property to accelerate the wound healing process. From Table 5.3, the water vapor transmission rates (WVTR) of BCWM-5.0 and 10.0 films was higher than that of the BC film. According to the hydrophilic property of *G. mangostana* aqueous extract which added into the BC film, the BCWM films could be permeated by water vapor better than the unmodified one. However, with the increase of the aqueous extract of *G. mangostana* addition from 5.0 to 10%w/v, no significant difference in WVTR was observed, which could be due to the decrease in pore volume and pore size of the film as seen in Figures 5.2-5.4.

The BC film exhibited the WVTR at 550.80 g/m^2/day, which was less than various commercial wound dressings (in Figure 5.10). However, the WVTRs of modified BC films with *G. mangostana* aqueous extract impregnation were increased to approximately 800 g/m^2/day, which were near to the WVTRs of some commercial

biomedical films such as Duoderm® (886 g/m^2/day), Metoderm® (829 g/m^2/day) and Gauze (800 g/m^2/day) (Wu *et al.*, 1995; Lowe, 2008).

Table 5.3 The water vapor transmission rate of BC and BCWM films (Mean value from duplicate testing)

Material	WVTR±S.D. (g/m^2/day)
BC	550.80±39.03
BCWM-5.0	808.80±18.33
BCWM-10.0	809.04±44.46

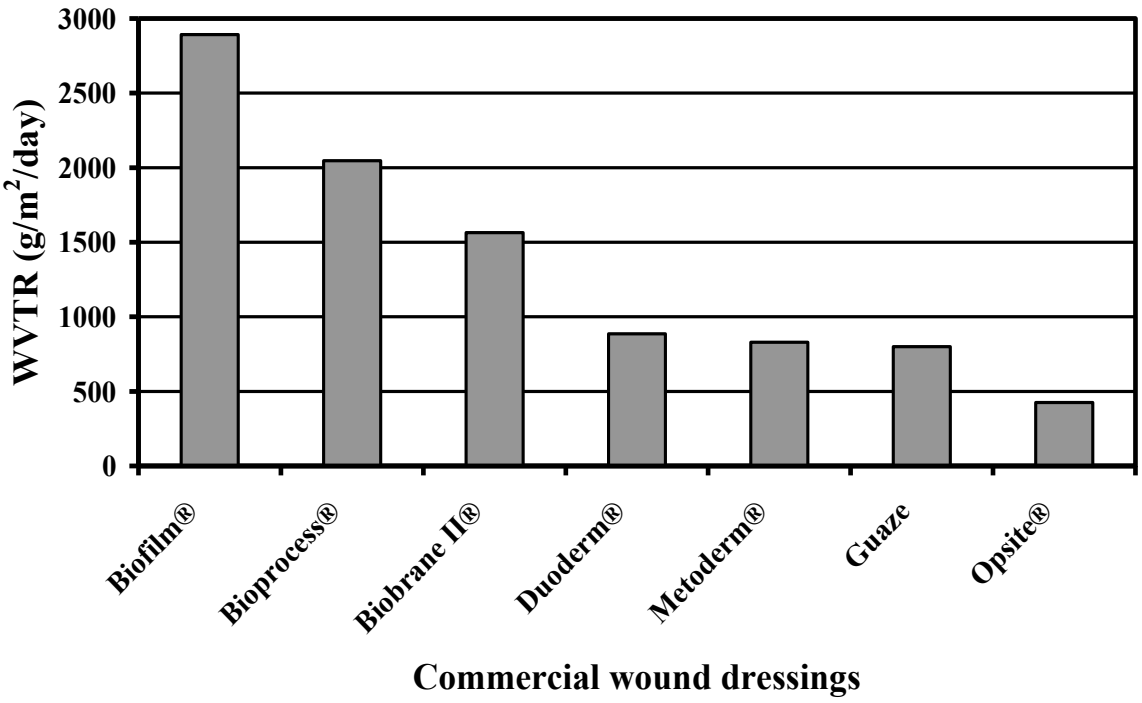


Figure 5.10 The water vapor transmission rate of commercial wound dressing (Wu *et al.*, 1995; Cardona *et al.*, 1996; Lowe, 2008).

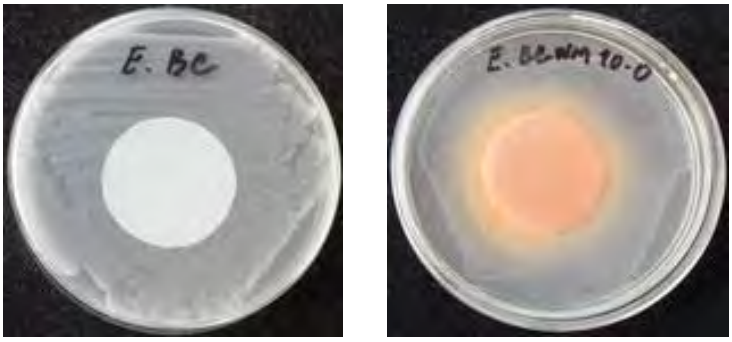
5.2.7 Antibacterial and Antifungal Ability

On the examination of antibacterial and antifungal ability of the modified BC films, *E. coli* and *S. aureus* were used as the representative gram negative and gram positive pathogenic bacteria, respectively. *S. epidermidis*, *P. acnes*, *C. albicans* and *A. niger* represented the pathogenic bacteria and fungus. As shown in Tables 5.4 and 5.5, the results indicated that the antibacterial and antifungal activities of the BCWM films were better than those of the BC film. According to former researches about the effects of *G. mangostana* aqueous extracts on the antimicrobial activities, *G. mangostana* aqueous extract exhibited the inhibitory abilities on *S. aureus*, *S. epidermidis* and *P. acnes* (Phadungkarn *et al.*, 2009; Phothitirat *et al.*, 2010). The BCWM films showed the inhibition of growths of *E. coli*, *S. aureus*, *S. epidermidis*, *P. acnes* and *A. niger*., whereas they had no inhibitory effect on the growth of *C. albicans*. Moreover, the BCWM-2.5 showed some antifungal activity; however, increasing more aqueous extract content (BCWM -5.0, -10.0) leaded to support the growth of *A. niger*.

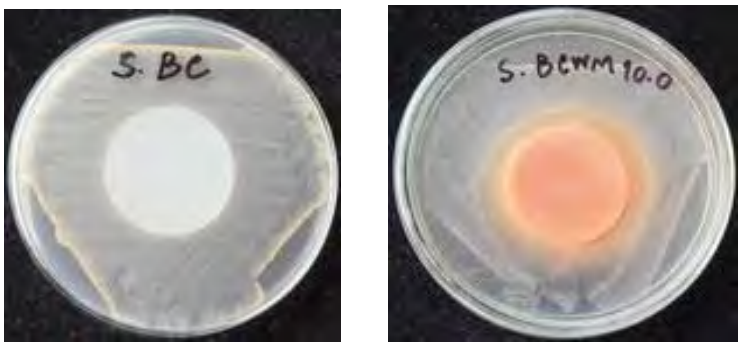
Typically, the antimicrobial activities were increased with the concentration of the *G. mangostana* aqueous extracts but not as a linear relationship. For antimicrobial activity tests of BCWM against *P. acnes*, the clear zone in a dose of 5 and 10%w/v is roughly the same size. The concentration of released extract and the diffusion length should be the most important factors affecting on the inhibition of microbial growths (observed by clear zone). Therefore, not only the initial concentration of impregnated *G. mangostana* aqueous extracts but also the rate of bioactive compounds release should be considered for the antimicrobial control of the modified BC films.

Table 5.4 Antimicrobial activities of BC and BCWM films against *E. coli*, *S. aureus*, *S. epidermidis*, *P. acnes*, and *C. Albicans*

Microorganisms	Sample	Clear zone (mm)
E. coli	BC	0
	BCWM-2.5	7.50±1.32
	BCWM-5.0	8.33±0.29
	BCWM-10.0	9.33±1.44
S. aureus	BC	0
	BCWM-2.5	5.00±1.00
	BCWM-5.0	5.50±0.50
	BCWM-10.0	6.50±0.87
S. epidermidis	BC	0
	BCWM-2.5	1.33±0.58
	BCWM-5.0	4.17±0.29
	BCWM-10.0	5.00±0.50
P. acnes	BC	0
	BCWM-2.5	5.50±0.87
	BCWM-5.0	6.00±0.00
	BCWM-10.0	6.00±0.00
C. albicans	BC	0
	BCWM-2.5	0
	BCWM-5.0	0
	BCWM-10.0	0



On *E. coli*

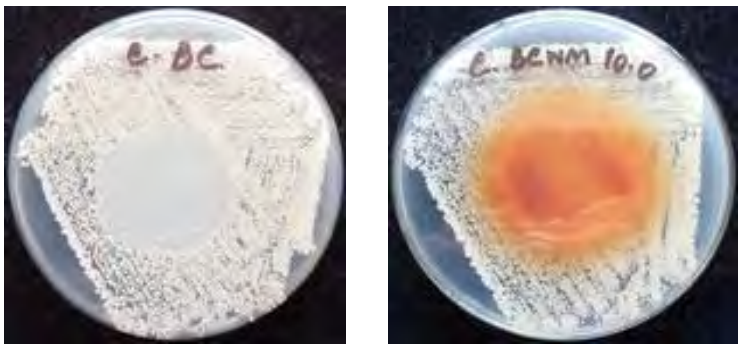


On *S. aureus*

On *S. epidermidis*

Figure 5.11 Inhibitory effect of the BC and BCWM samples on the growth of bacteria for 24 h incubated at 37^{o}C

On *P. acnes*



On *C. albicans*

Figure 5.12 Inhibitory effect of the BC and BCWM samples on the growth bacteria (*P. acnes*) for 72 h and yeast (*C. albicans*) for 24 h incubated at 37^{o}C

Table 5.5 Antifungal activity of BC and BCWM films against *A. niger*

Microorganisms	Sample	Observed growth
		Grade
A. niger	BC	5
	BCWM-2.5	3
	BCWM-5.0	4
	BCWM-10.0	5

*Grade was used as a measurement of fungal growth: 0 = none, 1 = only apparent under microscope, 2 = trace (<10%), 3 = light growth (10-30%), 4 = medium growth (30-60%) and 5 = heavy growth (> 60%)

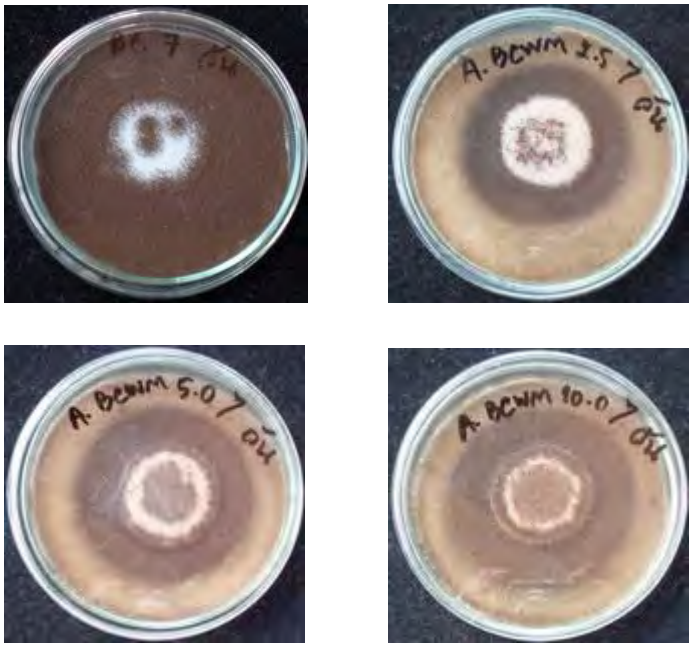


Figure 5.13 The growth of *A. niger* on the BC and BCWM specimens, at 30^{o}C at the end of the incubation 7 days

5.3 Characterization of BC Films Modified by Supplementation of *G. Mangostana* Ethanolic Extract (BCEM)

In this part, the BC films modified by ethanolic *G. mangostana* extract supplementation were analyzed for their physical and biological characterizations. BCEM-n was used as the representative BC films modified by *G. mangostana* ethanolic extract supplementation with various concentrations, where n is the concentration of *G. mangostana* ethanolic extract solution (%v/v).

5.3.1 Morphology

Figure 5.14 expressed the surface morphologies of BC and BCEM films at 10,000 magnifications. After immersed the purified BC films into *G. mangostana* ethanolic extract solution, the extract were sorbed into the film coating on BC fibrils and penetrated into empty space between network structures of BC fibrils. As shown in Figure 5.14(b-d), the void fractions of BCEM films were declined and BCEM fibrils appeared denser with the supplement of more extract concentration. However, when compared to the BCWM films, the BCEM films were more transparent. Figures 5.15 and 5.16 demonstrate the cross sectional morphologies of BC and BCEM films at 3,500 and 10,000 magnifications, respectively. The images showed that the layers of BC sheet in the BCEM films were thicker with supplement of *G. mangostana* ethanolic extract from 0.25-1.00 %v/v. However, the interlayer space between sheets was still observed.

From the SEM images (Figures 5.14-5.16), *G. mangostana* ethanolic extract could absorb on BC fibrils. Even cellulose is well-known to be hydrophilic conformation, its structure is possible to provide hydrophobicity due to the planar conformation of β-1,4-glucan chain (Yamane *et al.*, 2006).

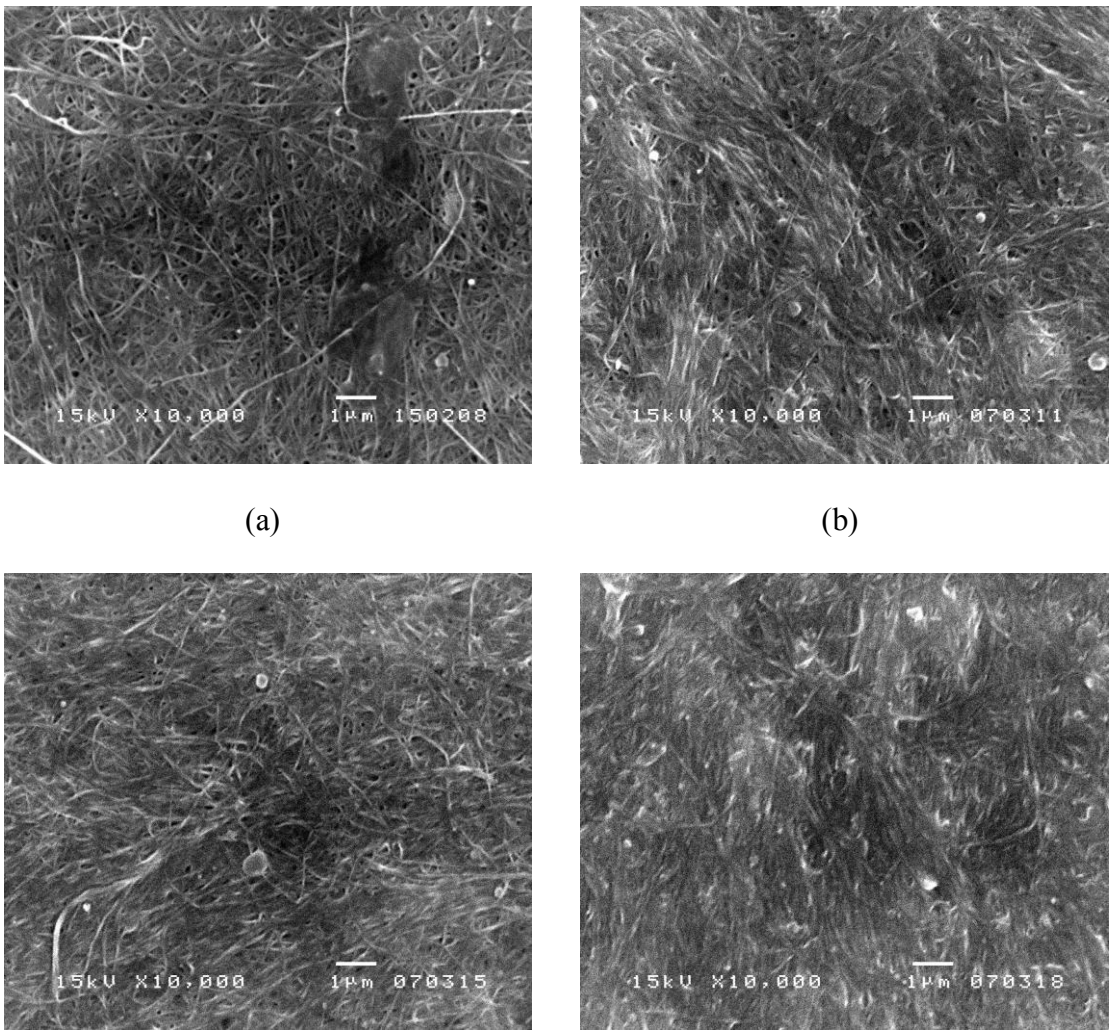
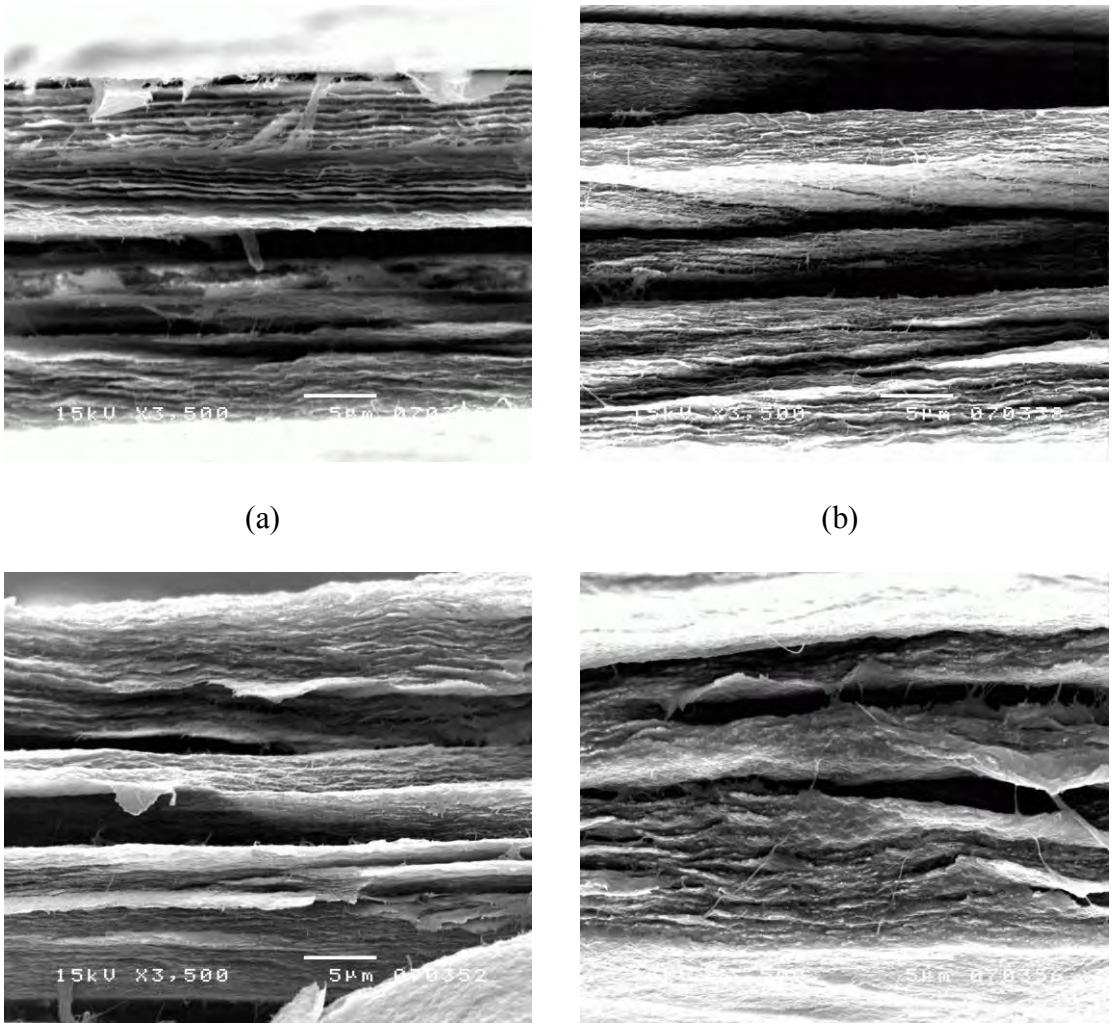


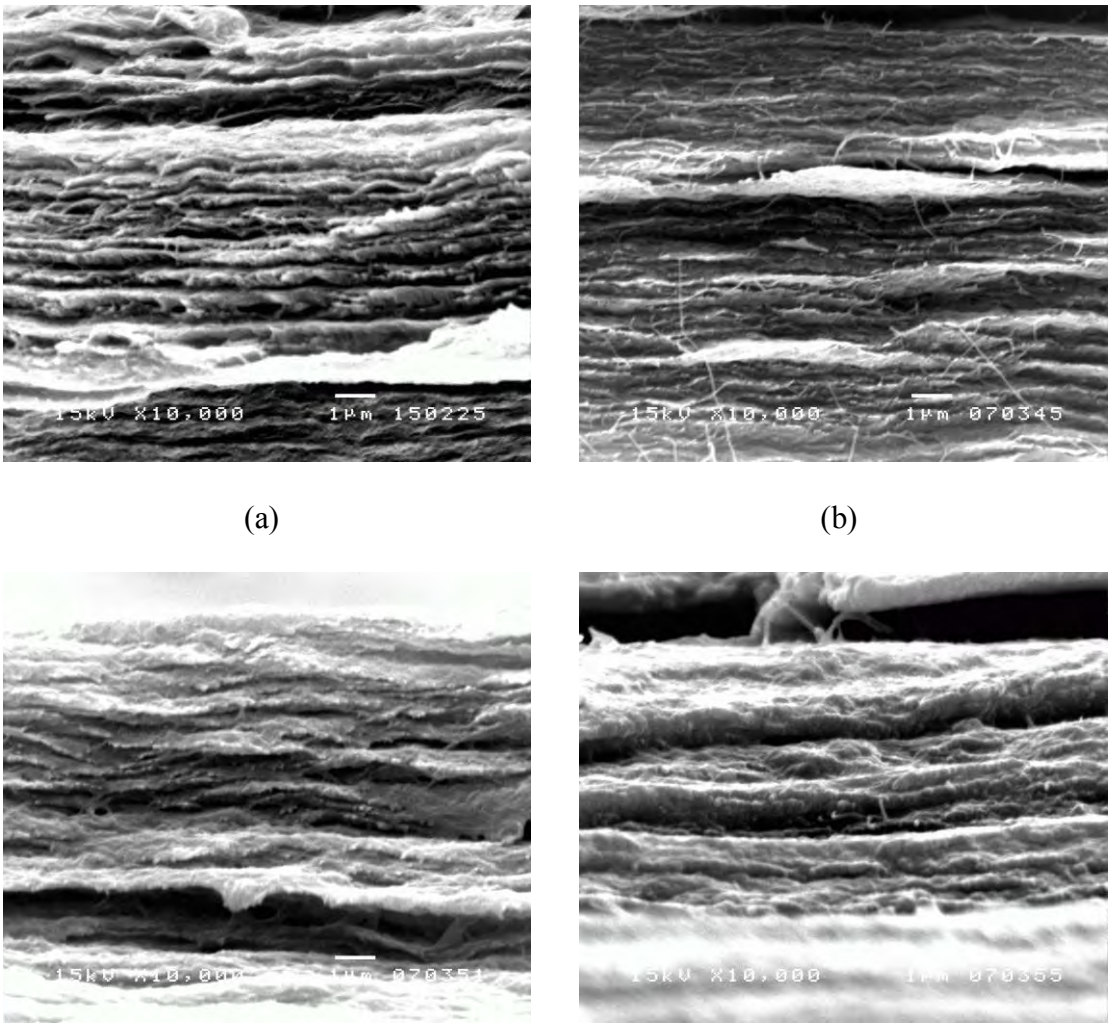
Figure 5.14 SEM images of surface morphology at magnification 10,000X: BC (a), BCEM-0.25 (b), BCEM-0.50 (c) and BCEM-1.00 (d)



(a) (b)

(c) (d)

Figure 5.15 SEM images of cross sectional morphology at magnification 3,500X: BC (a), BCEM-0.25 (b), BCEM-0.50 (c) and BCEM-1.00 (d)



(a) (b)

(c) (d)

Figure 5.16 SEM images of cross sectional morphology at magnification 10,000X: BC (a), BCEM-0.25 (b), BCEM-0.50 (c) and BCEM-1.00 (d)

5.3.2 FTIR Analysis

Functional groups or chemical bonds that exist in BC and *G. mangostana* ethanolic extract compounds structure were determined by Fourier transform infrared (FTIR) spectroscopy to estimate interactions which occurred in BCEM films with wavenumbers ranging from 4000 to 450 cm^{-1}. In Figure 5.17, the BC film showed a band at 3412 cm^{-1} of hydroxyl group (-OH) and 1643 cm^{-1} of glucose carbonyl group (C=O) of cellulose (Phisalapong and Jatupaiboon, 2008; Kanjanamosit *et al.*, 2009). The band of H–O–H bending vibration of absorbed water molecules consisting in the structure of BC also shows at ~1640 cm^{-1} (Proniewicz *et al.*, 2002; Lojewska *et al.*, 2005; Adel *et al.*, 2011). The characteristic absorptions of *G. mangostana* ethanolic extract were the bands at 1608, 1640 and 3391 cm^{-1} which attributed to double bond of carbon (C=C) in aromatic ring presenting in phenolic compounds structure, chelate carbonyl group in the structure of xanthone backbone (As shown in Figure 5.18) and to phenolic hydroxyl group, respectively (Ee *et al.*, 2008; Han *et al.*, 2009; Kumar *et al.*, 2010; Veerasamy *et al*, 2011). The FTIR results illustrated the specific bands of O-H and C=O stretching vibrations of BC film modified by the supplementation of *G. mangostana* ethanolic extract with different concentrations (0.25, 0.50 and 1.00%v/v), were slightly shifted from a wave number at 3412 cm^{-1} to a wave number at 3412, 3411 and 3409 cm^{-1}, respectively, and a wave number at 1643 cm^{-1} to a wave number at 1639, 1638 and 1637 cm^{-1}, respectively as showed in Figure 5.17(b-d). The peak which represented as double bond in aromatic ring (1608 cm^{-1}) consisting in *G. mangostana* ethanolic extract would be combined with the peak of chelate carbonyl group (1640 cm^{-1}), thus the band of double bond carbon did not show in the FTIR results of the BCEM films. These results could imply weakly interactions between the functional groups of BC film and *G. mangostana* ethanolic extract compounds.

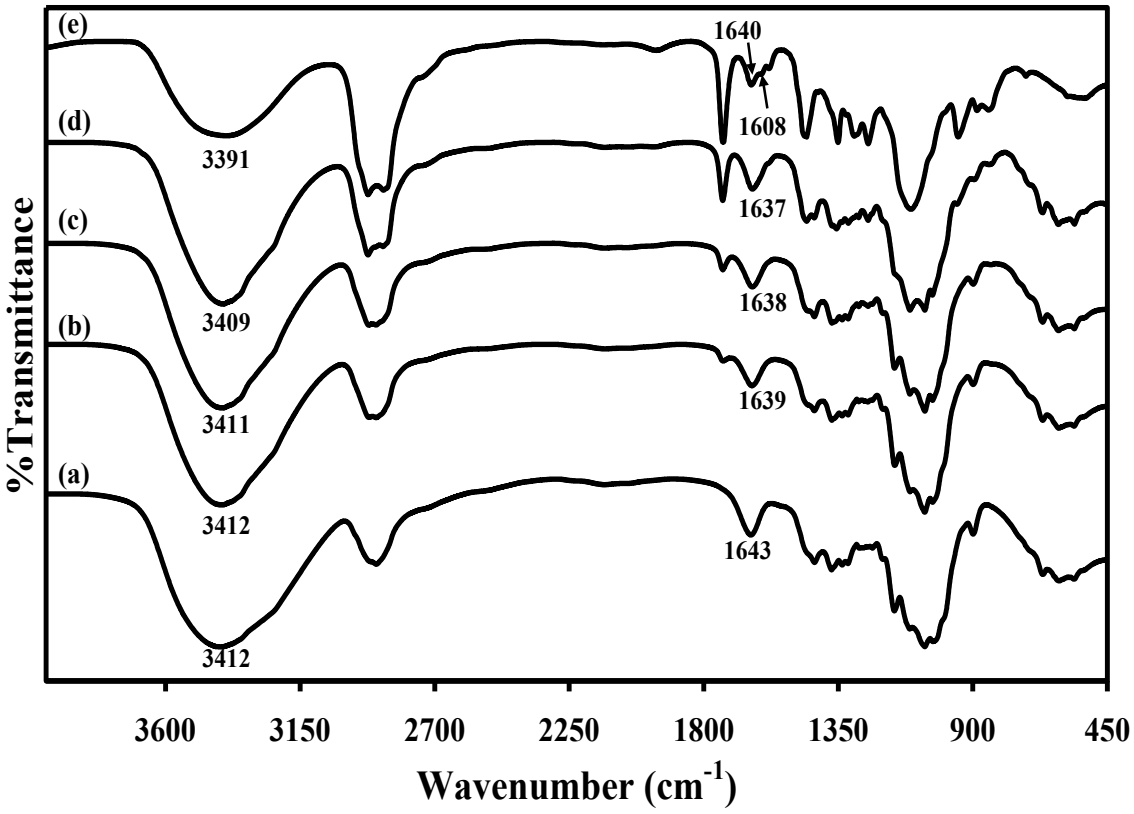


Figure 5.17 The FTIR spectra in wavenumber ranging from 4000 to 450 $cm^{-1}$of (a) BC, (b–d) BCEM films and (e) *G. mangostana* ethanolic extract. The supplementation of mangosteen extract (%v/v) in BCEM were: (b) 0.25%, (c) 0.50% and (d) 1.00%

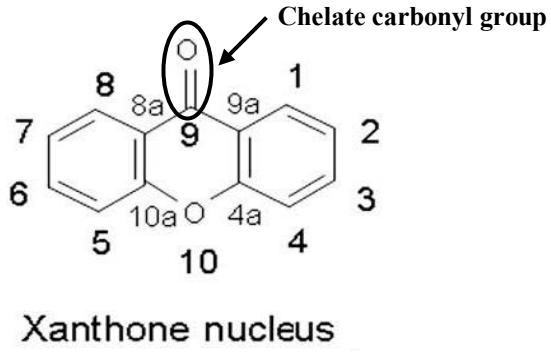


Figure 5.18 Chelate carbonyl group in the backbone of xanthone (Chaverri *et al.*, 2008)

5.3.3 Mechanical Property

In this study, the physical properties of BCEM films such as tensile strength, elongation at break and Young's modulus were analyzed for determining the effects of *G. mangostana* ethanolic extract supplementation on physical properties of the films. As shown in Figure 5.19, a certain interconnection of the ethanolic extract compounds and film fibers could improve the tensile strength of the films.

According to the results of morphology analysis in the previous part (5.3.1), The *G. mangostana* ethanolic extract coated on BC fibrils of the BCEM films might promote interconnections of BC fibrils resulted in an increase of the films' tensile strength.

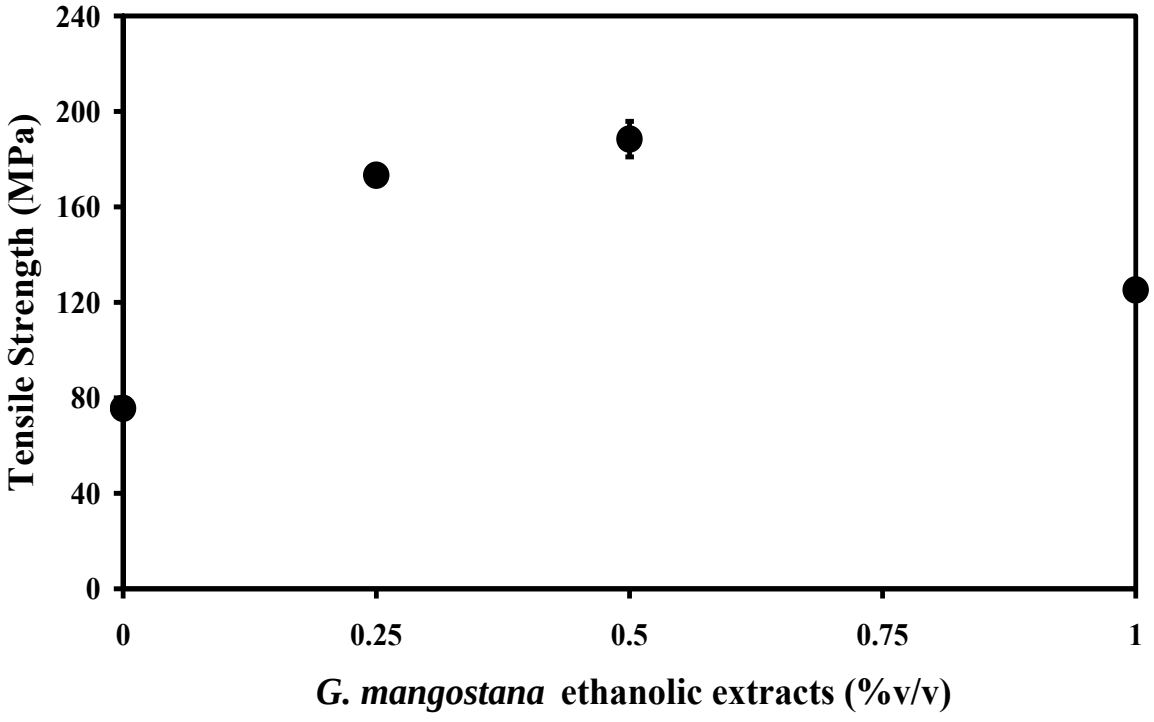


Figure 5.19 Tensile strength of BC and BCEM films as a function of *G. mangostana* ethanolic extract supplementation (%v/v)

As the tensile strengths of BC films was 75.55 MPa, the tensile strengths of BCEM films at concentrations of 0.25 and 0.50%v/v were raised to 173.24 and 188.39 MPa, respectively. Nevertheless, the tensile strength of the BCEM-1.00 was dropped to 125.19 MPa, but still higher than that of the BC film. Therefore, with the existence of an excessive ethanolic extract compounds, the BC fibril binding would become relatively weaker in comparison to the one with the optimal content of the extract compounds.

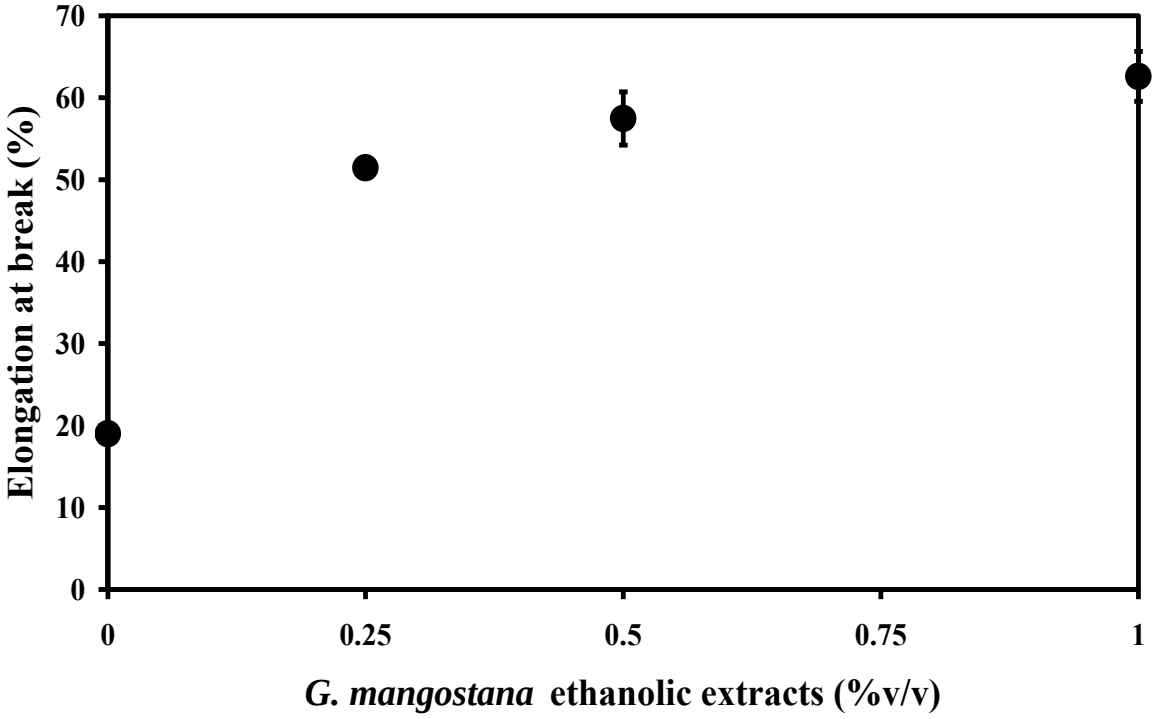


Figure 5.20 Elongation at break of BC and BCEM films as a function of *G. mangostana* ethanolic extract supplementation (%v/v)

As shown in Figure 5.20, the changes of elongation at break of the BCEM films became positive when adding the *G. mangostana* ethanolic extract into the BC films more than ones. The elongation at break of BC and BCEM-0.25, 0.50 and 1.00 films were 19.00, 51.44, 57.46 and 62.60%, respectively. The increase of BCEM films' elongation at break should be as a result of stronger binding between BC fibrils with *G. mangostana* ethanolic extract supplementation causing the BCEM films could endure to higher tension force at break point comparing with the BC film.

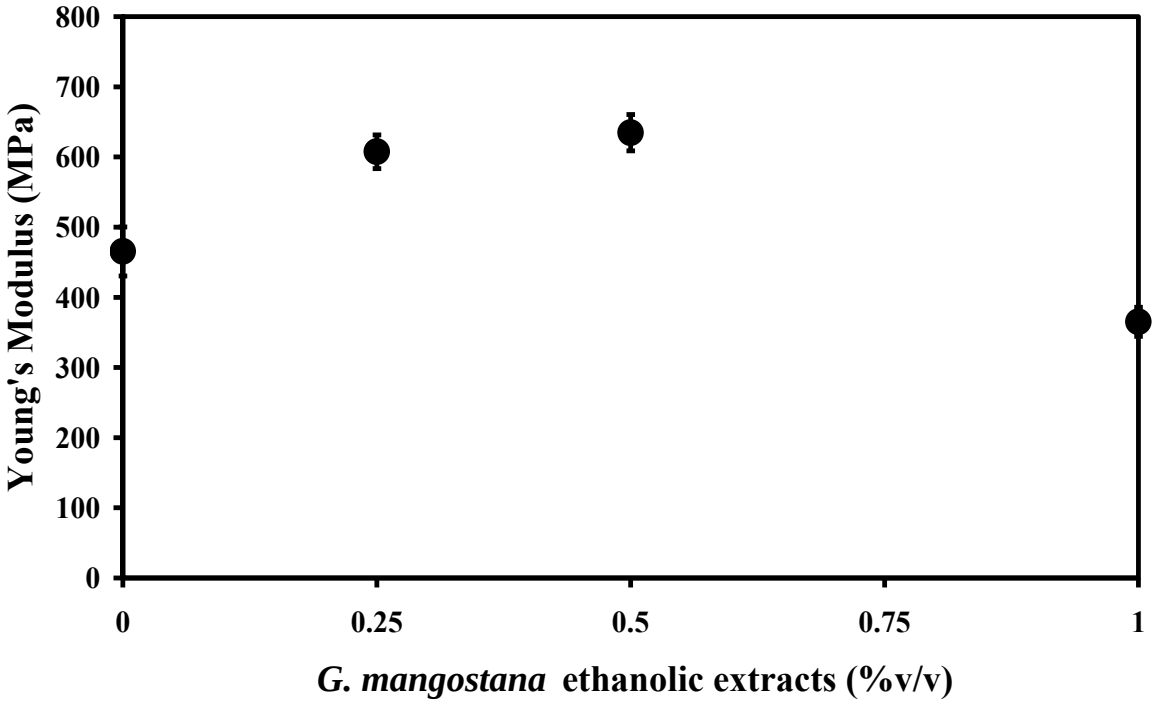


Figure 5.21 Young's modulus of BC and BCEM films as a function of *G. mangostana* ethanolic extract supplementation (%v/v)

Figure 5.21 demonstrates Young's modulus of BC and BCEM films (extract concentrations from 0.25-1.00%v/v). Similar to the results of the tensile strengths, the elastic modulus of BCEM films were increased by impregnation of *G. mangostana* ethanolic extract into the BC film.

After the modification of BC films by the immersion into *G. mangostana* ethanolic extract solution at concentrations of 0.25 and 0.50%v/v, the modulus of elasticity were raised to 607.42 and 634.59 MPa, respectively. Meanwhile, Young's modulus of BCEM-1.00 was dropped to 365.06 MPa which relatively less than unmodified BC film (465.36 MPa).

5.3.4 Water Absorption Capacity (WAC)

The water absorption capacities of BC and BCEM films were evaluated for the proportion of water holding in the BC and BCEM films which represented as abilities of wound exudates control and moisture retention.

According to Figure 5.22, it could imply that the WACs of the BCEM films were slightly lower than that of the BC film. The WACs of BCEM films with the supplementation of 0.25, 0.50, and 1.00%v/v of *G. mangostana* ethanolic extracts were 239.50, 277.14, and 354.87%, respectively. The hydrophobic nature of cellulose could be generated in the presence of hydrophobic substances, for example, hexane, toluene and dichloromethane. After drying process, they remain in cellulose domains (Yamane *et al.*, 2006). For the same reason, the hydrophobic nature of the modified films with various concentrations of *G. mangostana* ethanolic extract could be enhanced.

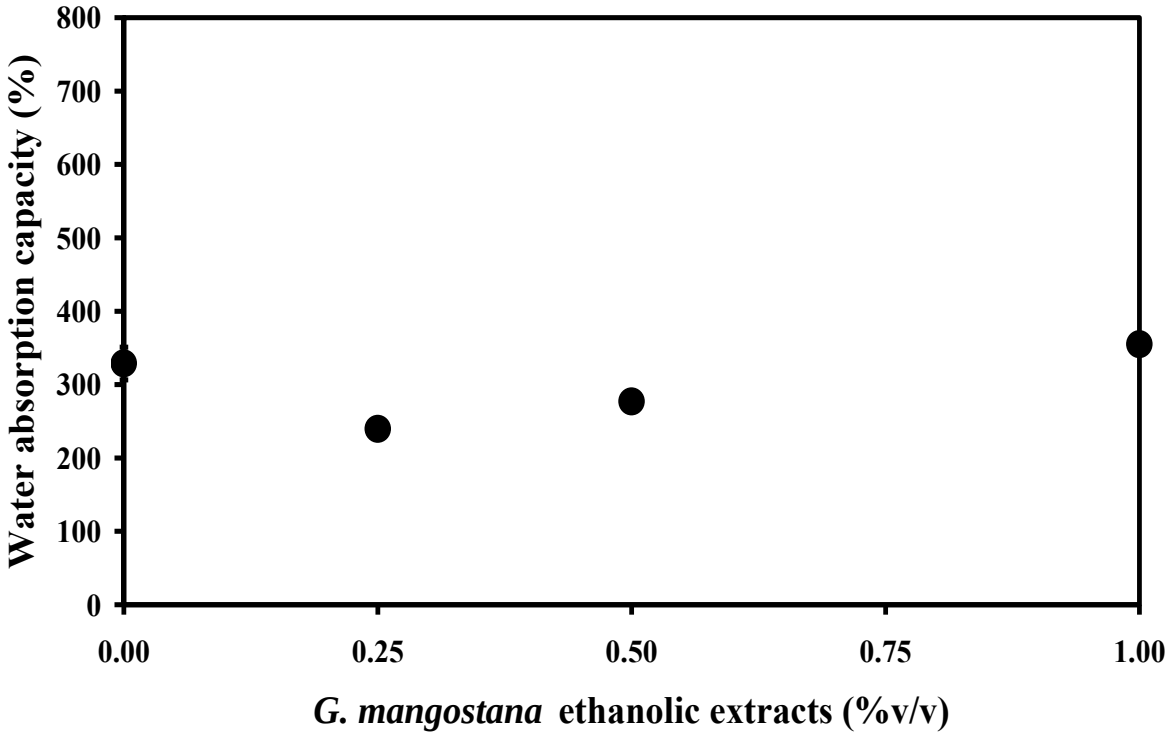


Figure 5.22 The water absorption capacity of BCEM films as a function of *G. mangostana* ethanolic extract supplementation (%v/v)

Due to the hydrophobic property of *G. mangostana* ethanolic extract compounds, compared to the BC film, the WACs of the BCEM films tended to decrease. However, the impregnation of the films with the extract compounds at high content could have constructive interfere in chemical bonds of the films and might result in a looser film structure. This could be a reason for relatively higher WAC of the BCEM film with the supplementation of 1.00%v/v *G. mangostana* ethanolic extract.

5.3.5 Antibacterial and Antifungal Ability

Tables 5.6 and 5.7 present the results of the antibacterial and antifungal activities of the BCEM films against 6 microbial strains: *E. coli*, *S. aureus*, *S. epidermidis*, *P. acnes*, *C. albicans* and *A. niger*. The BCEM films exhibited the inhibitory effects on the growths of *S. aureus, S. epidermidis*, *P. acnes*. and *A. niger*. Similarly, Pothitirat *et al.* (2010) conducted the experiment on the inhibitory effects against acne inducing bacteria by dose of mangosteen fruit rind extracts (hexane, dichloromethane ethanolic and aqueous extracts) and indicated that *G. mangostana* ethanolic extract could inhibit the growths of *S. epidermidis* and *P. acnes.* Chomnawang *et al.*, (2009) reported that *G. mangostana* ethanolic extract provided antibacterial activity against *S. aureus* and Methicillin-resistant *S. aureus* (MRSA). However, the BCEM films did not exhibited inhibitory activity on the growths of the *E. coli* and *C. albicans.*

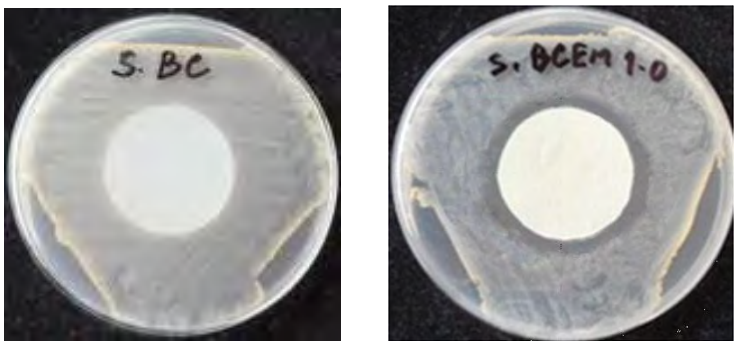
According to the study on antimicrobial activities of medicinal plants against *E. coli* by disc diffusion method (Voravuthikunchai *et al,*. 2004), *G. mangostana* ethanolic extract did not show the growth inhibition of *E.coli* .

The differences of bactericidal inhibition against gram negative (*E. coli*) and gram positive (*S. aureus*) films could be explained by their compositions and the arrangement of cell membrane. Peptidoglycan, an outer membrane of gram positive bacteria, is not a barrier to solutes. Conversely, the gram negative cell membrane does not permit lipophilic solutes into cytoplasm because of an outer phospholipidic layer. However, there are porins on the layer of the gram negative cell membrane causing the permeability of hydrophilic solutes (Palakawong *et al.*, 2010). Therefore, the BCWM films showed the inhibitory effect on *E. coli*, but the BCEM films did not.

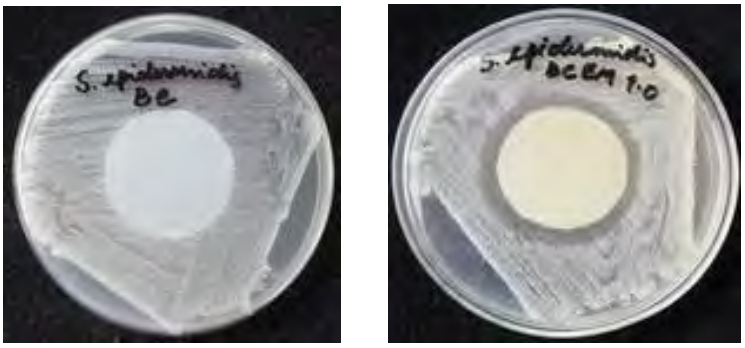
Table 5.6 Antimicrobial activities of BC and BCEM films against *E. coli*, *S. Aureus*, *S. epidermidis*, *P. acnes*, and *C. Albicans*

Microorganisms	Sample	Clear zone (mm)
E. coli	BC	0
	BCEM-0.25	0
	BCEM-0.50	0
	BCEM-1.00	0
S. aureus	BC	0
	BCEM-0.25	2.00±0.87
	BCEM-0.50	5.33±0.58
	BCEM-1.00	6.83±1.44
S. epidermidis	BC	0
	BCEM-0.25	2.17±1.26
	BCEM-0.50	2.33±0.29
	BCEM-1.00	3.50±0.00
P. acnes	BC	0
	BCEM-0.25	4.83±1.89
	BCEM-0.50	5.00±1.52
	BCEM-1.00	7.42±1.28
C. albicans	BC	0
	BCEM-0.25	0
	BCEM-0.50	0
	BCEM-1.00	0

On *E. coli*

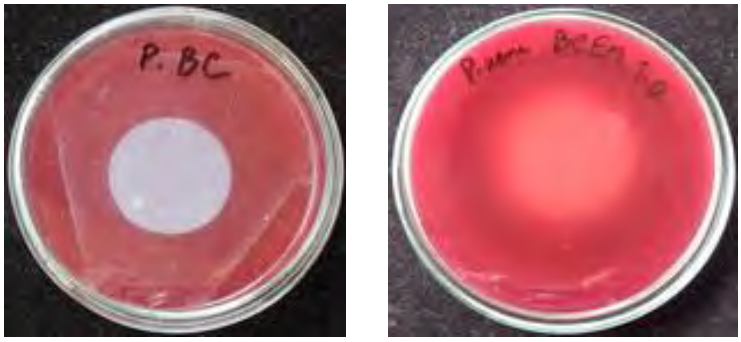


On *S. aureus*



On *S. epidermidis*

Figure 5.23 Inhibitory effect of the BC and BCEM samples on the growth of bacteria for 24 h incubated at 37^{o}C



On *P. acnes*

On *C. albicans*

Figure 5.24 Inhibitory effect of the BC and BCEM samples on the growth of bacteria (*P. acnes*) for 72 h and yeast (*C. albicans*) for 24 h incubated at 37^{o}C

Table 5.7 Antifungal activity of BC and BCEM films against *A. niger*

Microorganisms	Sample	Observed growth
		Grade
A. niger	BC	5
	BCEM-0.25	2
	BCEM-0.50	2
	BCEM-1.00	2

*Grade was used as a measurement of fungal growth: 0 = none, 1 = only apparent under microscope, 2 = trace (<10%), 3 = light growth (10-30%), 4 = medium growth (30-60%) and 5 = heavy growth (> 60%)

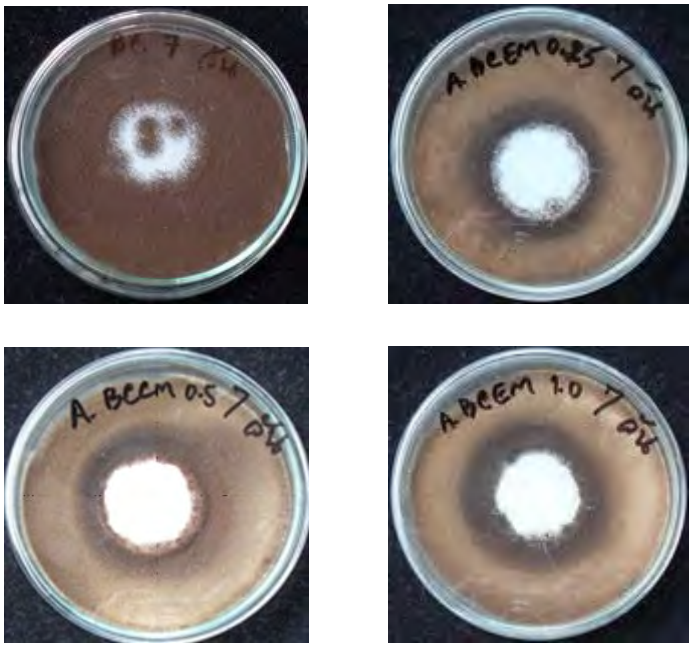


Figure 5.25 The growth of *A. niger* on the BC and BCEM specimens, at 30°C at the end of the incubation 7 days

5.4 Absorption and Release of Bioactive Compounds from BC Films containing *G. Mangostana* Aqueous and Ethanolic Extracts

Table 5.8 summarizes the actual amount of bioactive compounds were absorbed into BCWM and BCEM films. The adsorptions of bioactive compounds in BC film were measured to evaluate the extract contents loading capacity of BC film and to use for release characteristic analysis. After impregnation of *G. mangostana* aqueous and ethanolic extracts into BC film by 2 day immersion, BC films loading with *G. mangostana* aqueous extract at the concentrations of 2.5, 5.0 and 10.0%w/v, carried phenolic compounds (gallic acid equivalents) 47.88±0.28, 54.05±0.12 and 59.06±0.04 mg/cm^3 BCWM film, respectively.

Table 5.8 Actual amount of bioactive compounds were absorbed into BC wet (never dried) film

Films	Bioactive compounds	Actual amount in modified BC film (mg/cm^3)
BCWM-2.5	phenolic compounds (GAE)	47.88±0.28
BCWM-5.0		54.05±0.12
BCWM-10.0		59.06±0.04
BCEM-0.25	phenolic compounds (GAE)	1.769±0.061
BCEM-0.50		3.007±0.091
BCEM-1.00		4.580±0.086
BCEM-0.25	mangostins (AME)	1.488±0.007
BCEM-0.50		2.483±0.011
BCEM-1.00		4.054±0.012

The actual amount of mangostins in BCWM films was not analyzed because of the absence of mangostins in *G. mangostana* aqueous extract (see in part 5.1). Meanwhile, BC films loading with *G. mangostana* ethanolic extract at the concentrations of 0.25, 0.50 and 1.00%v/v, contained phenolic compounds (gallic acid equivalents) 1.769±0.061, 3.007±0.091 and 4.580±0.086 mg/cm^3 BCEM film, respectively. In addition, the actual amount of mangostins (alpha-mangostin equivalents) in BCEM films with loaded concentrations of 0.25, 0.50 and 1.00%v/v, were 1.488±0.007, 2.483±0.011 and 4.054±0.012 mg/cm^3 BCEM film, respectively. From Table 5.8, the change of bioactive compounds amount in BCWM films by increase *G. mangostana* aqueous extract in immersing solution was not obvious, thus at the concentration of 10%w/v aqueous extract in immersing solution should approach to the maximum load of BC film.

The release characteristics of bioactive compounds from BC films containing *G. mangostana* aqueous and ethanolic extracts were examined using the total immersion method modified by Suwantong *et al*. in 2007. Previously, Suwantong *et al*. used a B/T/M medium to study the release characteristics of curcumin from curcumin-loaded electrospun CA fiber mats and cast CA films. The release assay was conducted with immersion of BCWM and BCEM films in the acetate buffer solution (pH 5.5) composing of 0.5%v/v Tween80 and 3%v/v methanol at 37 ^{o}C. In this study, the cumulative release profiles of bioactive compounds from BC films containing *G. mangostana* aqueous and ethanolic extracts were reported as the percentage of the weight of bioactive compounds (phenolic compounds expressed as gallic acid equivalents (GAE) and mangostins expressed as alpha-mangostin equivalents (AME)) released divided by the actual weight of bioactive compounds containing in BCWM and BCEM films

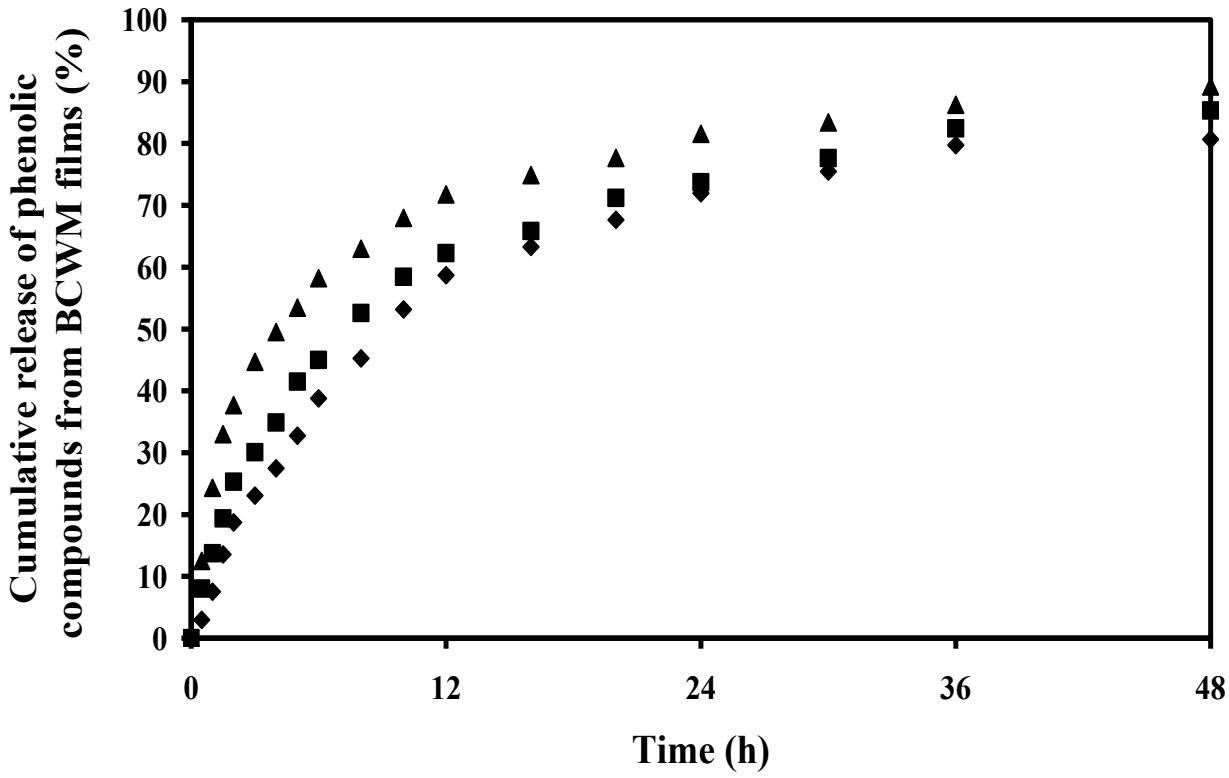


Figure 5.26 Cumulative release profile of phenolic compounds from BCWM films reported as the percentage of the weight of phenolic compounds (GAE) released divided by the actual amount of phenolic compounds

♦ BCWM film impregnated with 2.5%w/v of *G. mangostana* aqueous extract

■ BCWM film impregnated with 5.0%w/v of *G. mangostana* aqueous extract

▲ BCWM film impregnated with 10.0%w/v of *G. mangostana* aqueous extract

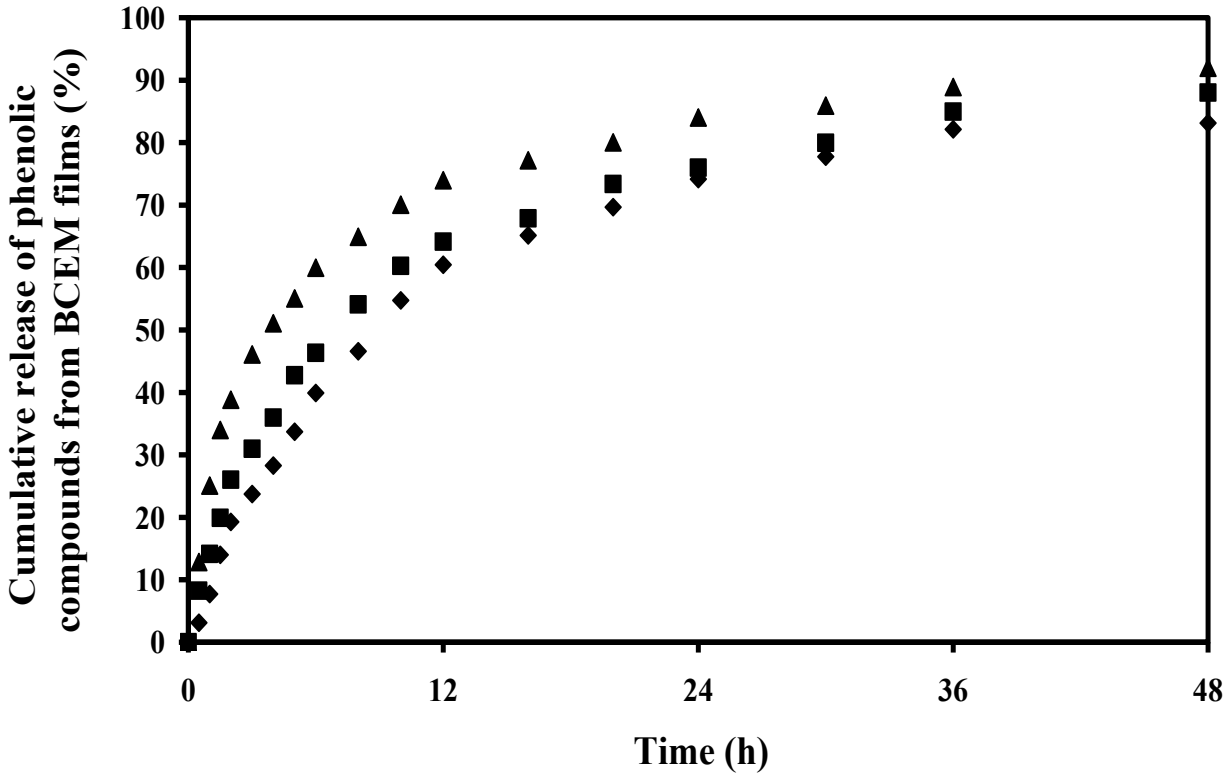


Figure 5.27 Cumulative release profile of phenolic compounds from BCEM films reported as the percentage of the weight of phenolic compounds (GAE) released divided by the actual amount of phenolic compounds

♦ BCEM film impregnated with 0.25%v/v of *G. mangostana* ethanolic extract

■ BCEM film impregnated with 0.50%v/v of *G. mangostana* ethanolic extract

▲ BCEM film impregnated with 1.00%v/v of *G. mangostana* ethanolic extract

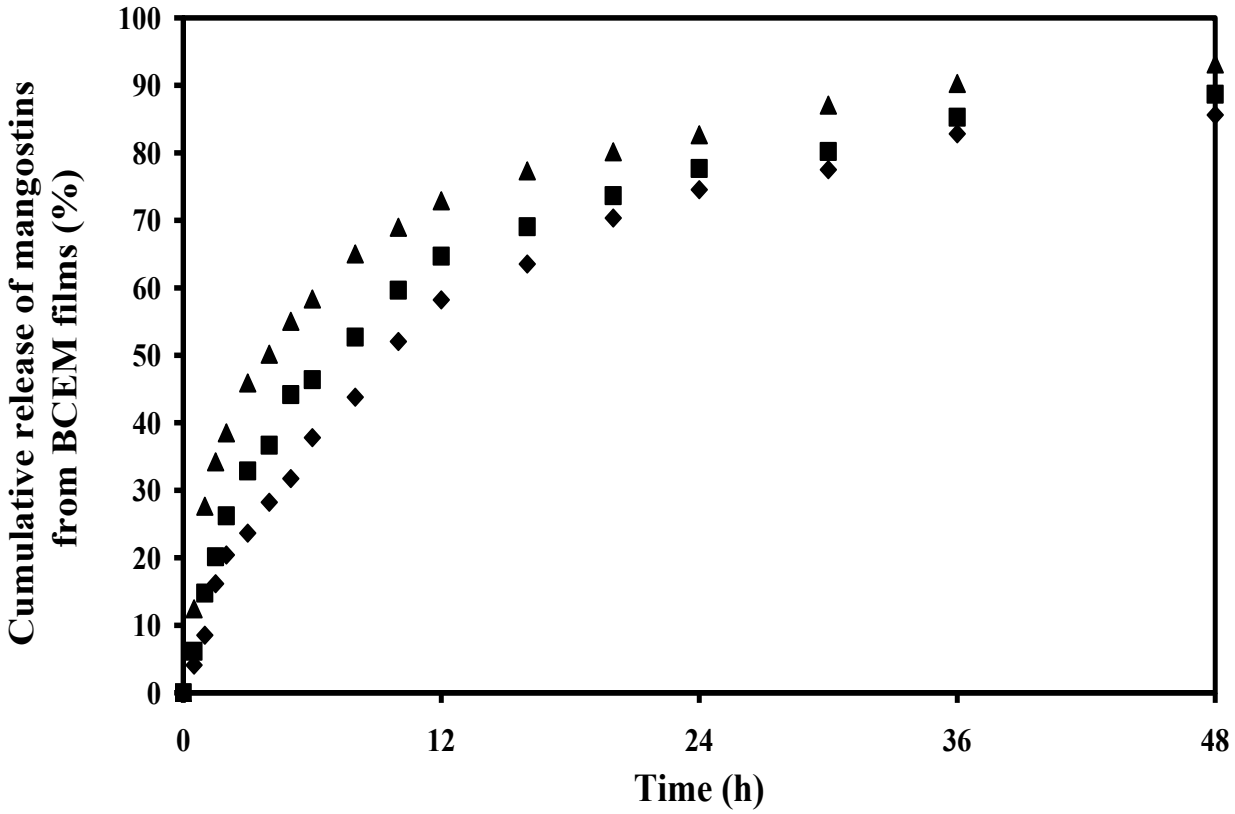


Figure 5.28 Cumulative release profile of mangostins from BCEM films reported as the percentage of the weight of mangostins (AME) released divided by the actual amount of mangostins

♦ BCEM film impregnated with 0.25%v/v of *G. mangostana* ethanolic extract

■ BCEM film impregnated with 0.50%v/v of *G. mangostana* ethanolic extract

▲ BCEM film impregnated with 1.00%v/v of *G. mangostana* ethanolic extract

The cumulative release of phenolic compounds from BCWM films was reported in figure 5.26. The BC film modified by supplementation of *G. mangostana* aqueous extract with various concentrations of 2.5, 5.0 and 10.0%w/v were investigated for the total amount of released phenolic compounds. The results showed that BCWM-2.5, 5.0 and 10.0 have the percentage of cumulative release equal to ~81, ~85 and ~89%, respectively. From Figures 5.27 and 5.28, the amount of released phenolic compounds from BCEM films after the end of total immersion (at 48 h) were ~83, ~88 and ~92% for BC film impregnated with *G. mangostana* ethanolic extract at several concentrations of 0.25, 0.50 and 1.00%v/v, respectively. As well as the total mangostins released from BCEM-0.25, 0.50 and 1.00 were expressed at ~86, ~89 and ~93%, respectively. Both BCWM and BCEM films showed a rapid increase in the cumulative release of bioactive compounds (phenolic compounds and mangostins) during the initial 24 h of total immersion. Afterwards, the cumulative release rate of bioactive compounds from BCWM and BCEM films became slower than the former and the total amount of released bioactive compounds into B/T/M medium gradually increased from 24 to 48 h of total immersion. Moreover, the results indicated that bioactive compounds which were absorbed into BC film could almost release into B/T/M solution.

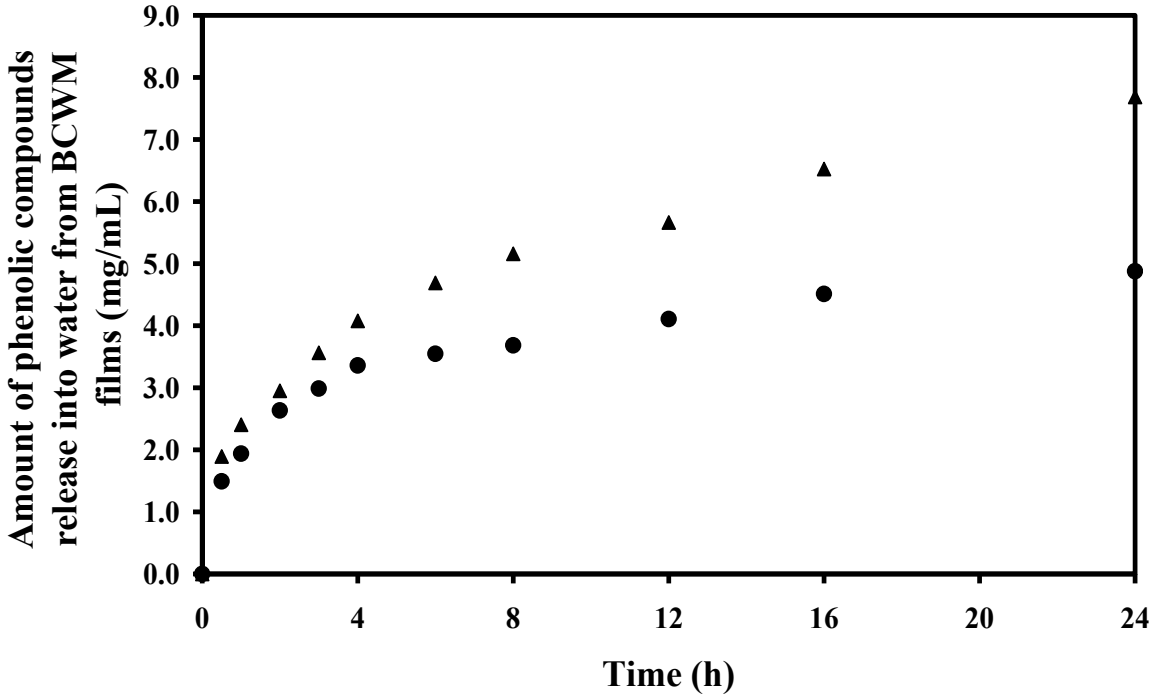


Figure 5.29 Amount of phenolic compounds release into water from BCWM films incubated at 37^{o}C

● BCWM film impregnated with 5.0%w/v of *G. mangostana* aqueous extract

▲ BCWM film impregnated with 10.0%w/v of *G. mangostana* aqueous extract

Figures 5.29 and 5.30 illustrate the amount of phenolic compounds release into water from BC films modified with *G. mangostana* aqueous and ethanolic extract, respectively. Meanwhile, Figure 5.31 indicates the amount of alpha-mangostin release into water from BC films modified with *G. mangostana* ethanolic extract. These release profiles were studied on the basis of bioactive compounds releasing condition in 24-well-plated which was used as cytotoxic effects of BC modified films assay in this study (see in part 5.5).

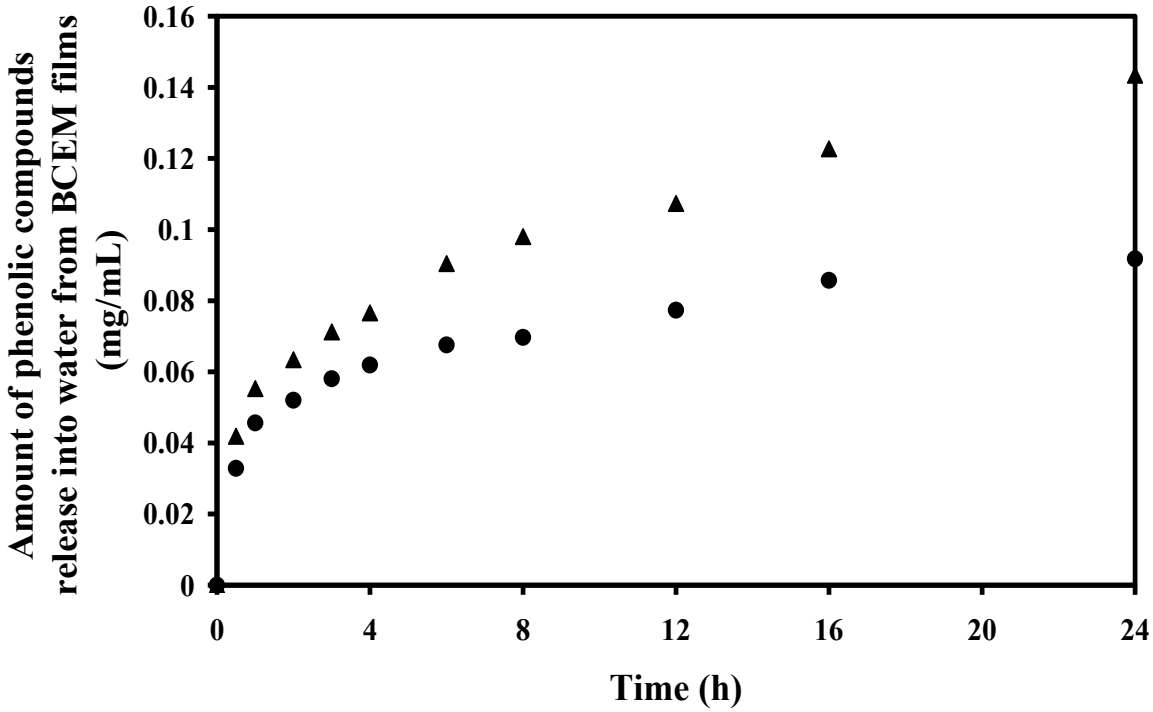


Figure 5.30 Amount of phenolic compounds release into water from BCEM films incubated at 37°C

● BCEM film impregnated with 0.50%v/v of *G. mangostana* ethanolic extract

▲ BCEM film impregnated with 1.00%v/v of *G. mangostana* ethanolic extract

As seen in Figures 5.29-5.31, the amount of phenolic compounds (gallic acid equivalents) release into water at 3 h incubation were 3.17 and 3.82 mg/mL for BCWM-5.0 and 10.0, respectively. Released phenolic compounds from BCEM-0.50 and 1.00 into water at 0.5 h incubation were 0.046 and 0.055 mg/mL, respectively, and released mangostins (alpha-mangostin equivalents) from BCEM-0.50 and 1.00 into water at 0.5 h incubation were 0.018 and 0.028 mg/mL, respectively.

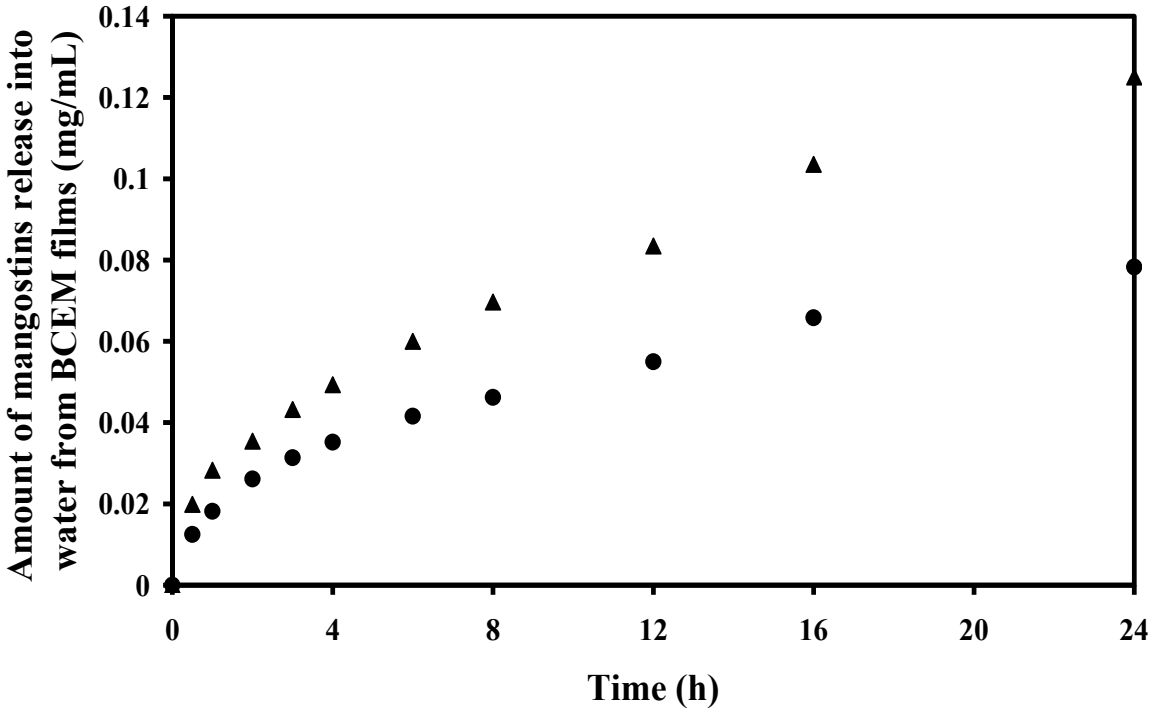


Figure 5.31 Amount of mangostins release into water from BCEM films incubated at 37^{o}C

● BCEM film impregnated with 0.50%v/v of *G. mangostana* ethanolic extract

▲ BCEM film impregnated with 1.00%v/v of *G. mangostana* ethanolic extract

5.5 Cytotoxic Effects against B16 Melanoma Cells

In order to investigate the cytotoxic effects of the BCWM and BCEM films on the mouse melanoma cells (B16), the viability of B16 melanoma cells was examined by the exposure of B16 melanoma cells in the media culture containing *G. mangostana* aqueous or ethanolic extracts, which were released from the BCWM and BCEM films, respectively, compared with the cells cultured in the medium of BC without extract (control). The amount of cell viability after B16 melanoma cells were treated with *G. mangostana* aqueous and ethanolic extracts was then evaluated by the MTT assay.

The MTT assay is basically active cell viability measurement and it was first developed by Mosmann in 1983. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is water soluble yellow salt which was conversed to purple formazan crystals, not soluble in aqueous solution, by mitochondrial succinic dehydrogenases secreted from viable cells. The proportion of metabolically active cells base on formazan formed (Hamid *et al.*, 2004; Young *et al.*, 2005).

Table 5.9 Cytotoxic effects of BCWM and BCEM films against B16 melanoma cells

Extracts	Samples	Time (h)	%Cell viability
Water	BCWM-5.0	3	84.97±11.26
	BCWM-10.0	3	81.41±12.77
Ethanol	BCEM-0.50	0.5	13.98±0.38
	BCEM-1.00	0.5	13.42±0.30

Table 5.9 expresses that both of the BCWM (5.0 and 10.0%w/v) and BCEM (0.50 and 1.00%v/v) inhibited the viability of dermal carcinoma cells. The antiproliferation activity of BCWM films showed that BCWM-5.0 and 10.0 exhibited antiproliferation activity against B16 melamona cells with the following inhibitory activities of 15.03 and 18.59%, respectively. Meanwhile, the anticancer activity of BCEM films indicated that BCEM-0.50 and 1.00 exhibited anticancer activity against B16 melamona cells with the corresponding inhibitory activities of 86.02 and 86.58%, respectively.

In this present work, Both of BCWM and BCEM films exhibited the antiproliferation activity against B16 mouse melanoma cells. The quantitative determination of bioactive compounds in *G. mangostana* aqueous and ethanolic extract as seen in part 5.1 could imply that the major coumpounds which should exhitbited the anticancer activity, were tannins for aqueous extract and mangostins (xanthones) for ethanolic extract.

From previous studies, *G. mangostana* ethanolic extract exhibited cytotoxic effects on U937, K562, HL60, NB4 and Molt4 leukemia cell lines, and Caco-2 human colorectal adenocarcinoma cells (Matsumoto *et al.*, 2003; Okonogi *et al.*, 2007; Sun *et al.*, 2009; Ampasavate *et al.*, 2010). The induction of apoptosis by xanthones from mangosteen in HL60 human leukemia cell lines was studied by Matsumoto *et al.* (2003). The results showed that xanthones extracted from the pericarp of mangosteen composing of α-mangostin, β-mangostin, γ-mangostin, mangostinone, garcinone E and 2-isoprenyl-1,4-dihydroxy-3-methoxyxanthone, have inhibitory effect against HL60 cell lines with IC_{50} equal to 6.8, 7.6, 6.1, 19.0, 15.0 and 23.6 μM, respectively. Moreover, the antioxidant effects on metal ion dependent (Cu^{2+}), independent (aqueous peroxyl radicals) oxidation of human LDL and the reactive oxygen species

(ROS) scavenging capacity were also provided by *G. mangostana* ethanolic extract (Williams *et al.*, 1994; Chaverri *et al.*, 2009). Futhermore, the antioxidant activities against DPPH radical, hydroxyl radical and lipid peroxidation of *G. mangostana* aqueous extract were reported and *G. mangostana* aqueous extract also showed the low cytotoxicity to human keratinocyte cells compared with *G. mangostana* extracted by other solvents (methanolic and hexane extracts) (Ngawhirunpat *et al.*, 2010). These results could indicate that the BC modified films with supplementation of *G. mangostana* aqueous and ethanolic extract have potential to be used for biomedical application.

G. mangostana ethanolic extract would have been expected to be superior competency against various human cancer cell lines; however, the dose of *G. mangostana* ethanolic extract which was contain in the BC film, should be also considered for the suitable content loaded into the BC film before further application on human normal cells. According to the cytotoxicity test in human normal cells by Ampasavate *et al.* (2010), the cytotoxic effect of *G. mangostana* ethanolic extract on peripheral blood mononuclear cells (PBMCs), which were frequently used as the model for human normal cells, was rather high with IC_{50} equal to 4.9 ± 0.2 μg/mL. Therefore, the content of *G. mangostana* ethanolic extract impregnated into the BC film should be further investigated in order to optimize the effects of the films carrying this extract compounds.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In this work, the modification of BC film was performed by immersing the BC films into aqueous and ethanolic extract solutions of *G. Mangostana*. The SEM images show that the surface of BC film became denser and the void fraction of the modified films decreased with increasing concentration of mangosteen extract compounds impregnated into the films. The amorphous layer of aqueous extract compounds coating on the BC film surface decreased the film's transparency. The FTIR result demonstrated the changes of peaks of the modified films, which implied the interactions between BC film and *G. mangostana* aqueous and ethanolic extract compounds. It was shown that the incorporation of *G. mangostana* aqueous extract into the films reduced the tensile strength, elongation at break and also elastic modulus of the BCWM films. On the other hand, the overall mechanical properties of BC films modified by *G. mangostana* ethanolic extract supplementation were improved, excepting the slightly reduced tensile strength and elasticity at excessive content of the ethanolic extract compounds. The WACs of BCWM films were increased owing to the more hydrophilic property, whereas the WACs of BCEM films were lower than that of the unmodified one. The water vapor transmission rates (WVTR) was raised by the increase of the mangosteen aqueous extract concentration, but the OTR was slightly decreased. It was shown that the BCWM films exhibited the antimicrobial action against *E. coli*, *S. aureus, S. epidermidis*, *P. acnes* and *A. niger*.

Meanwhile, the BCEM films exhibited the antimicrobial action against *S. aureus, S. epidermidis*, *P. acnes* and *A. niger*. In contrast, the growth of *C. albicans* did not be inhibited by BCWM and BCEM films. Finally, both BCWM and BCEM films exhibited the cytotoxicity against B16 mouse melanoma cells. The films loaded *G. mangostana* ethanolic extract showed significant potential in the cancer cells reduction. The results could indicate that the BC modified films with supplementation of *G. mangostana* aqueous and ethanolic extract have potential to be used for biomedical application.

6.2 Recommendations for Future Studies

Based on this study, further works for the improvement of the extract loaded films are recommended.

1. Modify the extract loaded films by the incorporation of other herbal extracts.
2. Study the method to adjust the film structure in order to control the release rate of the applied herbal extract at optimal level.
3. Investigate effects of the modified BC films on human normal cells and cancer cells for the suitable design of the films carrying herbal extracts.

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APPENDIX

APPENDIX

Table1 Data of Figure 5.6

G. mangostana aqueous extract supplementation (%w/v)	Tensile strength (MPa)						
	1	2	3	4	5	Average	S.D.
0	98.16	100.81	109.74	112.74	116.01	107.89	8.08
2.5	71.00	76.88	78.45	80.61	82.57	77.90	4.42
5.0	40.06	42.02	42.49	42.88	44.20	42.33	1.51
10.0	23.35	23.71	23.81	24.25	25.22	24.07	0.72

Table2 Data of Figure 5.7

G. mangostana aqueous extract supplementation (%w/v)	Elongation at break (%)						
	1	2	3	4	5	Average	S.D.
0	22.68	23.83	24.00	24.33	25.00	23.97	0.85
2.5	18.00	19.50	20.33	21.00	21.68	20.10	1.43
5.0	16.67	17.00	17.17	18.67	19.33	17.77	1.16
10.0	14.08	14.25	15.95	17.67	18.17	16.02	1.89

Table3 Data of Figure 5.8

G. mangostana aqueous extract supplementation (%w/v)	Young's modulus (MPa)						
	1	2	3	4	5	Average	S.D.
0	715.89	728.63	758.05	792.38	799.24	758.84	37.13
2.5	530.54	535.44	565.84	572.71	581.53	557.21	22.87
5.0	240.66	242.52	253.31	260.46	261.94	251.78	9.88
10.0	136.12	139.74	141.61	142.98	143.37	140.76	2.96

Table4 Data of Figure 5.9

G. mangostana aqueous extract supplementation (%w/v)	Water absorption capacity (%)						
	1	2	3	4	5	Average	S.D.
0	303.57	354.55	336.00	342.31	307.41	328.77	22.31
2.5	591.67	576.81	595.00	559.09	562.90	577.09	16.27
5.0	664.76	647.66	642.20	631.53	669.52	651.14	15.81
10.0	769.90	787.56	746.48	743.78	736.70	756.88	21.20

Table5 Data of Table 5.1

Extracts	Bioactive compounds	Amount of bioactive compounds (mg) in *G. mangostana* aqueous (per 1 g) and ethanolic (per 1 mL) extracts				
		1	2	3	Average	S.D.
Water	phenolic compounds	181.60	205.11	205.13	198.88	10.81
	mangostins	19.19	21.20	22.54	20.98	1.69
Ethanol	phenolic compounds	34.56	34.40	33.36	34.11	0.65
	mangostins	32.24	31.41	32.29	31.98	0.49

Table6 Data of Table 5.2

G. mangostana aqueous extract supplementation (%w/v)	OTR ($cc/m^2/day$)			
	1	2	Average	S.D.
0	2.53	2.53	2.53	0.00
5.0	1.70	1.72	1.71	0.01
10.0	1.45	1.45	1.45	0.00

Table7 Data of Table 5.3

G. mangostana aqueous extract supplementation (%w/v)	WVTR ($g/m^2/day$)			
	1	2	Average	S.D.
0	578.40	523.20	550.80	39.03
5.0	821.76	795.84	808.80	18.33
10.0	777.60	840.48	809.04	44.46

Table8 Data of Table 5.4

Microorganisms	*G. mangostana* aqueous extract supplementation (%w/v)	Clear zone (mm)				
		1	2	3	Average	S.D.
E. coli	0	0.00	0.00	0.00	0.00	0.00
	2.5	8.50	6.00	8.00	7.50	1.32
	5.0	8.50	8.50	8.00	8.33	0.29
	10.0	11.00	8.50	8.50	9.33	1.44
S. aureus	0	0.00	0.00	0.00	0.00	0.00
	2.5	6.00	5.00	4.00	5.00	1.00
	5.0	5.00	5.50	6.00	5.50	0.50
	10.0	6.00	6.00	7.50	6.50	0.87
S. epidermidis	0	0.00	0.00	0.00	0.00	0.00
	2.5	1.00	2.00	1.00	1.33	0.58
	5.0	4.50	4.00	4.00	4.17	0.29
	10.0	4.50	5.50	5.00	5.00	0.50
P. acnes	0	0.00	0.00	0.00	0.00	0.00
	2.5	6.00	4.50	6.00	5.50	0.87
	5.0	6.00	6.00	6.00	6.00	0.00
	10.0	6.00	6.00	6.00	6.00	0.00
C. albicans	0	0.00	0.00	0.00	0.00	0.00
	2.5	0.00	0.00	0.00	0.00	0.00
	5.0	0.00	0.00	0.00	0.00	0.00
	10.0	0.00	0.00	0.00	0.00	0.00

Table9 Data of Figure 5.19

G. mangostana ethanolic extract supplementation (%v/v)	Tensile strength (MPa)						
	1	2	3	4	5	Average	S.D.
0	72.18	72.77	74.43	78.65	79.73	75.55	3.44
0.25	168.97	170.05	173.28	174.56	179.36	173.24	4.11
0.50	180.34	180.93	190.25	193.68	196.72	188.39	7.44
1.00	121.31	125.03	126.11	126.51	127.00	125.19	2.29

Table10 Data of Figure 5.20

G. mangostana ethanolic extract supplementation (%v/v)	Elongation at break (%)						
	1	2	3	4	5	Average	S.D.
0	18.67	18.67	19.00	19.17	19.50	19.00	0.35
0.25	50.70	50.70	51.30	51.70	52.80	51.44	0.87
0.50	54.30	54.70	56.70	59.90	61.70	57.46	3.24
1.00	57.70	62.30	62.70	65.00	65.30	62.60	3.05

Table11 Data of Figure 5.21

G. mangostana ethanolic extract supplementation (%v/v)	Young's modulus (MPa)						
	1	2	3	4	5	Average	S.D.
0	437.87	441.79	446.30	481.11	519.75	465.36	34.93
0.25	580.55	591.34	601.15	625.66	638.41	607.42	24.05
0.50	601.64	614.29	641.35	652.73	662.93	634.59	25.86
1.00	334.60	361.96	364.32	371.97	392.46	365.06	20.83

Table12 Data of Figure 5.22

G. mangostana ethanolic extract supplementation (%v/v)	Water absorption capacity (%)						
	1	2	3	4	5	Average	S.D.
0	303.57	354.55	336.00	342.31	307.41	328.77	22.31
0.25	234.78	244.90	244.00	242.00	231.82	239.50	5.85
0.50	270.21	285.71	282.00	289.80	258.00	277.14	12.96
1.00	371.93	359.32	344.07	355.93	343.10	354.87	11.91

Table13 Data of Table 5.6

Microorganisms	*G. mangostana* ethanolic extract supplementation (%v/v)	Clear zone (mm)				
		1	2	3	Average	S.D.
E. coli	0	0.00	0.00	0.00	0.00	0.00
	0.25	0.00	0.00	0.00	0.00	0.00
	0.50	0.00	0.00	0.00	0.00	0.00
	1.00	0.00	0.00	0.00	0.00	0.00
S. aureus	0	0.00	0.00	0.00	0.00	0.00
	0.25	2.50	2.50	1.00	2.00	0.87
	0.50	5.00	6.00	5.00	5.33	0.58
	1.00	8.50	6.00	6.00	6.83	1.44
S. epidermidis	0	0.00	0.00	0.00	0.00	0.00
	0.25	2.00	1.00	3.50	2.17	1.26
	0.50	2.50	2.50	2.00	2.33	0.29
	1.00	3.50	3.50	3.50	3.50	0.00
P. acnes	0	0.00	0.00	0.00	0.00	0.00
	0.25	4.00	7.00	3.50	4.83	1.89
	0.50	4.00	6.75	4.25	5.00	1.52
	1.00	6.00	8.50	7.75	7.42	1.28
C. albicans	0	0.00	0.00	0.00	0.00	0.00
	0.25	0.00	0.00	0.00	0.00	0.00
	0.50	0.00	0.00	0.00	0.00	0.00
	1.00	0.00	0.00	0.00	0.00	0.00

Table14 Data of Table 5.8

Films	Bioactive compounds	Actual amount in modified BC film (mg/cm^3)				
		1	2	3	Average	S.D.
BCWM-2.5	phenolic compounds (GAE)	47.57	47.94	48.13	47.88	0.28
BCWM-5.0		53.93	54.06	54.17	54.05	0.12
BCWM-10.0		59.08	59.01	59.08	59.06	0.04
BCEM-0.25	phenolic compounds (GAE)	1.769	1.708	1.830	1.769	0.061
BCEM-0.50		3.086	2.908	3.206	3.007	0.091
BCEM-1.00		4.504	4.561	4.674	4.580	0.086
BCEM-0.25	mangostins (AME)	1.484	1.484	1.496	1.488	0.007
BCEM-0.50		2.483	2.472	2.494	2.483	0.011
BCEM-1.00		4.061	4.040	4.061	4.054	0.012

Table15 Data of Figure 5.26

		Cumulative release of phenolic compounds (%)		
	Films	BCWM-2.5	BCWM-5.0	BCWM-10.0
Time (h)	0.5	2.98	8.01	12.50
	1.0	7.50	13.72	24.32
	1.5	13.57	19.35	32.98
	2.0	18.71	25.29	37.69
	3.0	23.05	30.06	44.72
	4.0	27.46	34.89	49.50
	5.0	32.73	41.50	53.43
	6.0	38.78	44.98	58.22
	8.0	45.26	52.58	63.01
	10.0	53.15	58.45	67.99
	12.0	58.71	62.29	71.76
	16.0	63.27	65.86	74.88
	20.0	67.66	71.18	77.68
	24.0	71.94	73.72	81.57
	30.0	75.44	77.61	83.40
	36.0	79.72	82.42	86.28
	48.0	80.67	85.34	89.24

Table16 Data of Figure 5.27

		Cumulative release of phenolic compounds (%)		
	Films	BCEM-0.25	BCEM-0.50	BCEM-1.00
Time (h)	0.5	3.11	8.27	12.86
	1.0	7.73	14.16	25.10
	1.5	14.00	19.93	33.99
	2.0	19.26	26.02	38.84
	3.0	23.73	30.99	46.10
	4.0	28.29	35.96	51.09
	5.0	33.71	42.76	55.06
	6.0	39.93	46.35	59.96
	8.0	46.60	54.11	64.94
	10.0	54.73	60.25	70.05
	12.0	60.45	64.13	73.99
	16.0	65.16	67.86	77.17
	20.0	69.68	73.38	80.03
	24.0	74.14	75.99	84.04
	30.0	77.75	79.95	85.94
	36.0	82.12	84.95	88.91
	48.0	83.12	88.00	91.98

Table17 Data of Figure 5.28

		Cumulative release of mangostins (%)		
	Films	BCEM-0.25	BCEM-0.50	BCEM-1.00
เวลา (h)	0.5	4.10	6.13	12.46
	1.0	8.53	14.77	27.63
	1.5	16.15	20.16	34.22
	2.0	20.42	26.21	38.50
	3.0	23.64	32.86	45.91
	4.0	28.23	36.69	50.16
	5.0	31.73	44.18	55.04
	6.0	37.79	46.38	58.35
	8.0	43.79	52.68	65.02
	10.0	52.06	59.64	68.99
	12.0	58.22	64.69	72.92
	16.0	63.54	69.05	77.33
	20.0	70.36	73.66	80.17
	24.0	74.55	77.67	82.68
	30.0	77.50	80.21	87.09
	36.0	82.82	85.29	90.29
	48.0	85.62	88.68	93.17

Table18 Data of Table 5.9

Type of films	Time (h)	%Cell viability				
		1	2	3	Average	S.D.
BCWM-5.0	3.0	74.54	96.91	83.46	84.97	11.26
BCWM-10.0		68.28	82.19	93.78	81.41	12.77
BCEM-0.50	0.5	14.42	13.70	13.82	13.98	0.38
BCWM-1.00		13.70	13.46	13.11	13.42	0.30

Table19 Data of Figures 5.29-5.31

		The amount of released contents (mg/mL)					
Bioactive compounds		phenolic (GAE)		phenolic (GAE)		mangostin (AME)	
Type of films		BCWM-5.0	BCWM-10.0	BCEM-0.50	BCEM-1.00	BCEM-0.50	BCEM-1.00
Time (h)	0.5	1.491	1.894	0.033	0.042	0.012	0.020
	1	1.940	2.403	0.046	0.055	0.018	0.028
	2	2.635	2.952	0.052	0.063	0.026	0.035
	3	2.986	3.562	0.058	0.071	0.031	0.043
	4	3.358	4.078	0.062	0.077	0.035	0.049
	6	3.547	4.691	0.068	0.090	0.042	0.060
	8	3.684	5.158	0.070	0.098	0.046	0.070
	12	4.108	5.664	0.077	0.107	0.055	0.083
	16	4.511	6.524	0.086	0.123	0.066	0.104
	24	4.877	7.686	0.092	0.143	0.078	0.125

Table20 Standard calibration curve of phenolic compounds (gallic acid equivalents) analyzed by UV-Vis spectrophotometer

Concentration (mg/mL)	Absorbance (765 nm)			Average
0.03	0.340	0.337	0.339	0.339
0.04	0.422	0.424	0.424	0.423
0.05	0.550	0.549	0.552	0.550
0.06	0.661	0.660	0.660	0.660
0.07	0.758	0.761	0.763	0.761

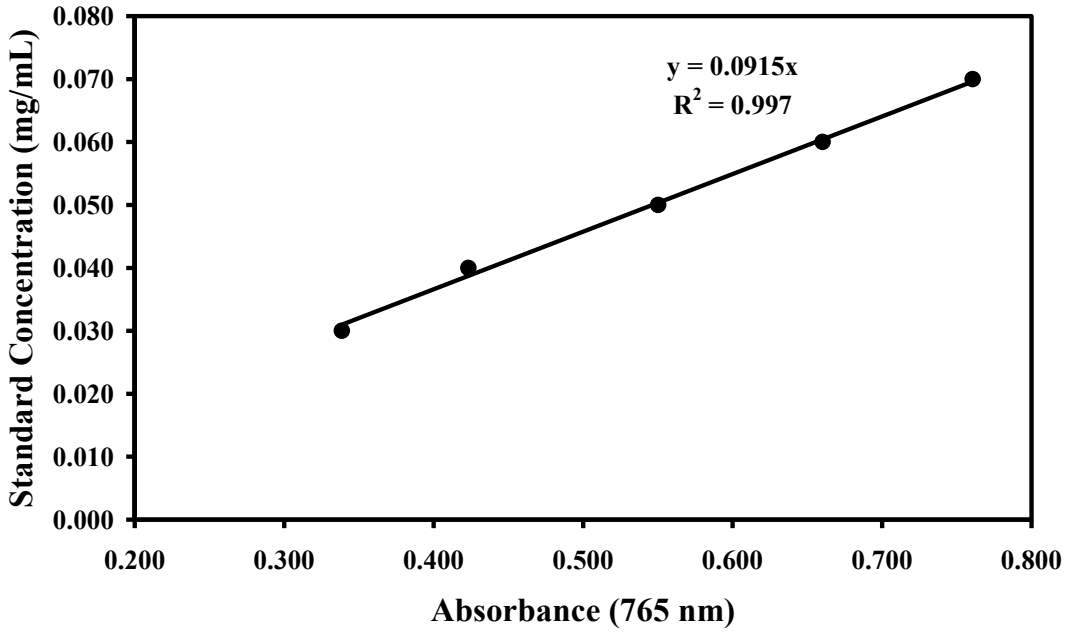


Figure1 Standard calibration curve of phenolic compounds

Table21 Standard calibration curve of mangostins data (alpha-mangostin equivalents) analyzed by UV-Vis spectrophotometer

Concentration (mg/mL)	Absorbance (320 nm)			Average
0.004	0.249	0.251	0.252	0.251
0.006	0.359	0.361	0.364	0.364
0.008	0.477	0.480	0.482	0.482
0.010	0.586	0.590	0.585	0.585
0.012	0.727	0.728	0.730	0.730

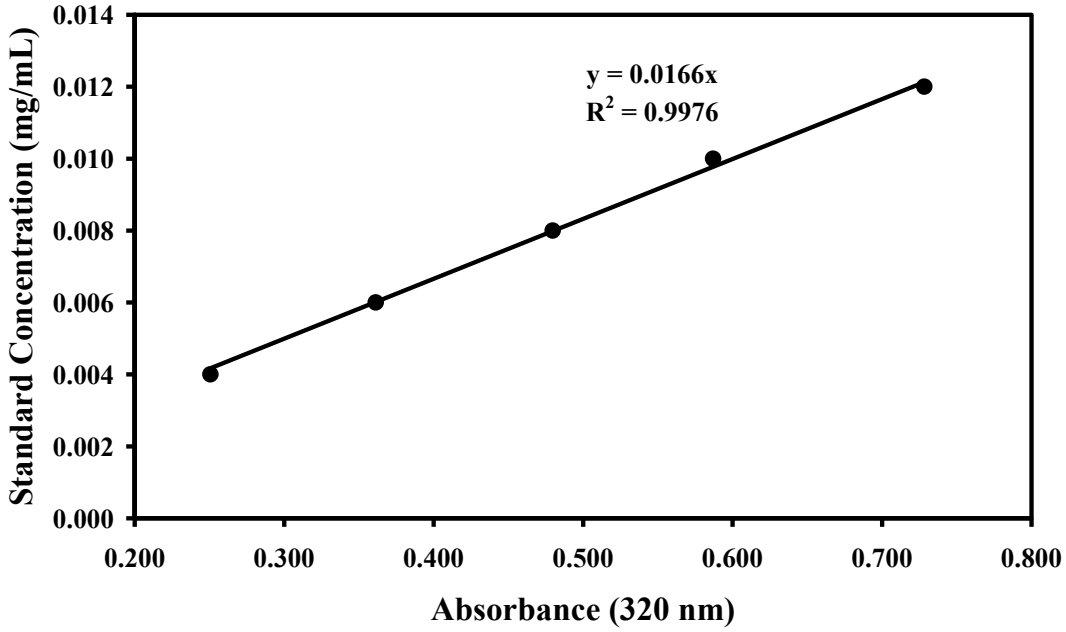
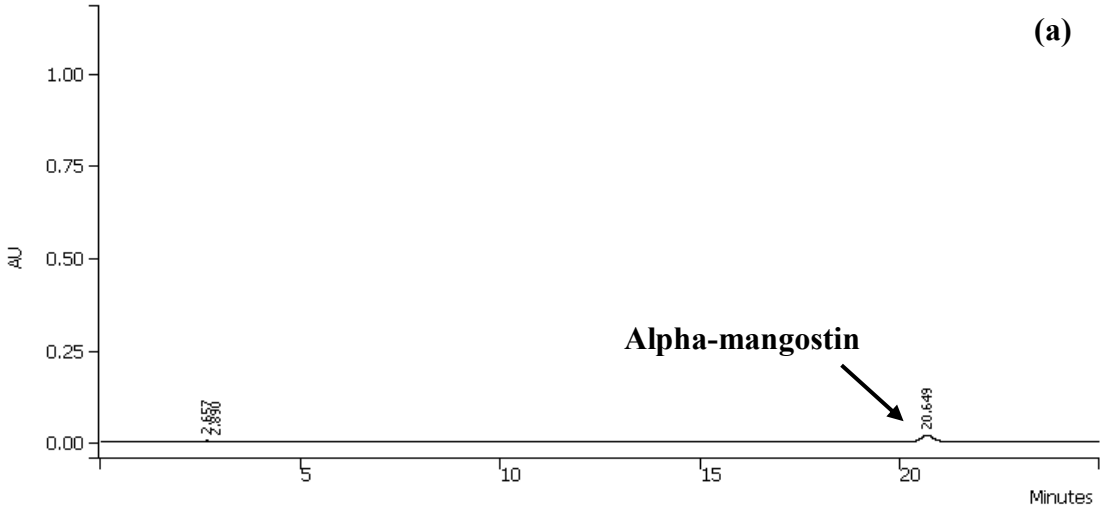
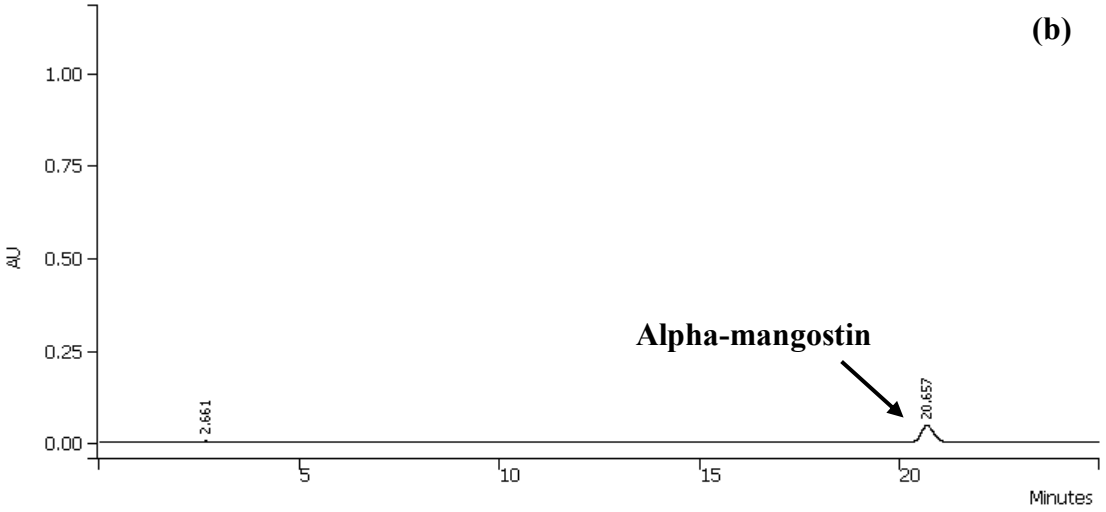


Figure2 Standard calibration curve of mangostins

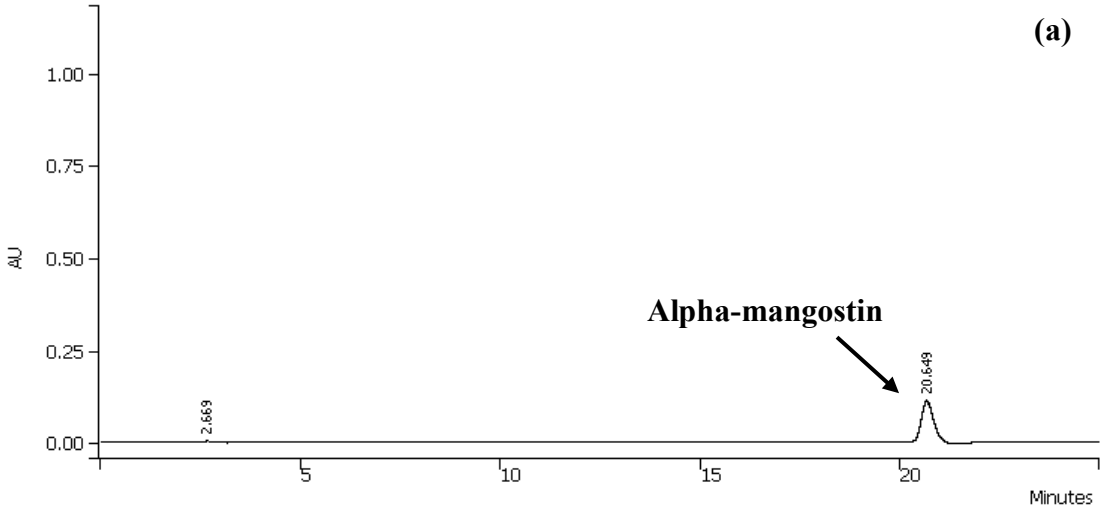


Peak No	Peak Name	Result (%area)	Ret. Time (min)	Area (counts)	Sep. Code	Width 1/2 (sec)
1		3.0328	2.657	123314	BP	3.6
2		1.3071	2.890	53148	PB	0.0
3		95.6600	20.649	3889536	BB	19.6
	Totals	**99.9999**		**4065998**		

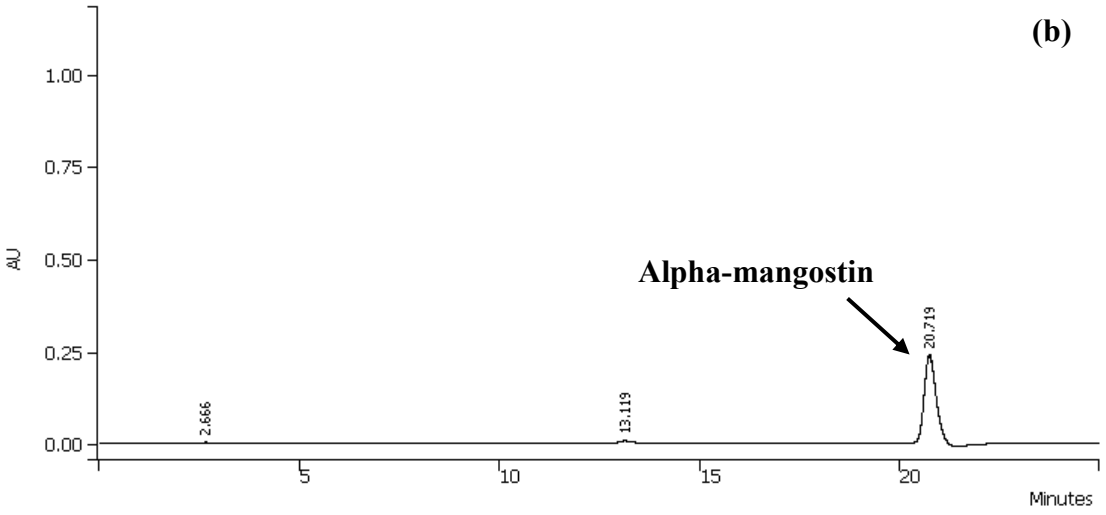


Peak No	Peak Name	Result (%area)	Ret. Time (min)	Area (counts)	Sep. Code	Width 1/2 (sec)
1		1.2052	2.661	118256	BB	3.7
2		98.7948	20.657	9694198	BB	19.6
	Totals	**100.0000**		**9812454**		

Figure3 HPLC chromatograms of α-mangostin standard at various concentrations: (a) 5 µg/mL and (b) 10 µg/mL

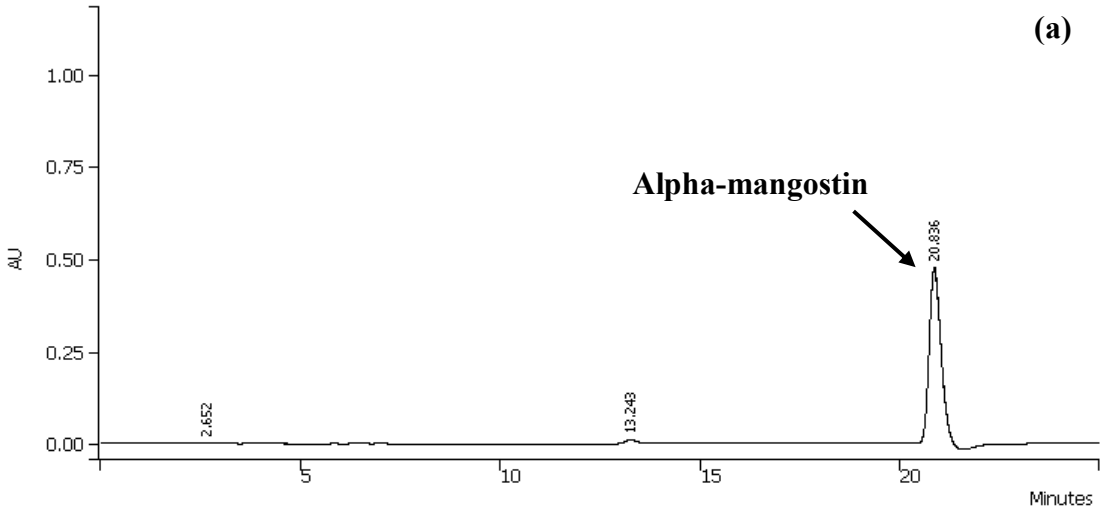


Peak No	Peak Name	Result (%area)	Ret. Time (min)	Area (counts)	Sep. Code	Width 1/2 (sec)
1		0.4269	2.669	103917	BB	3.5
2		99.5731	20.649	24237688	BB	19.3
	Totals	**100.0000**		**24341604**		

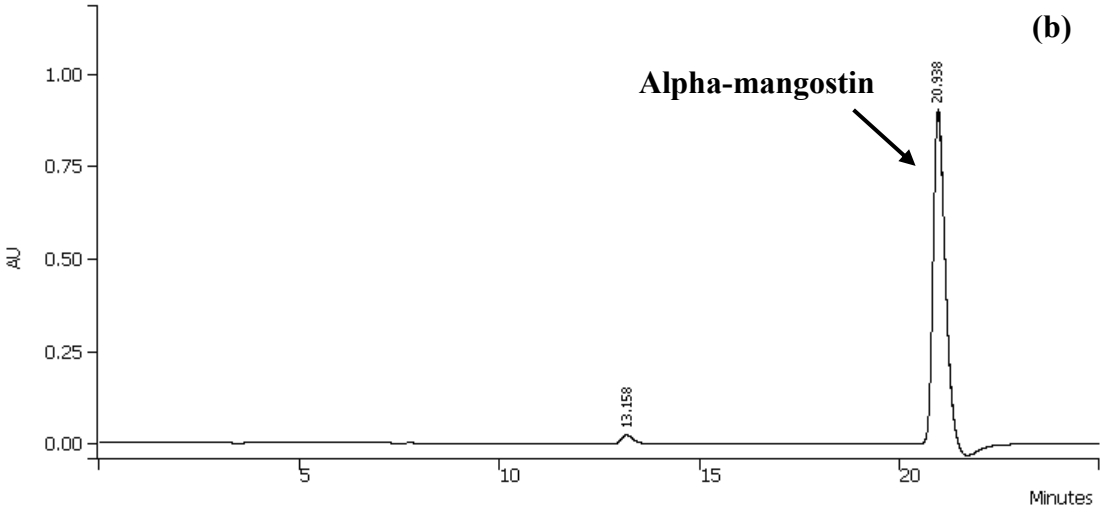


Peak No	Peak Name	Result (%area)	Ret. Time (min)	Area (counts)	Sep. Code	Width 1/2 (sec)
1		0.1906	2.666	99308	BB	3.2
2		1.7790	13.119	926979	BB	16.7
3		98.0304	20.719	51080996	BB	19.0
	Totals	**100.0000**		**52107284**		

Figure4 HPLC chromatograms of α-mangostin standard at various concentrations: (a) 25 µg/mL and (b) 50 µg/mL



Peak No	Peak Name	Result (%area)	Ret. Time (min)	Area (counts)	Sep. Code	Width 1/2 (sec)
1		0.0748	2.652	77902	BB	3.0
2		1.7456	13.243	1817444	BB	17.6
3		98.1796	20.836	102219760	BB	19.0
	Totals	**100.0000**		**104115104**		



Peak No	Peak Name	Result (%area)	Ret. Time (min)	Area (counts)	Sep. Code	Width 1/2 (sec)
1		2.1389	13.158	4296677	BB	17.4
2		97.8611	20.938	196585376	BB	19.2
	Totals	**100.0000**		**200882048**		

Figure5 HPLC chromatograms of α-mangostin standard at various concentrations: (a) 100 µg/mL and (b) 200 µg/mL

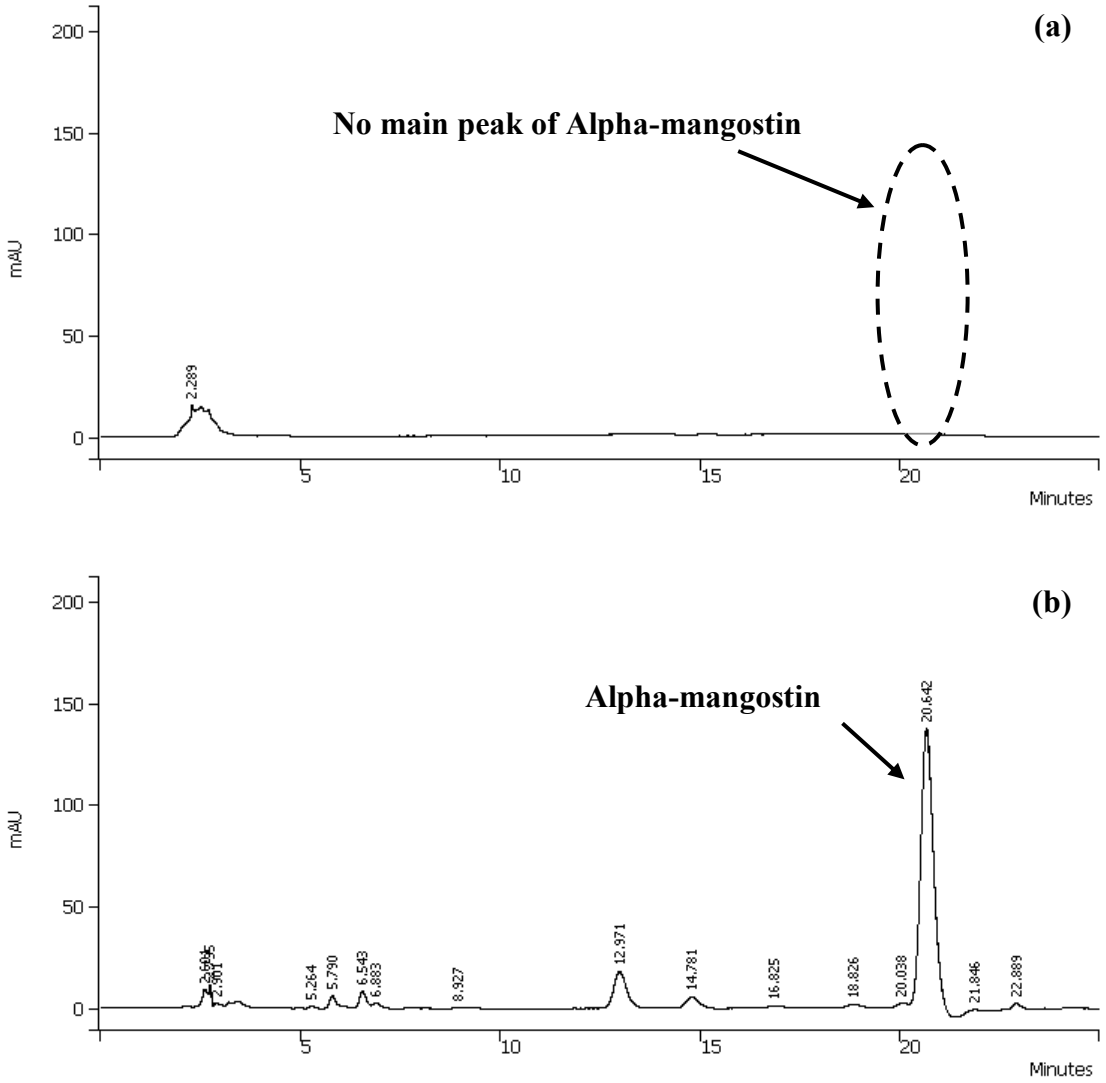


Figure6 HPLC chromatograms of *G. mangostana* extract by different extraction: (a) aqueous solvent and (b) ethanolic solvent

Table22 Standard calibration curve of mangostins data (alpha-mangostin equivalents) analyzed by HPLC

	Concentration (mg/mL)	Peak area
Standard (AME)	0.005	3889536
	0.010	9694198
	0.025	24237688
	0.050	51080996
	0.100	102219760
	0.200	196585376
Sample (diluted 1000-fold)	32.50	32500090
Sample (diluted 2000-fold)	31.41	15702950

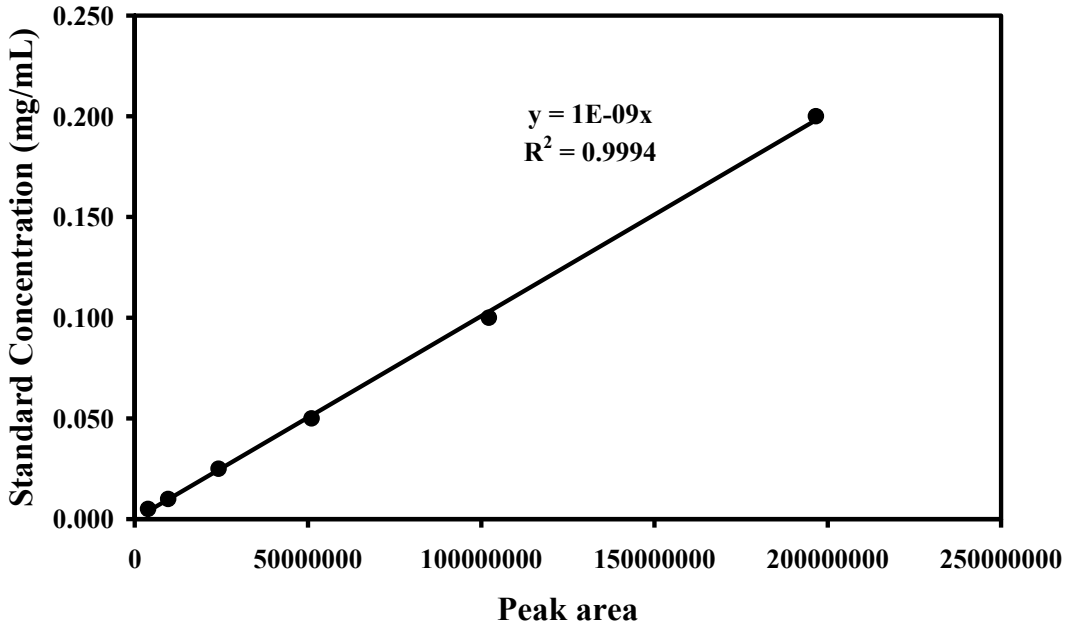


Figure7 Standard calibration curve of mangostins

VITAE

Mr. Natthawut Nunkaew was born on October 29th, 1986 in Chumphon, Thailand. He received the Bachelor Degree of Chemical technology from Faculty of Science, Chulalongkorn University in May, 2009. He continued Master degree in chemical engineering at Chulalongkorn University in June, 2009.

His academic publications are as follows:

1. CHARACTERISTICS OF BACTERIAL CELLULOSE FILM SYNTHESIZED BY *ACETOBACTER XYLINUM* CONTAININD EXTRACT FROM RIND OF *GARCINIA MANGOSTANA* in the 3th Pure and Applied Chemistry International Conference 2011 (PACCON2011) on January 5-7, 2011 at Miracle Grand Hotel, Bangkok, Thailand.
2. ANTIMICROBIAL ACTIVITIES OF BACTERIAL CELLULOSE MODIFIED BY SUPPLEMENTATION OF WATER AND ETHANOLIC EXTRACTS OF FRUIT RIND OF *GARCINIA MANGOSTANA* in the 3th Biochemistry and Molecular Biology International Conference 2011 (BMB2011) on April 6-8, 2011 at The Empress Convention Centre, Chiang Mai, Thailand, pp.290-294.