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THESIS

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF THAI HERB EXTRACTS IN COCONUT MILK

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A Thesis Submitted in Partial Fulfillment of
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Antimicrobial and antioxidant activities of twenty five Thai herb extracts were screened for their antioxidant capacity and growth inhibition of coconut milk spoilage microorganisms. The results indicated that ethanol extract from the selected plants showed different degree of growth inhibition of the test microorganisms and antioxidant capacities which were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and reducing power methods. Among crude ethanol extracts of all plants, the extract of cassod tree exhibited a significant ($P < 0.05$) high antimicrobial activity with inhibition zone ranging from 16.17±0.29 to 25.00±1.00 mm and high levels of total phenolic contents (345.64±6.24 mg GAE/g), IC_{50} values of 1.86±0.03 µg/ml and reducing power values of 0.40±0.00. Its MIC and MBC values were 0.3-1.2 mg/ml and 0.6-3.0 mg/ml, respectively, depending on the strain type. The inhibitory action of the extract against *B. licheniformis* KUB1 was also confirmed in nutrient broth and its antimicrobial efficiency was found to be affected by coconut oil concentration and pH. In model food, it displayed bacteriostatic effect in coconut milk and coconut milk cream for 12 h of incubation with total viable counts of ca. 6 log CFU/ml which was ca. 2 log CFU/ml lower than that in control. Furthermore, transmission electron microscopy clearly demonstrated that the cassod tree extract showed localized disintegration of cell envelope and cell wall, leaking of cytoplasm and irregular aggregation in the cytoplasm. Additionally, the major compound of the extract identified by HPLC-PDA, LC-TOF-MS and NMR spectroscopy was piceatannol at the amount of 722.88 µg/g of crude extract. This study suggests that cassod tree extract and its bioactive components have potential for application as natural food preservatives.

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Kriangkrai Phattayakorn

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

e.g.	=	*exempli gratia* (for example)
ca.	=	approximately
CFU/ml	=	colony forming unit per milliliter
h	=	hour
°C	=	degree celcius
µg/ml	=	microgram per milliliter
etc.	=	et cetara (and so on)
mg/l	=	milligram per liter
HPLC-MS	=	high performance liquid chromatography-mass spectrometry
NMR	=	nuclear magnetic resonance spectroscopy
TEM	=	transmission electron microscope
g	=	gram
w/v	=	weight per volume
v/v	=	volume per volume
ml	=	milliliter
µl	=	microliter
mm	=	millimeter
nm	=	nanometer
mg GAE/g	=	milligram of gallic acid equivalent
M	=	molar
µM	=	micro molar
MIC	=	minimum inhibitory concentration
MBC	=	minimum bactericidal concentration
MFC	=	minimum fungicidal concentration
rpm	=	round per minute
min	=	minute
ml/min	=	milliliter per minute
IC_{50}, EC_{50}	=	amount of extract necessary to inhibit DPPH radical concentration by 50%

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF THAI HERB EXTRACTS IN COCONUT MILK

INTRODUCTION

Coconut milk is a sweet, milky white cooking base derived from the meat of a mature coconut. It plays an important role in the cuisines of South East Asia countries including Indonesia, Malaysia, Philippines and Thailand (Simuang *et al*., 2004). It is a major ingredient in the preparation of a wide variety of dishes, desserts and beverages. Thailand was known as a major coconut producer in Southeast Asia. The copra exportes has been reported by the department of business economics with cooperation of the customes department (2003). In year 2000, top destination was Hong Kong with 50.84% followed by U.S.A. (23.17%), Singapore (17.57%), Japan (3.42%) and Canada (2.51%), respectively.

Fresh coconut milk is prone to rapid microbiological spoilage because it is a very nutrient-dense food containing calcium, Omega 3 fats, fiber and protein which supports the growth of microorganisms, usually introduced via contaminated shells, utensils, processing equipment and handlers. The common types of microorganism include bacteria (*Bacillus, Achromobacter*, *Microbacterium, Micrococcus* and *Brevibacterium*) and fungi (*Penicillium*, *Geotricum*, *Mucor*, *Fusarium*, and *Saccharomyces* spp.). Aerobic plate counts in coconut milk was observed to reach levels of 1.2×10^6 to 1.7×10^8 CFU/ml that could be expected to pose serious organoleptic defects within 6 h of storage at 35°C (Seow and Gwee, 1997).

Lipid oxidation and microbial contamination are two main factors that determine food quality loss and shelf-life reduction. Lipid oxidation is responsible for reduction in nutritional quality as well as changes in flavor, while microbial contamination can precipitate major public health hazards and economic loss in terms of food poisoning and food spoilage (Sallam *et al*., 2004). Therefore, delaying lipid oxidation and preventing microbial cross-contamination are highly relevant to food

processes. Generally, synthetic preservatives have been widely used in the food industry to inhibit the process of lipid oxidation and microbial growth. However, there has been increasing concern of the consumers about foods free or with lower level of synthetic preservatives because these could be toxic for humans. Moreover, uncontrolled use of chemical antimicrobial preservatives has been inducing factor for resistant of microbial strains (López *et al*., 2005; Souza *et al*., 2005).

It is realized that the possibility of using plants as natural preservatives was achievable. Spices and herbs have been added to food since ancient times as flavoring agents, folk medicines and food preservatives (Shan *et al*., 2007a). They contain a large number of substances that have been reported to possess a wide range of biological effects, including antioxidant, antimicrobial and anti-inflammatory actions (Dorman and Deans, 2000; Brunet *et al*., 2009). Major components with antimicrobial and antioxidant activities found in herb and spices are phenolic compounds, terpenes, aliphatic alcohols, aldehydes, ketones, acids and isoflavonoids (López *et al*., 2007). In Thailand, there are many herbs and spices with antioxidant and antimicrobial activity (Lipipun *et al*., 2003; Siripongvutikorn *et al*., 2005; Sithisarn *et al*., 2005; Maisuthisakul *et al*., 2007a; Phattayakorn and Wanchaitanawong, 2009). However, few studies have been reported on major components containing in the plants. In this study, twenty five Thai herbs were screened for their antioxidant and antimicrobial activity against coconut milk spoilage microorganisms to use as a new source of natural food preservatives to extend shelf-life of coconut milk and coconut products.

OBJECTIVES

The objectives of this study were to;

1. determine antioxidant activity and antimicrobial activity of Thai herb extracts against coconut milk spoilage microorganisms,

2. evaluate the antimicrobial efficiency of Thai herb extracts in broth media and a model food,

3. determine the major chemical compositions of selected Thai herb extract using HPLC-MS and NMR spectroscopy, and

4. investigate the mode of action against coconut milk spoilage microorganisms of selected Thai herb extracts using TEM.

LITERATURE REVIEWS

1. Coconut milk

Coconut milk is the aqueous extract of coconut (*Cocos nucifera* L.) endosperm and is widely used as an ingredient in many tropical countries (Tangsuphoom and Coupland, 2009). Coconut milk naturally contained about 54% moisture, 35% fat and 11% solid non-fat and is categorized as oil in water emulsion. The main carbohydrates present are sugars and some starch. The major minerals found in raw coconut milk appear to be phosphorus, calcium and potassium (Simuang *et al*., 2004). To be preserved, coconut milk in canned and dehydrated forms is well known in the world market. However, for domestic consumption, pasteurized coconut milk in soft plastic bags has more freshness and is more convenient for cooking. In the pasteurization process, the milk is heated to pasteurization temperature of between 72°C and 75°C for 20 min. The pasteurized coconut milk has a shelf-life of not more than 5 days at 4°C (Seow and Gwee, 1997).

Fresh coconut milk is prone to rapid microbiological spoilage even under chilled storage. The generation time for multiplication of bacteria in coconut milk was found to drop from 232 min at 10°C to 44 min at 30°C (Fernandez *et al*., 1970). Coconut milk is a rich medium which can support the growth of all common spoilage microorganisms, usually introduced *via* contaminated shells, utensils, processing equipment, handlers, etc. The common types of bacteria encounted include those from the genera *Bacillus*, *Achromobacter*, *Microbacterium*, *Micrococcus* and *Brevibacterium*, while *Penicillium*, *Geotricum*, *Mucor*, *Fusarium* and *Saccharomyces* spp. appear to be the predominant fungi isolated from coconut milk. Aerobic plate counts in coconut milk and coconut skim milk were observed to levels of 1.2×10^6 - 1.7×10^8 that could be expected to pose serious organoleptic defects within 6 h of storage at 35°C.

Apart from microbial spoilage, coconut milk is also highly susceptible to chemical (including enzymic) deterioration, primarily through lipid autooxidation and lipolysis which result in objectionable tastes and odours. The hydrolysis of acylglycerols can be particularly rapid when catalyzed by the enzyme, lipase. The release of short-chain fatty acids such as butyric, caproic, caprylic and capric acids gives rise to strong off-odours. On the other hand, medium-chain fatty acids such as lauric and myristic acids (which are typical of coconut oil) produce a distinctive soapy taste (Seow and Gwee, 1997).

2. Antimicrobial properties of herbs and spices

It is estimated that there are 250,000 to 500,000 species of plants on earth. A few percentages (1-10%) of them play an important role in promoting human health by their anticancer, antioxidative, anti-inflamatory and antimicrobial properties (Ceylan and Fung, 2004). Herbs and spices have been used in food for flavoring and preservative for thousands of centuries (Cowan, 1999; Peter, 2004). In addition, herbs and spices prolong the storage life of foods by preventing rancidity and spoilage through their antioxidant activity and through bacteriostatic or bactericidal activity. Herbs and spices and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies. In recent years, the extracts of many herbs and spices are known as excellent sources of natural antioxidants and antimicrobials which have become popular to use for food products.

Herbs and spices are important sources of antimicrobials and use in food for two main reasons: (1) to control natural spoilage processes and (2) to prevent/control growth of food spoilage and pathogenic microorganisms. Numerous studies have been published on the antimicrobial activities of plant extracts against different types of microorganisms, including spoilage and foodborne pathogen (Table 1). Five Australian native herb extracts (*Backhousia citriodora, Anetholea anisata, Eucalyptus staigerana, Eu. olida* and *Prostanthera incise*) were determined for their antibacterial

activities against pathogenic bacteria. The ethanol and hexane extracts of all herbs displayed antimicrobial activity against *Staphylococcus aureus* with minimum inhibitory concentration (MIC) ranging from 7.8 to 125 µg/ml (Dupont *et al*., 2006). According to Si *et al*. (2006), Chinese green tea (*Camellia sinensis* L.) extracts were found to strongly inhibit the growth of foodborne pathogens (*Escherichia coli* O157:H7, *Salmonella* Typhimurium DT104, *Listeria monocytogenes, S. aureus and Bacillus cereus*). In addition, bioactive polyphenol compounds, epigallocatechin gallate, had the lowest MIC values against *S. aureus* (58 mg/l). Furthermore, the extracts from Finnish plants were investigated for their inhibitory effect on the microorganisms (*Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *E. coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *S. aureus* and *S. epidermidis*). It was found that the most active plant extracts were purple loosestrife (*Lythrum salicaria* L.) against *C. albicans*, meadowsweet (*Filipendula ulmaria* (L.) Maxim.), willow herb (*Epilobium angustifolium* L.), cloudberry (*Rubus chamaemorus* L.) and raspberry (*Rubus idaeus* L.) against bacteria and white birch (*Betula pubescens* Ehrh.), pine (*Pinus sylvestris* L.) and potato (*Solanum tuberosum*. L.) against *S. aureus* (Rauha *et al*., 2000).

Spices are also important sources of antimicrobials for controlling microbial growth in foods. The extracts of five spices widely used in South India such as garlic, nutmeg, ginger, onion and pepper have been reported the antibacterial activity against *E. coli*, *Salmonella* sp., *L. monocytogenes* and *Aeromonas hydrophila*. It was found that garlic extracts showed the excellent antibacterial activity against all test strains, except *L. monocytogenes* and nutmeg showed good anti-listerial activity. Garlic and nutmeg extracts were effective against *A. hydrophila* (Indu *et al*., 2006). According to Shin *et al*. (2004), the wasabi (*Wasabia japonica*) leaves extracts showed the high bactericidal activities with the minimum bactericidal concentration (MBC) of 1.05-1.31 mg/ml against three strains of *Helicobacter pylori* (NCTC11637, YS27 and YS50). The root showed a little lower bactericidal activity with MBC of 2.61-4.17 mg/ml. Pandit and Shelef (1994) studied the antilisterial effect of 18 spices and observed inhibitory effect of rosemary (*Rosmarius officinalis*) (≥ 5% w/v) and clove

Table 1 Spices, herbs and essential oils with antimicrobial activity and their components

Plants	Compounds	Class	Activity
Alfalfa	-	-	Gram-positive organisms
Allspice	Eugenol	Essential oil	General
Aloe	Latex	Complex mixture	*Corynebacterium*, *Salmonella*, *Streptococcus*, *S. aureus*
Apple	Phloretin	Flavonoid derivative	General
Barberry	Berberine	Alkaloid	Bacteria, protozoa
Basil	Essential oils	Terpenoids	*Salmonella*, bacteria
Bay	Essential oils	Terpenoids	Bacteria, fungi
Betel pepper	Catechols, eugenol	Essential oils	General
Black pepper	Piperine	Alkaloid	Fungi, *Lactobacillus*, *Micrococcus*, *E. coli*
Blueberry	Fructose	Monosaccharide	*E. coli*
Cashew	Salicylic acids	Polyphenols	*P. acnes*, bacteria, fungi
Ceylon cinnamon	Essential oils	Terpenoids, tannins	General
Clove	Eugenol	Terpenoids	General
Cilantro	-	-	Bacteria, fungi
Cranberry	Fructose	Monosaccharide	Bacteria
Eucalyptus	Tannin	Polyphenol	Bacteria, viruses

Table 1 (Continued)

Plants	Compounds	Class	Activity
Garlic	Allicin, ajoene	Sulfoxide	General
Ginseng	-	Saponins	*E. coli*, *Staphylococcus*
Grapefruit peel	-	Terpenoid	Fungi
Green tea	Catechin	Flavonoid	General
Hops	Lupulone	Phenolic acid	General
Horseradish	-	Terpenoids	General
Legume	Alpinumisoflavone	Flavone	*Schistosoma*
Licorice	Glabrol	Phenolic alcohol	*S. aureus*, *M. tuberculosis*
Oak	Tannins	Polyphenols	-
Olive oil	Hexanol	Aldehyde	General
Onion	Allicin	Sulfoxide	Bacteria, *Candida*
Papaya	Latex	Mixture of terpenoids	General
Peppermint	Menthol	Terpenoid	General
Rosemary	Essential oils	Terpenoid Anthraquinone	General

Source: Cowan (1999)

(*Eugenia cariophylata*) (≥ 1% w/v) on *L. monocytogenes*.

According to Cheeptham and Towers (2002), six species, namely Phaeng-Pouy-Farang (*Catharanthus roseus*), Tao-Enn-Oorn (*Crytolepis buchanai*), ginkgo (*Ginkgo biloba*), Thong-Thaeng (*Physali minima*), wild betel (*Piper sarmentosum*), Queen's flower (*Lagerstroemia speciosa*) possessed antimicrobial properties against *S. aureus*. In addition, young garden celery (*Apium graveolens*) and guava (*Psidium guajava*) showed activities against *E. coli* and aloe vera (*Aloe barbadensis*), Khi-lek (*Cassia siamea*) and cinnamon (*Cinnamomum zeylanicum* L.) possessed activities against *E. coli* DC10.

Essential oils in the range of 0.05-0.1% are used by the food industry as natural agents for extending the shelf life of foods. The essential oils from various plants exert bacteriostatic/inhibition effects such as oregano, clove, cinnamon, garlic, coriander, rosemary, parsley, lemongrass, sage and vanillin (Tassou *et al*., 1995; Dorman and Deans, 2000; Tajkarimi *et al*., 2010). Anise oil, essential oils from sweet basil (*Ocinmum basilicum* L.), were examined the antimicrobial activity of against a wide range of foodborne Gram-positive and negative bacteria, yeast and moulds. The anise oil at concentration of 100-1,000 µg/ml was generally effective against *Lactobacillus curvatus* and *S. cerevisiae* (Lachowicz *et al*., 1998).

Several researchers studied antimicrobial activity in broth media. Yano *et al*. (2006) examined the antibacterial activities of eighteen plant species on *Vibrio parahaemolyticus*. It was found that the lowest MIC was 0.125% observed in clove and marjoram at 30°C in a nutrient rich medium. According to Moleyar and Narasimham (1992), the antibacterial activity of fifteen essential oil components against food pathogenic microorganisms (*Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp. and *Enterobacter* sp.) were investigated. It was found that cinnamic aldehyde with concentration of 500 µg/ml could completely inhibit the bacterial growth more than 30 days at 30°C. Moreover, culinary herbs including parsley and cilantro inhibited the growth of *B. subtilis* and *E. coli* in Luria–Bertani medium for 24

h of incubation (Wong and Kitts, 2006). Furthermore, ethanol extracts of leave, fruits and stem bark from the Siamese neem tree (*Azadirachta indica*) exhibited high free radical scavenging effect on the DPPH assay with IC_{50} at 26.5, 27.9 and 30.6 µg/ml, respectively (Sithisarn *et al*., 2005).

Factors affecting on antimicrobial activity of herb extract can be divided to: (i) the food composition (protein and fats) and its physicochemical (pH); (ii) extrinsic factor such as temperature and (iii) the nature of the microorganisms (Tassou *et al*., 1995). The antimicrobial efficiency of plant extracts is known to be reduced in a food system from the interaction of different food components, thus requiring much higher concentrations to reduce the bacterial growth. Gutierrez *et al*. (2008) observed that the presence of high concentrations of protein in beef extracts promoted the growth of *L. monocytogenes*, but the efficiency of oregano and thyme was also greater at these higher concentrations of protein. These authors explained that peptones with hydrophobic properties might display interaction with essential oils to facilitate their dissolution in beef extracts. In contrast, the antimicrobial efficiency of essential oils (lemon balm, marjoram, oregano and thyme) had the higher in lettuce and beef media than basal media on *Listeria* spp. observed from MIC values. The rich nutrients in broth media compared to lettuce media may enable bacteria to repair damaged cells faster (Gutierrez *et al*., 2009).

Lipids have been reported to decrease the antimicrobial activity of some antimicrobial agents in foods. Glass and Johnson (2004) evaluated the effect of fat on the antibotulinal activity of several preservatives. It was observed that 20% milk fat or soybean oil significantly decreased the anti-botulinal activity of nisin, whereas the effect of 10% fat was variable. Corresponding with Jung *et al*. (1992), the antilisterial activity decreased by ca. 50% and 88% of milk products containing 1.29% and 12.9% fat, respectively. Farbood *et al*. (1976) found that fat in food could form a protective coat around bacteria, thereby protecting them from antimicrobial agents. Furthermore, the reduced water content in food compared to laboratory media could hamper the transfer of antimicrobial agents to the active site in the microbial cell.

The susceptibility of bacteria to the antimicrobial effect of phenolics also appears to increase with a decrease in the pH of the food. Tassou and Nychas (1995) observed that the growth of *S.* Enteritidis was inhibited by oleuropein (phenolic compounds from olive) at low pH (pH 5.5 and 6). At low pH, the hydrophobicity of phenolics increases, enabling them to dissolve more easily in the lipids of the cell membrane of target bacteria (Juven *et al*., 1994; Holley and Patel, 2005). However, antimicrobial must be lipophilic and soluble in the aqueous phase to attach and pass through the cell membrane (Davidson and Harrison, 2002). On the other hand, Canillac and Mourey (2004) found that the antilisterial activity of spruce essential oil increased in high pH media (pH 8.7). These results could explain that essential oil might attack *Listeria* more easily because of a possible repulsion between negative charges of amino compounds and cell surface. While at pH 6-7, amino compounds are less negative charge that might be protect bacterial cells from spruce essential oil.

3. Antioxidant properties of herbs and spices

Natural antioxidants may function as free radical scavengers, reducing agents, chelating agents or as quenchers of singlet oxygen. Recently, many researchers have focused on crude extracts and pure compounds from natural antioxidants were previously reported to have antioxidant properties (Ozen *et al*., 2011; Wang *et al*., 2011). For example, *Ligaria cuneifolia* of Argentina medicinal plants exhibited the high values of total phenolic content of 100.2 mg gallic acid equivalence (GAE)/g and antioxidant capacity of 1,862 μmol Fe(II)/g, respectively (Borneo *et al*., 2009). Bouayed *et al*. (2007) evaluated the amount of total phenolic and flavonoid content from Iranian popular medicinal plants. It was concluded that leaves of *Lavandula officinalis* and *Melissa officinalis* exhibited high total phenolic content of 16.2-20.3 mg GAE/g and total flavonoid content of 6.18-10.0 mg CE/g.

Moreover, essential oil from aromatic and medicinal plants has been reported to possess biological activity including antioxidant properties. Essential oil of *Myrtus communis* var. *italic* leaf had high total phenolic content (33.67 mg GAE/g) and total

flavonoid content (5.17 mg CE/g) (Wannes *et al*., 2010). According to Politeo *et al*. (2007), basil essential oil had an antioxidant capacity with IC_{50} value of 1.378 g/l.

In Thailand, several researchers studied on phenolic content and antioxidant activity of extracts from many plants. According to Maisuthisakul *et al*. (2007a), they reported a potential of Thai indigenous plants for use as natural antioxidants. Results showed that seed extracts from *Antidesma velutinum* Tulas., *Cleistocalyx opercultatus* var. *paniala* (Roxb.) and *Eugenia siamensis* exhibited very high total phenolic content (123-180 mg GAE/g) compared to that obtained from other fruit and berry seeds (20-54 mg GAE/g). Fruits and vegetables are also reported to contain a wide variety of antioxidant components. Bitter gourd (*Momordica charantia* L.) or Mara (in Thai) leaf extract showed the highest value of antioxidant activity, based on DPPH radical scavenging activity (9.72$\pm$0.25 mg/ml) and ferric reducing activity (433$\pm$0.007 µmol $FeSO_4$/g) (Kubola and Siriamornpun, 2008). Phak-Paew (*Polygonum odoratum*), guava (*Psidium guajava*), rambutan (*Nephelium lappaceum*) and mangosteen (*Garcinia mangostana*) showed the high antioxidant capacity with IC_{50} of 315.4 µg/ml and trolox equivalent antioxidant capacity (TEAC) values of 4.908$\pm$0.050 mM/mg, 3.074$\pm$0.003 and 3.001$\pm$0.016 mM/mg, respectively (Tachakittirungrod *et al*., 2007; Nanasombat and Teckchuen, 2009).

In addition, antioxidant activities of plant extracts were found to depend on the type of solvents. In the report of Sarikurcu *et al*. (2009), the water extract of *Vitex agnus castus* L. fruits showed stronger antioxidant activity with DPPH scavenging activity (82.72%), total phenolic content (112.46 µg GAE/mg) and reducing power (0.751) than other solvent extracts (hexane, dichloromethane, ethyl acetate and methanol). The methanol extract of leaves of Chiangda (*Gymnema inodorum*) exhibited the highest level of antioxidant activity with antioxidant index of 14.8, followed by Chaplu (*Piper sarmentosum*) and Japanese mint (*Mentha arvensis*) with antioxidant index of 13 and 10.9, respectively (Chanwitheesuk *et al*., 2005). Furthermore, flower extract of *Cassia siamea* Britt. have been reported antioxidant

activity with total phenolic content of 28.90±1.95 mg GAE/g, EC_{50} of 1.49±0.04 mg and TEAC of 4.55±0.06 mM TE/g (Phomkaivon and Areekul, 2009).

4. Antimicrobial and antioxidant properties of Thai herbs

In Thailand, various medicinal plants have long been used for anti-inflammatory, antimicrobial, anticancer and anti-diarrheic (Sawangjaroen and Sawangjaroen, 2005; Wannissorn *et al*., 2005; Siriwatanametanon *et al*., 2010). Many of them exhibit the antimicrobial activity against food spoilage and pathogenic microorganisms.

Senna siamea (Lam.) Irwin & Barneby is found indigenously in Thailand and locally called "Khi Lek". It has a long history of use as a folk medicine and its therapeautic efficacy is well recognized. Different parts of *S. siamea* can be used for various medical purposes. Krasaekoopt and Kongkarnchanatip (2005) examined the antimicrobial properties of the crude flower extracts of cassod tree (*Senna siamea*) against *B. cereus*, *E. coli* and *S. aureus*. Among these three, *S. aureus* was found to be the most sensitive to the extracts with inhibition zone of 11.7 mm, followed by *E. coli* (10.2 mm) and *B. cereus* (10.0 mm). Bukar *et al*. (2009) revealed that water extracts of cassod tree leaf displayed the highest activity against *Pseudomonas aeruginosa* with inhibition zone of 30 mm followed by ethanol extract (16 mm).

Punica granatum L. (Pomegranate) has been used extensively in the folk medicine of India, Pakistan and many other countries. The peel extract is a rich source of hydrolysable tannins of the ellagitannin group. The extracts of rind and fruit peel exhibited a potent bacteriostatic effect against *Propionibacterium acnes*, Gram-positive anaerobe, with MIC of 15.6 µg/ml, and Gram-positive facultative anaerobic bacteria, *S. aureus* and *S. epidermidis*, with MIC of 7.8–15.6 µg/ml (Panichayupakaranant *et al*., 2010). Moreover, the growth of *L. monocytogenes* ATCC23715 and *S. aureus* FRI722 were inhibited by 0.25 and 1 mg/ml of pomegranate fruit peel and fruit extracts, respectively (Braga *et al*., 2005; Zoreky

2009). In addition, it has been reported that ellagitannins isolated from pomegranate exhibited antibacterial activity against both methicillin-resistant and methicillin-sensitive *S. aureus* (Machado *et al*., 2003). In addition, aqueous peel extract have been reported the high phenolic content of 161.25 mg/g (Kanatt *et al*., 2010). Negi and Jayaprakasha (2003) also reported similar phenolic content (140 mg/g) for peel extract. The flavonoid content of peel was 7.57 mg/g. Moreover, antioxidant potential of pomegranate *in vivo* and *in vitro* has been proved by Iqbal *et al*. (2008). Its peel extract exhibited 66.23% inhibition of peroxidation in sunflower oil at concentration of 800–850 ppm.

Phyllanthus emblica L. (Malacca tree) is a euphorbiaceous plant and use in many traditional medicinal systems, such as Chinese herbal medicine, Tibetan medicine and Ayurvedic medicine. According to Liu *et al*. (2008), fruit extract was found to have antioxidant properties with total phenolic content of 81.5 to 120.9 mg GAE/g and the flavonoid content of 20.3 to 38.7 mg quecetin equivalents (QE)/g and proanthocyanidin content of 3.7 to 18.7 mg catechin equivalents (CE)/g. MIC and MBC values of the extract were 21.8±0.6 mm and 13.97 mg/ml against *S. aureus*, respectively (Mayachiew and Devahastin, 2008)

Piper betle Linn. (Betle vine) belongs to the family Piperaceae. Its common names are betel vine, *phlu* and *see-keh*. Ethnomedicinal uses of *Piper betle* Linn. parts include treatment of disorders of physiological function, endo-parasites, skin diseases, eye diseases, urticaria, bronchial sputum occlusion and promotion of healthy teeth and skin. Jenie *et al*. (2001) reported that the whole extract (mixture of volatile and nonvolatile extract) of *Piper betle* L. at 0.025% (v/v) could inhibit *S. aureus* and *E. coli* while Nalina and Rahim (2007) found that the crude aqueous extract exhibited antibacterial activity towards *Streptococcus mutans*.

Carthamus tinctorius (Safflower) is a member of the family Asteraceae. It has been reported the total phenolic content of 18.02±139.98 mg of GAE/g and

antioxidant activity with FRAP value of 5.05±1,140.5 µmol/g and DPPH of 96.65% scavenging effect (Kruawon and Kangsadalampai, 2006).

Citrus aurantifolia (Lime), belonging to the family Rutaceae, has long been regarded as a food and also as a medicinal plants with biological activity. Ghafar *et al*. (2010) reported the total phenolic, flavonoid contents and hesperidine content of four species of citrus in Malaysia, namely *C. hystrix* (wild lime), *C. aurantifolia* (common lime), *C. microcarpa* (musk lime) and *C. sinensis* (orange), ranging from 105±3 to 490.74±1.75 mg of GAE/100 ml, 2.99±0.09 to 22.25±0.20 mg of hesperidine equivalent/100 ml and 5.58±0.66 to 16.67±2.57 mg/100 ml, respectively. Furthermore, the antimicrobial effect of crude lime extract against different bacterial species was observed by Jayana *et al*. (2010). The highest inhibition zone of 28 mm was observed in *Vibrio cholerae* followed by *Enterobacter* species (9 mm), *Citrobacter* species (8 mm) and *E. coli* (8 mm). While, *Shigella*, *Salmonella* and *Klebsiella* species were found to resist to the extract.

Cyperus rotundus (Nutgrass) is a medicinal plant belonging to the family of the Cyperaceae and natural drugs used as home remedy against spasms, stomach disorders and irritation of bowel. Rhizomes extract exhibited its antioxidant effects in total phenolic content of 73.27±4.26 mg GAE/g and hydroxyl radical scavenging activity of 0.021 mg/ml (Nagulendran *et al*., 2007). Antimicrobial activities have also reported by Parekh and Chanda (2008), methanol extract could inhibit *Trichosporon begelli* with inhibition zone of 10 mm.

Boesenbergia rotunda (L.) Mansf. (Krachai) is a perennial herb of the family Zingiberaceae. The fresh rhizome is used in cooking, also in folk medicine as an aphrodisiac, and for the treatment of colic disorder. The 0.9 mg/disc ethanol extract of *B. pandurata* has inhibitory effects on *S. mutan* and *Lactobacillus sp*. (Taweechaisupapong *et al*., 2010). In addition, essential oil obtained by hydrodistillation extraction could inhibit growth of *E. coli*, *S. aureus, B. cereus* and *L. monocytogenes* (Natta *et al*., 2008).

Garcinia mangostana Linn. (Mangosteen), family Guttiferae, is an indigenous plant in Thailand and some other Southeast Asian countries. It has been known to be good medicinal value and is traditionally used in folk medicines for treatment of abdominal pain, diarrhea, dysentery, infected wounds, suppuration, chronic ulcer, leucorrhoea and gonorrhoea. Mangosteen extract contained phenolic compounds at 60.2 mg GAE, IC_{50} of 84.35 µg/ml, while ascorbic acid had an IC_{50} of 65.7 µg/ml (Kosem *et al*., 2007) and TEAC values of 3.001±0.016 mM/mg (Tachakittirungrod *et al*., 2007). Palakawong *et al*. (2010) investigated antimicrobial properties of mangosteen. The MIC values of peel, leaves, and bark extracted against Gram-positive bacteria were ranged from 0.025-0.78 mg/ml. While, the MBC values were between 0.05-0.39 mg/ml.

Morus alba L. (Mulberry) has been known to show antiviral and antimicrobial effect against *S. aureus* (Yogisha and Raveesha, 2009). Hypoglycemic and antioxidant potency of some phenolic compounds (Flavonoids, stilbenes and 2-arylbenzofurans) have been reported by Nikkhah *et al*. (2009).

Azadirachta indica A. Juss (Siamese neem tree) is a large evergreen tree which belongs to the Meliaceae family. This plant is abundantly found in every part of Thailand and has been used as traditional medicine for many purposes. Crude extract gave the most effective DPPH-scavenging activity (EC_{50} 31.4 µg/ml) (Sithisarn *et al*., 2006). Nahak and Sahu (2010) have also reported antioxidant acivity with total phenolic contents of 360 µg/mg and IC_{50} of 0.008 µg/mg. Leave extracts showed the antimicrobial activity with zone of inhibition of 5-10 mm (Sukanya *et al*., 2009 and MIC of 0.07 and 0.5 mg/ml against *S. aureus* and *P. aeruginosa* (Patel *et al*., 2009). Furthermore, *K. pneumoniae* were susceptible to the extract that reported by El-Kamali *et al*. (2009).

Morinda citrifolia L. (Noni Indian mulberry) is a tree in the family Rubiaceae. Parts of the plant have been traditionally used for treatment of various complaints leaves showed antioxidant properties, giving DPPH radical scavenging activity with

IC_{50} values of 0.20-0.35 mg/ml (Thani *et al*., 2010). Methanol extract showed antibacterial activities against *B. subtilis, S. aureus, E. coli, K. pneumoniae* and *V. cholera* with inhibition zone of 7.7±0.6 to 11.3±0.6 mm (Jayaraman *et al*., 2008).

Senna alata (Ringworm bush) is an ornamental shrub which belonging to the Fabaceae family. It is locally used in the treatment of several infections, which include ringworm and parasitic skin diseases. Methanol crude extract showed antimicrobial activity against *P. aeruginosa* and *S. aureus* with inhibition zone of 12-17 mm (Owoyale *et al*., 2005). Okoro *et al*. (2010) found that the extract showed the antioxidant activity with total phenolic content of 23.19±0.89 mg GAE/g and antioxidant activities of 37.02±0.45 ammonium thiocyanate assay % of plants. Some microorganisms (*S. aureus* and *C. albicans*) were susceptible to the polyphenol extracts with MIC values between 1.25 to 5.00 mg/ml.

Andrographis paniculata Nees. (Kalmegh) is an herbaceous plant, commonly known as “king of bitters” belongs to the family Acanthaceae. Mostly the leaves and roots have been traditionally used over centuries for different medicinal purposes in Asia and Europe. The chloroform extracts were found to inhibit the growth of *S. aureus, B. subtilis, E. coli, Proteus vulgaris, A. niger* and *Penicillium chrysogenum* with inhibition zone of 10-12 mm (Radhika *et al*., 2010).

Curcuma longa (Turmeric) is widely used as a spice and colouring agent and is known for its medicinal properties. Rhizome extracts have been reported to inhibit pathogenic strains of Gram-positive (*S. aureus* and *S. epidermidis*) and Gram-negative (*E. coli, P. aeruginosa* and *S.* Typhimurium) bacteria (Singh *et al*., 2002). Pundir and Jain (2010) found that methanol extract showed activity with zone of inhibition ranged between 13 mm and 24 mm. While, MIC values ranged from 3.0 to 20.6 mm in diameter have been investigated in *B. subtilis* (Naz *et al*., 2010).

Syzygium aromaticum (Clove) is a tree in the family Myrtaceae. Clove is used in Ayurveda, Chinese medicine and Western herbalism. The extract showed the

highest antibacterial activity against some strains such as *S.* Typhimurium (15 mm), and *S. marcescens* (22 mm) (Nanasombat and Lohasupthawee, 2005). The essential oil could inhibit the growth of *E. coli* O157:H7 and *L. monocytogenes* (Mytle *et al*., 2006; Moreira *et al*., 2007).

Cinnamomum zeylanicum Nees. (Cinnamon) is belonging to the family Lauraceae. Cinnamon oil in the range of 10-150 µg/ml could inhibit *E. coli, S. aureus, P. aeruginosa, C. albican* and *S. cerevisiae* with inhibition zone of 25-53 mm (Hili *et al*., 1997).

5. Bioactive compounds present in herbs and spices

Phenolic compounds are commonly found in herbs and spices and they have multiple biological effects, including antioxidant and antimicrobial activities. The structure consisted of hydroxybenzoic or hydroxycinnamic acids. The natural phenolic compounds may be classified into (1) simple phenols and phenolic acids (e.g. p-creseol, 3-ethylphenol, vanillic, gallic, ellagic, hydroquinone); (2) hydroxycinnamic acid derivatives (e.g. p-coumaric, caffeic, ferulic, sinapic); (3) flavonoids (e.g. catechins, proanthocyanins, anthocyanidins and flavones, flavonols and their glycoside); and (4) tannin. In plants, phenolics mainly occur as glycosylated forms through *o*-glycosidic bonds with a number of different sugars such as glucose, galactose, rhamnose, arabinose, xylose and rutinose (Chirinos *et al*., 2008). Furthermore, Zheng and Wang (2001) found the various phenolic compounds in medicinal plants. For example, quercetin-3-O-rhamnosyl-(1→2)-rhamnosyl-(1→6)-glucoside, kaempferol-3-O-rhamnosyl-(1→2)-rhamnosyl-(1→6)-glucoside hesperetin, acacetin, diosmetin, apigenin, luteolin, ferulic acid, rosemarinic acid and caffeic acid were present in *Salvia officinalis, Thymus vulgaris, Origanum majoricum* and *Poliomintha longiflora*.

The most widespread and diverse phenolics are the flavonoids. The basic structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three

rings (C_6-C_3-C_6), labeled A, B and C. Various classes of flavonoids differ in the level of oxidation and saturation of ring C, while individual compounds within a class differ in the substitution pattern of rings A and B. The differences in the structure and substitution will influence the phenoxyl radical stability and thereby the antioxidant properties of the flavonoids (Wojdylo *et al*., 2007). Flavonoids exhibited a wide range of biological activities such as anti-inflammatory, anti-thrombotic, anti-hypertensive, anti-arrhythmric activity, spasmolytic and cancer chemopreventive (Yilmaz and Toledo, 2004; Jiang *et al*., 2005; Podsędek, 2007). Chinese traditional herbs are a rich source of isoflavonoids. Four major compounds (daidzein, daidzin, puerarin and 5-hydroxypuerarin) were found from *Pueraria lobata* (Wild.), Ohwi (Ye-Ge) and *Pueraria thomsonii* Benth (Fe-Ge) (Proestos *et al*., 2005). Moreover, Ye *et al*. (2011) reported that hesperidin was found to be the major flavonoids in mandarin fruits and followed by narirutin, nobiletin and tangeretin. Their concentrations ranged from 142$\pm$2 to 261$\pm$1 mg/g, 5.8$\pm$0.1 to 46.9$\pm$0.3 mg/g, 0.45$\pm$0.01 to 13.7$\pm$0.4 mg/g and 0.07$\pm$0.01 to 12$\pm$0.2 mg/g, respectively. Wang *et al*. (2003) found apigenin-7-rutinoside and narirutin as the bioactive compounds in artichoke.

Most antioxidants isolated from higher plants are polyphenol and more than 4,000 phenolic and polyphenolic compounds have been identified (e.g. phenolic acids, tannins, coumarins, anthraquinones, flavonoids) (Tawaha *et al*., 2007). Chlorogenic acid, dicaffeoylquinic acid, ferulic acid derivatives isolated from Teaw (*Cratoylum formosum* Dyer) leaves showed the IC_{50} values of 6.26-8.96 µg/ml by DPPH method and 2.67-3.06 mmol of trolox/g sample of TEAC (Maisuthisakul *et al*., 2007b). Gallic acid, ellagic acid, mucic acid 1,4-lactone-3-o-gallate, isocorlagin, chebulanin, chebulagic acid and mallotusinin were isolated and purified from *Phyllanthus emblica*. All of phenolic compounds showed strong radical scavenging activity (IC_{50} 3.99-23.72 µM), good potency to chelate Fe^{2+} (IC_{50} 0.22-1.27 µM) and good inhibition ability of lipid peroxidation (IC_{50} 11.4-71.3 µM) (Luo *et al*., 2011).

6. Mechanism of antioxidant and antimicrobial activities

6.1 Mechanism of antimicrobial activity

Antimicrobial agents can be effective to preserve food products by either controlling the overall growth of microorganisms or directly destroying the microorganisms. Bacteriostasis or bacteriocidal properties depend on the reversibility of action mode of the antimicrobial agents. There are three action mode: (1) reaction with the cell membrane, causing increased permeability and loss of cellular constituents; (2) inactivation of essential enzyme; or (3) destruction or functional inactivation of genetic material. The action mode can be helpful to determine the efficiency and usefulness of an antimicrobial. The activity of very hydrophobic antimicrobials may be limited by Gram-negative organisms, which have the ability to screen the antimicrobial from the transporting membrane by the lipopolysaccharide layer of the cell wall (Davidson and Branen, 1993). Major classes of antimicrobial compounds and their action mode from plants were summarized in Table 2.

The action of preservatives on the cells of spoilage and poisoning microorganisms is based on a multiplicity of individual influences. These include not only physical and physico-chemical mechanisms but also biochemical reactions. Frequently, several individual factors may produce a cumulative effect, but sometimes only one single stage in a reaction in the microorganism cell is blocked. In the case of spore-forming bacteria, different preservatives develop their inhibitory action at varying stages of spore germination (Figure 1) (Lück and Jager, 1997).

Action mode of some chemical compounds from plants has been reported by several researchers. Phenolic compounds are the main phytochemical in many plants. There are hydrophobic in nature and membrane active (Sikkema *et al*., 1994). They have been shown to inhibit DNA, RNA and protein synthesis (Nes and Eklund, 1983) and glucose uptake (Munoz *et al*., 1987; Kreydiyyeh *et al*., 2000). Recently, vanillin, major constituent of vanilla bean, exhibits strong antimicrobial properties

against *E. coli* and *L. innocua*. Addition of 10-40 mmol/l vanillin inhibited respiration of their bacteria. Increasing of vanillin concentrations, bacteria completely dissipated potassium ion gradients and loss of pH homeostasis (Fitzgerald *et al*., 2004). The antimicrobial action can be explained by the following phenomena (1) influence on the DNA, (2) influence on protein synthesis, (3) influence on the cell wall and (4) influence on transport mechanisms for nutrients (Figure 2).

Essential oils are aromatic oily liquids obtained from different part of plant material such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots (Burt, 2004). The oils can be obtained by various method including compression, fermentation and extraction but steam distillation method is most commonly used for commercial production. Generally, the essential oils possessing the strongest antibacterial properties against foodborne pathogens contain a high percentage of phenolic compounds such as carvacrol, eugenol (2-methoxy-4-(2-propenyl) phenol) and thymol (Pol and Smid, 1999; Mann *et al*., 2000; Michiels *et al*., 2007). Antibacterial activity of essential oils is not attributable to one specific mechanism but there are several targets in the cell because a large number of different groups of chemical compounds. The locations or mechanisms in the bacterial cell thought to be sites of action for essential oils components are indicated in Figure 3. Their mechanism of action would to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport and coagulation of cell contents.

The major components of oregano and thyme are carvacrol and thymol, respectively. Thymol is structurally very similar to carvacrol, having the hydroxyl group at a different location on the phenolic ring. According to Michiels *et al*. (2007), the carvacrol and thymol showed similar antimicrobial properties. They are able to disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane. Their activity against lactobacilli was higher in the stomach than in the small intestine. In other research, eugenol destabilizes the cytoplasmic membrane, acts as a

Table 2 Major classes of antimicrobial compounds and their mode of action from plants

Class	Subclass	Examples	Mode of action
Phenolics	Simple phenols	Catechol	Substrate deprivation
		Epicatechin	Membrane disruption
	Phenolic acids	Cinnamic acid	-
	Quinones	Hypericin	Bind to adhesions, complex with cell wall, inactive enzyme
	Flavonoids	Chrysin	Bind to adhesins
	Flavones		Complex with cell wall
		Abyssinone	Inactive enzyme Inhibit HIV reverse transcriptase
	Flavonols	Totarol	-
	Tannins	Ellagitannin	Bind to proteins - Bind to adhesins - Enzyme inhibition - Substrate deprivation - Complex with cell wall - Membrane disruption; Metal ion complexation

Table 2 (Continued)

Class	Subclass	Examples	Mode of action
	Coumarins	Warfarin	Interaction with eukaryotic DNA (antiviral activity)
Terpenoids, essential oils		Capsaicin	Membrane disruption
Alkaloids		Berberine Piperine	Intercalate into cell wall and/or DNA
Lectins and polypeptides		Mannose-specific agglutinin Fabatin	Block viral fusion or adsorption Form disulfide bridges
Polyacetylenes		8S-Heptadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol	-

Source: Cowan (1999)

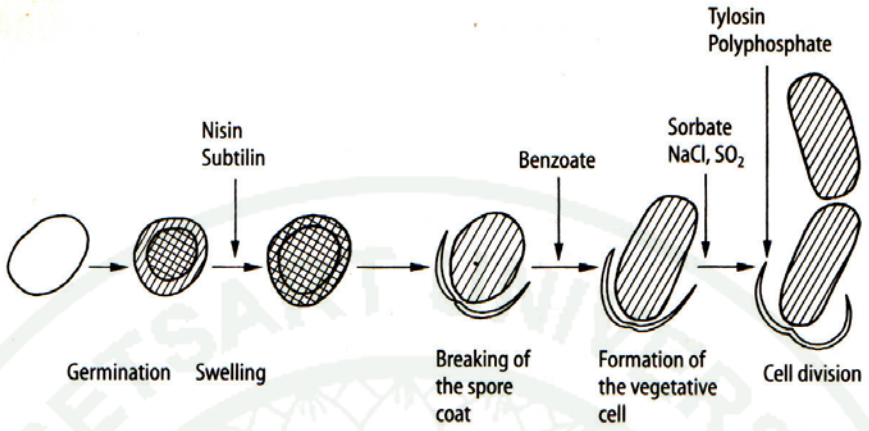


Figure 1 Possible points of attack for antimicrobial substances during germination of bacterial spores

Source: Lück and Jager (1997)

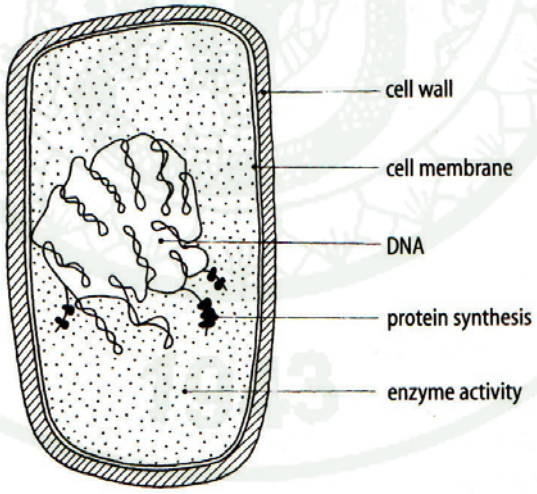


Figure 2 Possible attack points for antimicrobials in microorganism cell

Source: Lück and Jager (1997)

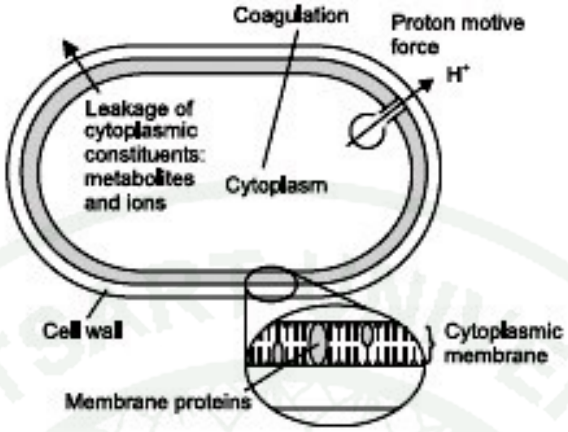


Figure 3 Locations and mechanisms in the bacterial cell thought to be sites of action for essential oil components: degradation of the cell wall; damage to cytoplasmic membrane; damage to membrane proteins; leakage of cell contents; coagulation of cytoplasm and depletion of the proton motive force

Source: Burt (2004)

proton exchanger and reducing the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP lead to cell death (Ultee *et al*., 2002).

In addition, terpinen-4-ol and 1,8-cinole are the major components in tea tree oil (the essential oil of *Melaleuca alternifolia*) has long be used as antiseptic, antifungal agent and mild solvent (Mann, 2000; Lahijani, 2006). It acts as membrane damaging agents on *Pseudomonas aerogenosa* (Mann *et al*., 2000). *S. aureus* cells in the stationary phase of growth were killed by tea tree oil and its components. Electron microscopy of terpinen-4-ol-treated cells showed the formation of mesosomes and the loss of cytoplasmic contents (Carson *et al*., 2002).

6.2 Mechanism of antioxidant activity

Lipid oxidation is a complex phenomenon induced by oxygen in the presence of initiators such as heat, free radicals, light, photosensitizing pigments and metal ions. It occurs *via* three reaction pathways: (i) non-enzymatic chain autoxidation mediated by free radicals, (ii) non-enzymatic and non-radical photooxidation and (iii) enzymatic oxidation. The first two types of oxidation consist of a combination of reactions involving triplet oxygen ($^3O_2$), which could be considered as a ground-state biradical (·OO·) and the singlet oxygen ($^1O_2$), which corresponds to an excited state of the molecule. There are many sources of singlet oxygen but its presence is often coupled with UV photonic impact in presence of photosensitizers. The singlet oxygen can bind directly to double bonds (C=C), leading to hydroperoxide formation. However, this so-called nonradical photooxidation seems to be a minor reaction in comparison to $^3O_2$-induced radical chain autoxidation. It has been suggested that photooxidation mainly generates hydroperoxides that break down into free radicals that could initiate autoxidation reactions (Laguerre *et al*., 2007). Autoxidation therefore seems to be a key mechanism in lipid oxidation. It mainly generates hydroperoxides and volatile compounds, generally through a three phase process (initiation, propagation and termination).

Initiation

From a mechanistic standpoint, the initiation phase involves homolytic breakdown of hydrogen in a position relative to the fatty acid chain double bond (LH). Reaction occurs spontaneously with $^3O_2$ because of the very high activation energy arising from the spin barrier between lipids and $^3O_2$.

$$LH + {}^3O_2 \rightarrow LOOH \qquad (1)$$

The reaction can be initiated *via* external physical agents such as heat, ionizing radiation or a photonic impact in the ultraviolet spectrum and also by chemical agents such as metal ions, free radicals and metalloproteins.

$$L_1H \xrightarrow{\text{initiator}} L_1\cdot + H\cdot \qquad (2)$$

Free radical ($L_1\cdot$) are highly unstable, short-lived intermediates that stabilize by abstracting a hydrogen from another chemical species. The oxidation process remains slow during this phase. At the end of the initiation period, oxidation suddenly accelerates, oxygen consumption becomes high and the peroxide content increases substantially. Lipid oxidation is primarily initiated by hydroxyl ($\cdot OH$) and hydroperoxyl ($HOO\cdot$) radicals, as well as lipid alkoxyl ($LO\cdot$) and peroxyl ($LOO\cdot$) radicals. Hydroxyl radicals are by far the highest reaction rate with lipids.

Propagation

In aerobic environments, the $L_1\cdot$ radical centered on the carbon molecule and formed during the initiation phase reacts very quickly with triplet oxygen to generate different radical species including peroxyradicals ($L_1OO\cdot$).

$$L_1\cdot + {}^3O_2 \rightarrow L_1OO\cdot \qquad (3)$$

Reaction (3) has very low activation energy and a high rate constant, so the $L_1OO\cdot$ concentration becomes much higher than the $L_1\cdot$ content in all oxygen-bearing systems. The peroxyradical then abstracts a hydrogen atom from another unsaturated lipid molecule to form hydroperoxide (primary oxidation compound) and $L_2\cdot$ radical (reaction (4)), thus replenishing reaction (3).

$$L_1OO\cdot + L_2H \rightarrow L_1OOH + L_2\cdot \qquad (4)$$

Termination

The oxidation process then continues with the transformation of hydroperoxides into secondary non-radical oxidation compounds. The main hydroperoxide decomposition mechanism involves scission of the double bond adjacent to the hydroperoxyl group, leading to the formation of hydrocarbons, aldehydes, alcohols and volatile ketones. Other non-volatile secondary compounds are also formed including non-volatile aldehydes, oxidized triacylglycerols and their polymers. Decomposition of primary oxidation compounds is a complex mechanism in which a single hydroperoxide can generate several types of volatile or nonvolatile molecules. The type of by-products obtained after fatty acid oxidation is determined by the hydroperoxide composition and by the type of scission of double bonds in the fatty acid chain. The reaction can also terminate after polymer formation.

Antioxidants counteract oxidation in two different ways (Pokorny *et al*., 2001); (1) by protecting target lipids from oxidation initiators and (2) by stalling the propagation phase.

(1) Preventive antioxidants

There are many different 'preventive' antioxidation pathways because of the diverse range of available oxidation initiators. These pathways include chelation of transition metals, singlet oxygen deactivation, enzymatic Reactive oxygen species (ROS) detoxification, UV filtration, inhibition of proxidant enzymes and antioxidant enzyme cofactors.

(a) Transient metal chelators

Chelators of transition metals such as copper and iron can prevent oxidation by forming complexes or coordination compounds with the metals. These are proteins such as transferrin, ferritin and lactalbumin that sequester iron, or

ceruloplasmin and albumin that sequester copper. Polyphosphates, ethylenediaminetetraceticacid (EDTA), citric acid, phenolic acids and flavonoids are also known for their transition metal chelation capacity. This mechanism of action is minor for lipid peroxidation inhibition as compared to anti-radical activity *via* ROS scavenging but paramount in the inhibition of DNA strand breakage (Sestili *et al*., 1998).

(b) Singlet oxygen quenchers

To current knowledge, carotenoids are the most efficient molecules for $^1O_2$ quenching. This latter mechanism of action occurs through deactivation of $^1O_2$ into $^3O_2$.

$$^1O_2 + \beta\text{-carotene} \rightarrow {}^3O_2 + \beta\text{-carotene}^*$$

Through the long conjugated polyenic system of these molecules, the excess energy generated in their excited state (β-carotene*) is dissipated *via* vibrational and rotational interactions with the solvent or the environment.

(2) Chain-breaking antioxidants

In lipid peroxidation, chain-breaking antioxidants usually lose a hydrogen radical to LOO·, thus halting radical oxidation propagation.

$$A\text{-}H + LOO\cdot \rightarrow A\cdot + LOO\text{-}H \qquad (5)$$

This primarily involves mono- or poly-hydroxylated phenol compounds (tocopherols, tocotrienols, flavonoids, phenolic acids and alcohols, stilbenes, etc.) with different substituents on one or several aromatic rings. Theoretically, the capacity of a phenol to dispose of an H atom could be quantified by the homolytic

dissociation energy of the O–H bond, i.e. bond dissociation energy (BDE). The donor capacity of an H atom increases as the phenol BDE decreases. This fate is generally dictated by the capacity of the antioxidant to stabilize unpaired electrons by delocalization. From this standpoint, the aromatic structure and the potential presence of bulky groups able to extend this delocalization and increased the stability of phenol radicals.

The ability of flavonoids to inhibit lipid oxidation has been reported by Pokorny *et al*. (2001). Flavonoid may act as antioxidants by scavenging radicals that include superoxide anions, lipid peroxyl radicals and hydroxyl radicals. Other mechanisms of action of flavonoids include singlet oxygen quenching, metal chelation and lipoxygenases inhibition. The glycosides are less effective as antioxidants than aglycones.

7. Application of herbs and spices extract in food

7.1 Herb and spices as antimicrobial agent in food

Herbs and spices have been added to food since ancient time as flavoring agents, folk medicine and food preservatives. They prolong the storage life of foods by bacteriostatic or bacteriocidal activity (Shan *et al*., 2007a). The extracts of many plant species have become popular in recent years for food processing applications. Numerous studies have been published on the antimicrobial activities of plant extracts against different types of microbes, including foodborne pathogens in food products (Gutierrez *et al*., 2008) (Table 3).

7.1.1 Meat products

Microbial contamination in meat is an important factor associated with meat quality. It has been found that bacterial contamination, such as *L. monocytogenes*, *Psuedomonas* sp., *E. coli* O157:H7, *Campylobacter* sp. and

Salmonella sp., impacted meat safety (Yin and Cheng, 2003). Plant extracts are useful for reduction of pathogens associated with meat products. The contamination of *Campylobacter* species in muscle foods such as poultry, beef and pork has been widely reported (Wong *et al*., 2007). Furthermore, *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are foodborne pathogen and common cause of bacterial gastroenteritis in human. In ground beef stored at 15°C for 6 days, roselle calyx extract inhibited the survival and growth of Campylobacter species. The addition of roselle calyx extract did not affect cooking loss, pH value and content of fat, protein and moisture of beef samples (Yin and Chao, 2008).

According to Zhang *et al*. (2009), extracts of clove, rosemary, cassia bark and liquorice showed strong antimicrobial activity against four types of microbes (*L. monocytogenes, E. coli, P. fluorescens* and *Lactobacillus sake*). In modified atmosphere packaged fresh pork and vacuum-packaged ham slices, mixed rosemary/liquorice extracts exhibited the great antimicrobial activity with *L. monocytogenes* decreased 2.9, 3.1 and 3.6 log CFU/g, the mesophilic aerobic bacteria by 2.7, 2.9 and 3.1 log CFU/g. The *Pseudomonas* spp. count decreased 1.6, 2.1 and 2.6 log CFU/g and the total coliform count decreased 0.6, 0.8 and 1.2 log CFU/g. The number of *L. monocytogenes* on ham slices decreased 2.5, 2.6 and 3.0 log CFU/g, the mesophilic aerobic bacteria plate counts decreased 2.9, 3.0 and 3.2 log CFU/g and the lactic acid bacteria counts decreased 2.4, 2.6 and 2.8 log CFU/g stored at 4°C.

The ability of plant essential oils to protect foods against pathogenic and spoilage microorganisms have been reported by several researchers. At 5-20 µl/g of eugenol, coriander, clove, oregano and thyme oils showed high antimicrobial activity against *L. monocytogenes, A. hydrophila* and autochthonous spoilage flora in meat products. Moreover, thyme and oregano have also reduced the total viable counts of coliform and *Pseudomonas* spp. when applied on ground beef patties (Emiroğlu *et al*., 2010) and essential oil of mustard could reduce the *B. thermophacta*, aerobic mesophilic bacteria and lactic acid bacteria after 2 day of

Table 3 Applications of herb and spice extracts as antimicrobial agents in food products

Food Products	Microorganisms	Herb and spices	References
Meat products			
-Pork	*E. coli* O157:H7	Garlic	Park and Chin, 2010
	L. monocytogenes, E. coli	Liqurice	Zhang *et al*., 2009
	P. fluoresces, L. sake		
-Ground beef	*Campylobacter* sp.	Roselle calyx	Yin and Chao, 2008
	Pseudomonas sp.	Thyme, Oregano	Lemay *et al*., 2002
-Hotdogs	-	Thyme, clove	Singh *et al*., 2003
Fish product			
-Fish	*L. monocytogenes*	Pomegranate	Zoreky, 2009
	Photobacterium phosphoreum	Oregano	Burt, 2004
Dairy products			
-Chocolate	*E. coli* O157:H7	Lemon	Kotzekidou *et al*., 2008
-Cream milk, skim milk	*L. monocytogenes*	*Eremophila alternifolia, E. duttonii*	Owen and Palombo, 2007
-Pasteurized milk	*E. coli*	Mango seed	Abdalla *et al*., 2007
-Yohurt	*S.* Enteritidis	Cinnamon, cardamom, clove	Burt, 2004

Table 3 (Continued)

Food Products	Microorganisms	Herb and spices	References
Vegetable and fruits			
-Orange juice	-	*Eupatarium lindleyanum* DC	Ji *et al*., 2008
-Fresh cut mango	Aerobic bacterium, yeast	Vanilla	Ngarmsak *et al*., 2006
-Fruit purées	Spoilage yeasts	Vanilla	Cerrutti and Alzamora, 1996

storage (Lemay *et al*., 2002).

Antimicrobial activity of essential oils in meat products can be reduced by high levels of fat. According to Singh *et al*. (2003), 1 mg/l thyme essential oil reduced bacterial populations in zero- and low-fat hotdogs, but not in full-fat hotdogs. At 10 ml/l thyme essential oil, it reduced the bacterial population >1.3 log CFU/g in zero-fat hotdogs, but was less effective in low- and full-fat hotdogs. Clove essential oil also exhibited antimicrobial activity at 1 ml/l in all hotdogs, and was more effective than thyme at 5 ml/l. However, increasing concentration to 10 ml/l did not result in reduction of bacterial population.

Pandit and Shelef (1994) studied the antilisterial effect of eighteen spices and observed inhibitory effect of rosemary (*Rosmarius officinalis*) (≥ 5% w/v) and clove (*Eugenia cariophylata*) (≥ 1% w/v) on *L. monocytogenes*. Rosemary (0.5% w/w) and its essential oil (1% v/w) were used for pork sausage during storage at 5ºC for 50 days.

According to Mytle *et al*. (2006), the inhibitory effect of clove oil (1% v/w) applied to the surface of ready to eat chicken frankfurters was determined on seven strains of *L. monocytogenes* inoculated at low (2-3 log CFU/g) or high cell numbers (4-6 log CFU/g), and stored at 5°C for 2 weeks or at 15°C for 1 week. Treatment with 1% clove oil reduced *L. monocytogenes* populations by 0.2-1.3 log CFU/ml in frankfurters inoculated with low levels of the bacterium. In case of high cell numbers, a decrease of 0.4-3.4 log CFU/ml was observed with 1% treatment.

7.1.2 Fish products

Spoilage of fresh fish and fish products are caused by the growth of specific spoilage microorganisms that produce metabolites causing off-flavors or off-odors leading to consumer rejection. *L. monocytogenes* is a major concern in fish products because of its lower infective dose and higher mortality. According to

Zoreky (2009), pomegranate fruit peels extracts (PE) revealed that PE dipping (0.26% as GAE) possessed an immediate inhibition (<3 log CFU/g at zero time) against *L. monocytogenes* in fish. Moreover, the mixture of oregano and cranberry extracts were decreased the *L. monocytogenes* with 2.3 log CFU/g on fish slices (Lin *et al*., 2004).

7.1.3 Dairy products

Contamination of *Salmonella* Typhimurium, *L. monocytogenes* and *E. coli* has been reported in dairy products (Kapperud *et al*., 1990; Pearson and Marth, 1990; Baylis *et al*., 2004). Lemon essences have strong inhibition effect (reduction of ca. 1.4 log) against *S. aureus* in chocolate at 20ºC (Kotzekidou *et al*., 2008).

According to Owen and Palombo (2007), ethanol extracts of two native medicinal Australian plants (*Eremophila alternifolia* and *E. duttonii*) have been found to inhibit the growth of *L. monocytogenes* in full cream milk and skim milk. Time kill experiments indicated that the extracts were able to inhibit the growth of *L. monocytogenes* at 4ºC and 37ºC. However, components in the full cream milk and skim milk appeared to inhibit the anti-listerial activity of the extracts, requiring higher concentration to control bacterial growth. Furthermore, essential oils of clove, cinnamon, bay and thyme were tested against *L. monocytogenes* and *S.* Enteritidis in soft cheese which clove oil was found more effective against *S.* Enteritidis in full fat cheese than in cheese slurry (Burt, 2004).

Extract of mango seed kernel could reduce total bacterial count, inhibit coliform growth, exert remarkable antimicrobial activity against an *E. coli* strain and extend the shelf life of pasteurized cow milk (Abdalla *et al*., 2007). Cinnamon, cardamom and clove oils inhibited the growth of yohurt starter cultures more than mint oils. Similarly, it have been reported that mint oil was effective against *S.* Enteritidis in low fat yohurt and cucumber salad (Burt, 2004).

7.1.4 Vegetable and fruits

Vegetable products, including fresh produce, pre-cut vegetables and fresh juices are typically preserved by minimal processing (Muñoz *et al*., 2009). Fresh cut products were easily contaminated by microorganisms including bacteria, yeasts and moulds and even pathogens such as *Salmonella* and *Shigella* spp. *Aeromonas hydrophila, Yersinia enterocolitica* and *L. monocytogenes*. These current trends in fresh cut products have led to interest in investigating natural antimicrobial agents (Scifò *et al*., 2009). Extracts from *Eupatorium lindleyanum* DC (0.4 mg/ml) was exhibited against eight food spoilage and foodborne pathogens (*S. aureus, B. subtilis, B. cereus, E. coli, S.* Typhimurium, *Enterococcus faecium, Proteus vulgaris* and *P. fluorescens*) in commercial orange juice (Ji *et al*., 2008).

Cerrutti and Alzamora (1996) reported that vanillin and essential oil of mint have ability to inhibit the growth of spoilage yeasts in fruit purées. Growth of *S. cerevisiae, Zygosaccharomyces rouxii, Debaryomyces hansenii* and Z. *bailii* was inhibited in apple purée containing 2,000 ppm of vanillin for 40 days storage at 27°C. Similarly, vanillin at concentration of 80 mM could delay the development of total aerobic bacteria and yeast population on fresh-cut mango (Ngarmsak *et al*., 2006).

7.2 Herb and spices as antioxidant agent

7.2.1 Meat and meat products

Lipid oxidation is a major cause of muscle food deterioration. It affects the quality of the product through loss of desirable colour, odour and flavor and reduces shelf life (Shan *et al*., 2009). Cooked meat is more susceptible than raw meat to lipid oxidation during chilled and frozen storage. The heating process leads to increase of oxidative reactions in lipids in meat. The antioxidant activity of plant extracts containing phenolic compounds against lipid oxidation has been investigated

Table 4 Applications of herb and spice extracts as antioxidant agents in food products

Food	Herb and spices	References
Meat products		
-Pork	Rosemary, ginseng, fenugreek, aloe vera	McCarthy *et al*., 2001
-Pork sausage	Rosemary	Georgantelis *et al*., 2007
-Beef	Oregano, Grape seed	Fasseas *et al*., 2007; Mielnik *et al*., 2006
-Lamb	Mint	Kanatt *et al*., 2007
-Goat	Peony, sappanwood	Han and Rhee, 2005
	Kinnow, pomegranate	Devatkal *et al*., 2010
Edible oils		
-Corn, soybean oils	Rosemary, Harng Jyur	Basaga *et al*., 1997; Duh, 1999
-Peanut oil	*Cortex fraxini*	Pan *et al*., 2007
-Lard	*Alkanna tinctoria*	Assimopoulou *et al*., 2004
-Edible oil	Citrus	Rehman, 2006

in cooked meat products. Non-culinary herbs from Korea consist of white peony, red peony, sappanwood, moutan peony and rehmania were tested for antioxidant activity in cooked goat meat. Results showed that 0.5-2.0% (g/100 g meat sample) reduced lipid oxidation in cooked goat meat with low 2-thiobarbituric acid reactive substances (TBARS) values (less than 0.4 mg malonaldehyde equivalent/kg) compared to the control (Han and Rhee, 2005). In addition, lipid oxidation in cooked beef could inhibit by 1.6 g/kg grape seed extract and 3% (w/w) oregano essential oil (Mielnilk *et al*., 2006; Fasseas *et al*., 2007).

Minced meats undergo oxidative changes more quickly from grinding exposes lipid membranes to metal oxidation catalysts. Kinnow rind (KR), pomegranate rind (PR) and pomegranate seed (PS) reduced TBARS values during storage of goat meat patties. Observations on percent reduction of TBARS values during refrigerated storage (4ºC) indicated that greater reduction of TBARS by PR (67%) followed by PS (40%) and KR (36%) (Devatkal *et al*., 2010). Food ingredients were screened the antioxidant activity. Results revealed that aloe vera (0.25%), fenugreek (0.01%), ginseng (0.25%), mustard (0.10%), rosemary (0.10%) and tea catechins (0.25%) were most effective antioxidants giving lower TBARS values than the control over 9 days period in frozen pork patties (McCarthy *et al*., 2001).

Combination of rosemary extracts and chitosan gave the best antioxidative effect against fresh pork sausage which the values of peroxide value (PV) and malondialdehyde (MDA) were lower at 20 days (Georgantelis *et al*., 2007). Sebranek *et al*. (2005) also reported that rosemary extract at 1,500 ppm and 2,500 ppm was effective in maintaining low thiobarbituric acid-reactive substances (TBARS) values of precooked-frozen sausage and preventing loss of red color in raw frozen sausage.

Moreover, mint extract had the good total phenolic content and flavonoid content. It exhibited excellent antioxidant activity and retarded lipid oxidation in radiation-processed lamb meat (Kanatt *et al*., 2007). Furthermore,

traditional medicinal plants in various countries have also been investigated for their antioxidant properties such as Ye-Ge (*Pueraria thomsonii*), Fen-Ge (*P. lobata*), hawthorn (*Crataegus oxyacantha*), *Acacia nilotica* and *Limoniastrum monopetalum* (Jiang *et al*., 2005; Singh *et al*., 2010; Trabelsi *et al*., 2010).

7.2.2 Edible oil

Edible oil is very susceptible to rancidity during storage and shelf life. Some antioxidant active compounds of rosemary leaves have been reported to prevent autoxidation of corn and soybean oils with 0.1 g/kg rosemary. At the end of a 4 days period, the peroxide value in corn oil and soybean reached 13 and 10. Moreover, when added as mixtures of 75:25, 50:50 and 25:75 had a synergistic effect on preventing oxidation of soybean oil (Basaga *et al*., 1997).

Dichloromethane extracts of *Alkanna tinctoria* root extracts (0.02% and 0.04% w/w) showed satisfactory antioxidant activity in lard. Moreover, a mixture of dichloromethane extract (0.02% w/w with caffeic acid 0.02% w/w) showed very high antioxidant activity, indicating a synergistic effect (Assimopoulou *et al*., 2004).

Methanol extract of citrus exhibited very strong antioxidant activity in edible oils. After 6 months of storage at 45°C, corn oil containing 1,600 and 2,000 ppm citrus peel extract, showed lower free fatty acid (FFA) contents (1.5% and 1.0%), and peroxide values (8.38 and 7.0 milliequivalents (meq)/kg) and higher iodine values (81, 89) than the control sample (Rehman, 2006).

Chinese medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess antioxidant activity. *Cortex fraxini* extract (CFE), a commonly used Chinese medicine, have been reported the reduction of lipid oxidation. Addition of CFE at a concentration of 0.01, 0.02, 0.05 and 0.08 mg/ml could reduce lipid oxidation by

25.30%, 38.78%, 43.97% and 55.78% in peanut oil, respectively. The results indicated that CFE had strong antioxidant activity in peanut oil (Pan *et al*., 2007). According to Duh (1999), four Harng Jyur varieties (Huang Harng Jyur, Bai Harng Jyur, Gan Harng Jyur and Kung Harng Jyur) could inhibit the MDA formation by 31.5-87.2% of soybean (50:50 w/v) emulsion and Bai Harng Jyur showed the greatest inhibition of MDA formation among all the test samples.

MATERIALS AND METHODS

Materials

1. Microorganisms

Spoilage microorganisms were isolated from coconut milk by our laboratory and identified by laboratory of the National Institute of Health, Department of Medical Science, Bangkok, Thailand as *Bacillus licheniformis* KUB1, KUB2, KUB3, KUB4 and KUB5; *Klebsiella pneumoniae* KUK1 and KUK2, *Enterobacter cloacae*, *Trichosporon mucoides*, *Candida lusitaniae* and *C. tropicalis*.

2. Culture media

2.1 Nutrients broth (NB)

2.2 Yeast malt extract (YM) medium

3. Chemicals

3.1 Methyl alcohol (CH_3OH) (Univar, Australia)

3.2 95% Ethyl alcohol (C_2H_5OH) (Univar, Australia)

3.3 Ethyl acetate (CH_3CHOCH_3) (Lab-Scan, Thailand)

3.4 Acetone ($(CH_3)_2CO$) (Univar, Australia)

3.5 Hexane (C_6H_{14}) (Lab-Scan, Thailand)

3.6 Chloroform ($CHCl_3$) (Univar, Australia)

3.7 Acetonitrile (CH_3CN) (Nacalai Tesque, Inc., Japan)

3.8 Trifluoroacetic acid (TFA) (Wako, Japan)

3.9 Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Fluka, Switzerland)

3.10 2,2-diphenyl-1-picrylhydrazyl (DPPH) Sigma and Aldrich (St. Louis, MO, USA)

3.11 Folin-Ciocalteu reagent (Fluka, Switzerland)

3.12 Piceatannol (Cayman chemical, MI, USA)

4. Equipments

4.1 Filter paper (Whatman No.4) (Whatman International Ltd., Maidstone, England)

4.2 Balance 2 digit (Sartorius, Model ED32023, Germany)

4.3 Balance 4 digit (Ohaus, NJ, USA.)

4.4 Autoclave (Tomy, Model ES-315, Japan)

4.5 UV-Vis spectrophotometer (Shimadzu Co., Kyoto, Japan)

4.6 Rotary evaporator (BÜchi Rotavapor, R200, Switzerland)

4.7 Water bath (Memmert, Germany)

4.8 Hot air oven (Memmert, Model UNE 200, Germany)

4.9 Incubator (Memmert, Model UNE 200, Germany)

4.10 Vortex mixer (Genie, Scientific Industries, USA)

4.11 Orbital incubator shaker (n-Biotek, NB205, Korea)

4.12 Lyophilizer (Virtis, NewYork, USA)

4.13 Larminar flow clean bench (NuAire, NU440, USA)

4.14 Liquid chromatography-mass spectrometry (LC-MS) (Agilent Technologies, Germany)

4.15 Nuclear magnetic resonance spectroscopy (NMR) (JOEL, Japan)

4.16 Transmission electron microscope (TEM) (JOEL, Japan)

Methods

1. Plant preparation

Twenty five Thai herbs listed in Table 5 were purchased from local market and drugstore in Bangkok, Thailand. Betel vine leave were washed with tap water, cut into small pieces and dried in incubator at 50°C for 36 h. Then, the material was

pulverized into fine powder. Other Thai herbs were purchased in powder. All powder materials were stored at 4°C.

Table 5 List of some Thai herbs used in the experiment

Scientific name	Common name	Plant part
Syzygium aromaticum Linn.	Clove	Stem
Piper betle Linn.	Betle vine	Leaf
Curcuma longa Linn.	Turmeric	Tuber
Punica granatum Linn.	Pomegranate	Fruit peel
Garcinia mangostana Linn.	Mangosteen	Fruit peel
Andrographis paniculata Nees.	The creat	Stem, Leaf, Flower
Senna alata (Linn.) Roxb.	Ringworm bush	Seed
Boesenbergia pandurata Holtt.	Kachai	Tuber
Cassia angustifolia Vahl.	Senna	Leaf
Cinnamomum zeylanicum	Cinnamon	Bark
Caesalpinia sappan Linn.	Sappan tree	Core
Curcuma xanthorrhiza Roxb.	Wan-chak-mot-luk	Tuber
Carthamus tinctorius Linn.	Safflower	Flower
Derris scandens Benth.	Fabaceae	Vine
Cyperus rotundus Linn.	Nutgrass	Tuber
Acanthus ebracteatus Vahl.	Sea holly	Stem, Leaf
Tinospora crispa (L.) Miersex Hook.	Kalmegh	Vine
Eclipta prostate	Trailing eclipta	Stem, Leaf, Flower
Phyllanthus emblica Linn.	Malacca tree	Fruit
Azadirachta indica A. Juss	Siamese neem tree	Leaf
Morinda citrifolia	Noni Indian mulberry	Fruit
Senna siamea (Lam) lrwin et Barneby	Cassod tree	Core
Morus alba Linn.	Mulberry tree	Leaf
Citrus aurantifolia	Lime	Fruit peel
Piper retrofractum Vahl.	Java long pepper	Flower

2. Preparation of crude extracts

Each herb powder (10 g) was individually extracted with 100 ml of 95% ethanol (1:10 w/v) at 60°C for 24 h. The extract solution was filtered using Whatman filter paper (No. 4). The solvent was removed from the sample by using a rotary vacuum evaporator (BÜchi Rotavapor, R200, Switzerland). The sample was rotary vacuum evaporated at 40°C until it reached ¼ its volume. Distilled water (10 ml) was added to the sample and the content was continuously rotary vacuum evaporated until it reached 10 ml and kept at 4°C until used (Jaturapronchai, 2003). The dried extracts were obtained by freeze-dried.

3. Determination of antimicrobial activity

Single colony of the test bacteria and yeasts were transferred into NB and YM medium, and the cultures were incubated overnight at 37°C and 30°C, respectively. Each culture (250 µl) of bacteria/yeast with a cell concentration of approximately 8 log colony forming units/ml (CFU/ml) was mixed with 25 ml of melt nutrient agar/YM agar medium at about 45°C and poured onto sterile Petri-dishes. Wells (8 mm-diameter) were punched out of the solid agar using sterile cork borer. The crude extracts (50 µl) were introduced into the wells. Ethanol (95%) was used as a control (Fazeli *et al*., 2007). The plates were incubated at 37°C and 30°C for 24 h for bacteria and yeasts, respectively. The diameters of the inhibition zones were measured in millimeters (mm). Each experiment was repeated in triplicate and the results were expressed as mean values $\pm$ standard deviation (SD).

4. Determination of antioxidant activity

4.1 Determination of total phenolic content

The total phenolic content of the Thai herb extracts was determined using the Folin-Ciocalteu reagent, according to method of Maisuthisakul *et al.* (2007a).

Each powder extract (0.1 g) was diluted with 50 ml distilled water. The sample of each herb extract solution (0.5 ml) was transferred into a test tube containing distilled water (10 ml) and then mixed thoroughly with 0.5 ml of Folin-Ciocalteu reagent. After mixing for 5 min, 7.5% (w/v) sodium carbonate (8 ml) was added. The mixture was mixed using vortex mixer and then allowed to stand for 30 min in the dark. The absorbance of herb extracts was measured in spectrophotometer at 765 nm. The blank consisted of a solution only with the Folin-Ciocalteu reagent (without the extract). This experiment was carried out in triplicate and calculated from a calibration curve obtained with gallic acid. Total phenolic content was expressed as milligrams of gallic acid equivalent (mg GAE/g extract). Data were expressed as mean values $\pm$ standard deviation (SD).

4.2 Determination of reducing power

Reducing power of the Thai herb extracts was determined by the method of Yen and Chen (1995) with slightly modification. The capacity of the extracts to reduce the ferric-ferricyanide complex to the ferrous-ferricyande complex of Prussian blue was determined. One ml of each extracts (0.08% w/v) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. 10% trichloroacetic acid (2.5 ml) was added to the mixture, and then centrifuged at 5,000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm. The assay was carried out in triplicate and the results were expressed as mean values $\pm$ standard deviation (SD).

4.3 Evaluation of DPPH radical-scavenging

The free radical-scavenging activity of the Thai herb extracts was evaluated using the stable radical DPPH, according to the method of Yoshiki *et al*. (2009) with modification. Briefly, 400 µl solution of a series of extract concentration with different ratios of extracts to ethanol, i.e. 1:10, $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$

were prepared, then was added to 1.6 ml of 0.1 M 2-morpholinoethanesulfonic acid buffer (pH 6.8) and mixed with 1.2 ml of 400 μM DPPH-ethanol solution at room temperature. After incubation for 5 min, the absorbance of the mixtures at 517 nm was determined using a UV-1200 spectrophotometer (Shimadzu Co., Kyoto, Japan). The control was prepared without herb extract, while blank contained DPPH solution. The percentage of DPPH radical scavenging activity of each plant extracts was calculated as:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

The percentage of DPPH radical-scavenging activity was plotted against the plant extract concentration to determine the amount of extract necessary to inhibit DPPH radical concentration by 50% (IC_{50}). The assay was carried out in triplicate and the results were expressed as mean values $\pm$ standard deviation (SD).

5. Effect of solvent types on antimicrobial activity

Various solvents (water, methanol, ethyl acetate, chloroform and hexane) were used for extraction of each Thai herb. Extraction and antimicrobial activity assay were carried out in the same manner as described in section on 3.

6. Determination of minimum inhibitory concentration (MIC); minimum bactericidal inhibitory (MBC) and minimum fungicidal concentration (MFC)

The crude extracts of selected Thai herbs were prepared at concentration of 0.0375, 0.075, 0.15, 0.3, 0.6, 1.2, 2.4 and 3 mg/ml in distilled water. MICs and MBCs were determined by broth dilution method (Davidson and Parish, 1989). The nutrient broth and YM medium (10 ml) were inoculated with different concentration of the crude extracts ad with 100 μl of active inoculums of microorganisms (approximately 8 log CFU/ml) for 24 h at 37°C for bacteria and 30°C for yeast. The viable plate

counts were determined by spreading a 0.1 ml sample of each treatment was spread on the surface of NA for bacteria and YM agar for yeast and the colonies were counted after incubation. The MIC was defined as the minimum level of the extract that produced a 90% reduction in growth of the test microorganisms. The MBCs/MFCs was the lowest concentration that killed at least 99.9% of the initial inoculums (Ponce *et al*., 2003).

The MIC/MBC values of bioactive compound were determined by microtitre plate method (Yano *et al*., 2006). A 100 µl serial dilution of fraction (0.125–4 mg/ml) were prepared in sterile polystyrene 96-well plates. Overnight culture of each test bacteria (100 µl) was added to the wells to achieve the final concentration of approximately 6 log CFU/ml and incubated at 37ºC for 24 h. Cell numbers of test strain were determined by colony plate counts. The MIC was defined as the minimum level of the extract that produced a 90% reduction in growth of the test microorganisms. The MBCs/MFCs was the lowest concentration that killed at least 99.9% of the initial inoculums (Ponce *et al*., 2003). Each treatment was carried out in duplicate. Distilled water and crude extract were used as the negative and positive control.

7. Effect of Thai herb extracts on microbial growth in broth media

The effect of selected Thai herb extracts on the growth of nine strains of coconut spoilage microorganisms was evaluated using broth dilution assay (Moreira, 2005). The nutrient broth (10 ml) was inoculated with each herb extracts at its MIC value and 100 µl of active inoculum of test bacteria/yeast (approximately 8 log CFU/ml). The samples were incubated at 37°C for bacteria and 30°C for yeast. Sampling for viable cells was carried out at 0, 15, 30, 45, 60 min; 5 and 20 h of incubation. The viable plate counts were serially diluted in dilution water and then 0.1 ml was plate on NA for bacteria and YM agar for yeast and the colonies were counted after incubation. Sample without herb extracts as control were tested in the same way. The results were expressed in log CFU/ml.

8. Factors affecting on antimicrobial efficiency of Thai herb extracts

8.1 Coconut oil

The effect of coconut oil on the antimicrobial efficiency against *B. licheniformis* KUB1 as indicator strain was performed (Gutierrez *et al*., 2008). Each extract of selected plants at their MIC values were added to nutrient broth (10 ml), inoculated with *B. licheniformis* KUB1 (approximately 8 log CFU/ml). Then, the coconut oil (10%, 20%, 30% and 40% v/v) was added. The samples were incubated at 37°C. Sampling for viable cells was carried out at 0, 1, 3, 6, 9, 12 and 24 h of incubation. The viable plate counts were serially diluted in dilution water and then 0.1 ml was plate on NA at 37°C after 24 h of incubation. Sample without herb extracts as control were tested in the same way. The results were expressed in log CFU/ml.

8.2 pH

The effect of pH on the antimicrobial efficiency against *B. licheniformis* KUB1 as indicator strain was performed (Gutierrez *et al*., 2008). Each extract of selected plants at their MIC values were added to nutrient broth (10 ml) adjusted pH to 5, 6 and 7 with 0.1 N HCl solution and 0.1 N NaOH and then inoculated with *B. licheniformis* KUB1 (approximately 8 log CFU/ml). The samples were incubated at 37°C. Sampling for viable cells was carried out at 0, 1, 3, 6, 9, 12 and 24 h of incubation. The viable plate counts were serially diluted in dilution water and then 0.1 ml was plated on NA at 37°C after 24 h of incubation. Sample without herb extracts as control were tested in the same way. The results were expressed in log CFU/ml.

9. Antimicrobial efficiency of Herb extracts in model food

9.1 Antimicrobial efficiency of herb extracts in coconut milk

Coconut milk was used as a model food for evaluation of the antimicrobial efficiency of selected herb extracts against *B. licheniformis* KUB1. Each extract of selected plants at their MIC values were added to sterile coconut milk (10 ml), inoculated with *B. licheniformis* KUB1 (approximately 8 log CFU/ml). The samples were incubated at 37°C. Sampling for viable cells was carried out at 0, 1, 3, 6, 9, 12 and 24 h of incubation. The viable plate counts were serially diluted in dilution water and then 0.1 ml was plate on NA at 37°C after 24 h of incubation. Coconut milk without herb extracts as control was tested in the same way. The results were expressed in log CFU/ml.

9.2 Antimicrobial efficiency of herb extracts in coconut milk cream

Coconut milk cream was used as a model food for evaluation of the antimicrobial efficiency of herb extracts. The ingredients for coconut milk cream consisted of 4% rice flour and 1.3% salt in coconut cream. All of ingredients were gently boiled and sterile. Each extract of selected plants at their MIC values were added to sterile coconut milk cream (10 ml), inoculated with *B. licheniformis* KUB1 (approximately 8 log CFU/ml). The samples were incubated at 37°C. Sampling for viable cells was carried out at 0, 1, 3, 6, 9, 12 and 24 h of incubation. The viable plate counts were serially diluted in dilution water and then 0.1 ml was plate on NA at 37°C after 24 h of incubation. Coconut milk cream without herb extracts as control was tested in the same way. The results were expressed in log CFU/ml.

Growth inhibition of the extract was also carried out in unsterile coconut milk cream. Each extract of selected plants at their MIC values were added to unsterile coconut milk (10 ml). The samples were incubated at 37°C. Sampling for viable cells was carried out at 0, 1, 3, 6, 9, 12 and 24 h of incubation. The viable plate

counts were serially diluted in dilution water and then 0.1 ml was plate on NA at 37°C after 24 h of incubation. Coconut milk cream without herb extracts as control was tested in the same way. The results were expressed in log CFU/ml.

10. Transmission electron microscopic analysis

TEM was used to examine morphological changes of bacteria cells before and after treatment of crude extracts (Massilia *et al*., 2009). Bacterial cultures (6 log CFU/ml) were prepared in sterile eppendroff tubes and were treated with the crude extract to final concentration of 1 mg/ml. Treatments were incubated for 15 min at 37°C and the cells were washed three times with phosphate buffer solution (pH 7.4) by centrifugation at 8,000x g for 10 min at 4°C.

The cell pellets were pre-fix with 2.5% (w/v) glutaraldehyde at 4°C for overnight. Cells were again washed three times with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7-7.4) for 10 min and postfixed with 1% osmium tetroxide for 2 h. Cells were rinsed three times in distilled water (10 min) and dehydrated with a series of acetone solutions (10%, 30%, 50%, 70% and 90%) for 10 min. Next, the cells were dehydrated three times with 100% acetone for 10 min. Cells were infiltered in a mixture of acetone and Spurr's resin (2:1, 1:1 and 1:2) for 6 h, followed by pure Spurr's resin for 6 h and polymerized at 70°C for 8 h. The polymerized samples were sliced into 60 nm thin sections with ultramicrotome (Leica, Wetzler, Germany) and collected on uncoated copper grids. Following staining with 5% uranyl acetate and lead citrate, the sections were examined in JOEL 1220 (JOEL, Japan) operating at 80 kV.

11. Identification of Thai herb extracts compounds

11.1 HPLC analysis

The dried extract powder of selected Thai herb (4 mg) was dissolved with 1 ml of 10% acetonitrile and 0.1% (v/v) TFA in water and filtered through 0.45 μm membrane filter, before HPLC analysis. HPLC experiments were performed in a Shimadzu LC-10AD (Shimadzu Co., Kyoto, Japan) with a JASCO MD-1510 photodiode-array detector (PDA). Compound separation was achieved using a 250 mm x 4.6 mm i.d., 5 μm (Cosmosil 5C18-AR-II column) with 250 mm x 4.6 mm i.d., 5 μm, guard column (Nacalai Tesque Inc, Kyoto, Japan). A mobile phase was a mixture of 10% acetonitrile and 0.1% (v/v) TFA in water (A) and 70% acetonitrile and 0.1% (v/v) TFA in water (B). The elution program involved gradient elution of 0% B for 0–5min, 70% B for 5-50 min, 100% B for 50-55 min, 100% B for 55-60 min and 0% B for 60-65 min. The flow rate of mobile phase was constant and kept at 1 ml/min. A volume of 10 μl sample was manually injected. Column temperature was maintained at 35°C. Fractions were collected separately for multiple chromatographic runs. To prevent degradation of the purified composition, the acetonitrile was removed by rotary evaporation at 37°C and stored at -20°C.

11.2 LC-MS analysis

LC-MS experiments were performed with an Agilent 1200 series LC system (Agilent Technologies, Germany). Chromatographic separation was carries out at 40°C on Cadenza CD-18 (150 mm x 2.0 mm i.d.; 3 μm) column. A mobile phase was a mixture of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The elution program involved gradient elution at 0% B for 0-5 min, 100% B for 5-50 min, 100% B for 50-55 min, and 0% B for 55-60 min. The flow rate of mobile phase was constant and kept at 0.2 ml/min. A volume of 10 μl sample was automatically injected. Mass spectra were recorded on a micrOTOF-QII mass spectrometer (Bruker, Germany) in negative modes. The ESI source conditions were

as follows: capillary temperature, 200°C; capillary voltages -4500 V; Scan range of 50-1000 amu.

11.3 Nuclear magnetic resonance (NMR) spectroscopy analysis

The structure of purified compound in selected Thai herb was determined by NMR spectroscopy. Sample was dissolved in methanol-*d4* in MNR tube to record spectra. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on JNM-ECP400 spectrophotometer (JEOL, Japan). Chemical shifts were reported in ppm (δ) using tetramethysilane as an internal standard at 30°C and coupling constants were expressed in Hertz.

12. Statistical analysis

The data subject to a one way analysis of variance and the significance of the difference between means determine by Duncan s multiple range test ($P < 0.05$) using the SPSS. Values express are mean of triplicate determination $\pm$ SD. Correlation and regression analysis of antioxidant activity versus the total phenolic content was carried out using SigmaPlot 2003.

RESULTS AND DISCUSSION

1. Screening of Thai herbs for biological activities

Twenty five herbs listed in Table 5 were extracted by using ethanol and the crude extracts were then used to determine the antimicrobial and antioxidant activities. The percentage yields of these ethanol crude extracts were ranged from 2.34±0.19% to 21.04±3.31% (Table 6). The stem of clove (*Syzygium aromaticum* L.) gave the highest percentage yield (%) whereas the fruit peel of lime (*Citrus aurantifolia*) gave the lowest.

1.1 Antimicrobial activity

The antimicrobial activities of the ethanol Thai herb extracts by agar diffusion method were shown in Table 7 and 8. The results indicated that the plant extracts showed growth inhibition of test microorganisms with various degrees. Generally, most of the test microorganisms were sensitive to the Thai herb extracts. Of twenty-five plants, crude ethanol extracts of three, namely malacca tree (*Phyllanthus emblica* Linn.), cassod tree (*Senna siamea* (Lam) Irwin et Barneby) and sappan tree (*Caesalpinia sappan* Linn.) exhibited inhibitory effect against all test bacterial strains except *Enterobacter cloacae*, *Candida lusitaniae* and *C. tropicalis*.

Among *Bacillus licheniformis* strains, the difference antimicrobial activities were observed in various herb extracts. KUB5 was very sensitive to many of herb extracts, followed by KUB3, KUB1, KUB2 and KUB4, respectively. Similar result was obtained in *Klebsiella pneumoniae* strains that KUK2 was more sentitive to herb extracts than KUK1. The ethanol extract of malacca tree, cassod tree and pomegranate (*Punica granatum* Linn.) showed high antimicrobial activity with zones of inhibition ranging from 12.33±0.58 to 25.00±1.73 mm. Moreover, the ethanol extracts could inhibit *Trichosporon mucoides* with zones of inhibition ranging from 16.17±0.29 to 17.50±0.50 mm.

Table 6 The percentage yields of ethanol Thai herb extracts

Scientific name	Common name	Plant part	Yield (%) (w/w)[a]
Syzygium aromaticum Linn.	Clove	Stem	21.04±3.31
Piper betle Linn.	Betle vine	Leaf	9.53±1.23
Curcuma longa Linn.	Turmeric	Tuber	6.09±0.20
Punica granatum Linn.	Pomegranate	Fruit peel	20.44±3.14
Garcinia mangostana Linn.	Mangosteen	Fruit peel	17.23±3.23
Andrographis paniculata Nees.	The creat	Stem, Leaf, Flower	5.46±0.23
Senna alata (Linn.) Roxb.	Ringworm bush	Seed	8.54±0.42
Boesenbergia pandurata Holtt.	Kachai	Tuber	8.07±0.26
Cassia angustifolia Vahl.	Senna	Leaf	9.17±0.63
Cinnamomum zeylanicum	Cinnamon	Bark	10.46±1.76
Caesalpinia sappan Linn.	Sappan tree	Core	5.78±0.45
Curcuma xanthorrhiza Roxb.	Wan-chak-mot-luk	Tuber	7.78±0.08
Carthamus tinctorius Linn.	Safflower	Flower	5.45±0.50
Derris scandens Benth.	Fabaceae	Vine	5.20±0.40
Cyperus rotundus Linn.	Nutgrass	Tuber	6.13±0.78

Table 6 (Continued)

Scientific name	Common name	Plant part	Yield (%) (w/w)[a]
Acanthus ebracteatus Vahl.	Sea holly	Stem, Leaf	2.72±0.22
Tinospora crispa (L.) Miersex Hook	Kalmegh	Vine	4.02±0.34
Eclipta prostate	Trailing eclipta	Stem, Leaf, Flower	5.44±0.49
Phyllanthus emblica Linn.	Malacca tree	Fruit	14.55±0.34
Azadirachta indica A. Juss	Siamese neem tree	Leaf	8.10±0.33
Morinda citrifolia	Noni Indian mulberry	Fruit	11.24±0.64
Senna siamea (Lam) lrwin et Barneby	Cassod tree	Core	5.58±0.42
Morus alba Linn.	Mulberry tree	Leaf	9.14±0.66
Citrus aurantifolia	Lime	Fruit peel	2.34±0.19
Piper retrofractum Vahl	Java long pepper	Flower	10.79±0.18

[a]Values expressed are mean ± SD of three experiments.

Table 7 Antimicrobial activity of ethanol Thai herb extracts against spoilage bacteria using agar well diffusion method

Herbs	Zone of Inhibition (mm)							
	KUB1	KUB2	KUB3	KUB4	KUB5	KUK1	KUK2	*E. cloacae*
Betle vine	11.33±0.58f	14.33±1.15bcd	20.00±1.00cd	14.17±0.29cd	20.00±0.00c	12.67±0.58c	15.33±4.16b	13.83±0.03
Malacca tree	12.33±0.58ef	14.00±1.73bcd	21.00±2.00bc	16.67±2.52ab	21.00±1.73bc	16.00±0.00b	14.00±0.00b	-
Siamese neem tree	-[a]	-	22.67±1.53ab	-	12.00±0.00f	-	-	-
Lime	-	-	14.00±0.00e	11.00±0.00e	13.33±0.58ef	-	-	-
Cassod tree	23.00±1.00a	22.67±0.58a	25.00±1.00a	16.67±0.58ab	23.00±1.00ab	18.33±0.58a	23.00±0.00a	-
Pomegranate	20.00±0.00b	16.33 ± 2.89b	25.00±1.73a	15.67±1.53bc	22.33±2.08abc	-	-	-
Mulberry tree	-	-	18.00±1.00d	-	12.00±0.00f	-	-	-
Noni Indian mulberry	-	11.33±1.15e	19.67±1.53cd	-	15.33±0.58de	-	-	-
Clove	14.67±1.53de	16.00±0.00bc	14.33±0.58e	10.67±1.15e	-	-	13.00±1.73bc	-
Sappan tree	16.00±1.00cd	15.67±0.58bc	20.67±2.08bc	15.67±1.53bc	22.33±2.52abc	11.00±0.00d	16.33±2.87b	-
Kachai	-	-	-	-	-	-	-	-
Wan-Chak-Mot-Luk	-	-	-	-	-	-	-	-
Java long pepper	-	-	-	-	17.00 ± 3.46d	-	-	-
Safflower	-	-	-	-	25.00±0.00a	-	-	-
Senna	13.33±1.15ef	14.33±0.58bcd	-	18.33±0.58a	21.3 ±2.89bc	-	16.33±1.53b	-
Fabaceae	-	-	-	-	-	-	-	-
Mangosteen	16.33±0.58cd	-	-	13.33±0.58d	15.00±0.00de	-	13.00±0.00bc	-

Table 7 (Continued)

Herbs	Zone of Inhibition (mm)							
	KUB1	KUB2	KUB3	KUB4	KUB5	KUK1	KUK2	*E. cloacae*
Cinnamon	17.67±3.21c	13.67±0.58cd	-	-	11.67±0.58f	-	10.00±0.00c	-
Nutgrass	13.33±0.58ef	12.00±1.00de	-	-	25.00±0.00a	-	-	-
Sea holly	-	-	-	-	-	-	-	-
Turmeric	-	-	-	-	-	-	-	-
Ringworm bush	-	-	20.00±0.00cd	-	-	-	-	-
Kalmegh	-	-	-	-	-	-	-	-
Trailing eclipta	-	-	-	-	-	-	-	-
The creat	-	-	-	-	-	-	-	-

[a] - : not detected (diameter of wells were 8 mm).

Values expressed are mean ± SD of three experiments.

Mean values with different letter in a column are significantly different ($P \leq 0.05$).

Table 8 Antimicrobial activity of ethanol Thai herb extracts against spoilage yeast using agar well diffusion method

Herbs	Zone of Inhibition (mm)		
	T. mucoides	*C. lusitaniae*	*C. tropicalis*
Betle vine	14.67±1.15c	-	-
Malacca tree	17.50±0.50a	-	-
Siamese neem tree	-	-	-
Lime	11.17±0.29e	-	-
Cassod tree	16.17±0.29b	-	-
Pomegranate	16.33±0.58b	-	-
Mulberry tree	-	-	-
Noni Indian mulberry	13.00±0.00d	-	-
Clove	14.33±0.58c	-	-
Sappan tree	12.17±1.04d	-	-
Kachai	-	-	-
Wan-Chak-Mot-Luk	-	-	-
Java long pepper	-	-	-
Safflower	-	-	-
Senna	16.67±0.29ab	-	-
Fabaceae	10.67±0.29e	-	-
Mangosteen	-	-	-
Cinnamon	-	-	-
Nutgrass	10.83±0.29e	-	-
Sea holly	15.67±0.58b	-	-
Turmeric	-	-	-
Ringworm bush	10.67±0.29e	-	-
Kalmegh	-	-	-
Trailing eclipta	-	-	-
The creat	-	-	-

[a] - : not detected (diameter of wells were 8 mm).

Values expressed are mean ± SD of three experiments.

Mean values with different letter in a column are significantly different ($P \leq 0.05$).

Results also showed that the inhibitory effect depended on type of microorganisms, Gram-positive bacteria were more sensitive to many medicinal plants. This may relate with difference in the structure of their cell wall. Gram-positive do not have the outer membrane and their cell walls are made up of twenty times as much peptidoglycan than Gram-negative bacteria. Also, antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and resulted in a leakage of the cytoplasm (Shan *et al*., 2007a). According to the results obtained by Panichayupakaranant *et al*. (2010), the extract of pomegranate rind exhibited inhibitory effect against the Gram-positive bacteria including *P. acnes*, *S. aureus* and *S. epidermidis*. Furthermore, flower of cassod tree exhibited antimicrobial activity against *E. coli*, *B. cereus* and *S. aureus* with inhibition zone ranging from 10.0 to 11.7 mm (Krasaekoopt and Kongkarnchantip, 2005).

1.2 Antioxidant capacity

Ethanol extracts of twenty five Thai herbs from various plant parts (fruit, peel, seed, leaf, flower, tuber, vine, stem, bark and heartwood) were determined for the antioxidant activity by total phenolic content, reducing powder and DPPH free radical scavenging assay. The results were shown in Table 9.

1.2.1 Total phenolic content

Total phenolic contents of all plant extracts were determined by Folin-Ciocalteu reagent. The assay is based on the reduction of molybdenum ions in an electron-transferbased reduction process (Ebrahimabadi *et al*., 2010). Folin-Ciocalteu assays are easy to perform and have been frequently used to estimate the total phenolic content in fruits, vegetables and plant extracts (Ozen *et al*., 2011). In this study, there is large variation in the total phenolic content of the plant species investigated, ranging from 21.88$\pm$0.73 to 345.64$\pm$6.24 mg GAE/g. Many plant species showed remarkably high total phenolic content (GAE > 200 mg/g). The extract of cassod tree showed the highest total phenolic content (345.64$\pm$6.24 mg GAE/g

extract), followed by the sappan tree (*Caesalpinia sappan*) (335.03±1.38 mg GAE/g extract), mangosteen (*Garcinia mangostana*) (262.58±4.23 mg GAE/g extract), malacca tree (*Phyllanthus emblica*) (223.35±7.87 mg GAE/g extract) and cinnamon (*Cinnamomum zeylanicum*) (222.43±5.76 mg GAE/g extract).

Total phenolic content in other parts of cassod tree have been reported. Leaves extract showed high total phenolic contents with 384±0.11 mg GAE/g. However, flower extract had the low total phenolic contents of 28.90±1.95 mg GAE/g (Chanwitheesuk *et al*., 2005; Phomkaivon and Areekul, 2009). In addition, aqueous pomegranate peel and malacca tree fruit extracts have been reported the high phenolic content of 161.25 and 81.5-120.9 mg/g (Liu *et al*., 2008; Kanatt *et al*., 2010). Generally, extracts with a high amount of phenolic compounds also exhibit high antioxidant activity (Wong *et al*., 2006; Ye *et al*., 2011). The Folin-Ciocalteu assay gives crude of the total phenolic compounds present in an extract. It is not specific to polyphenols, but many interfering compounds may react with the reagent, giving elevated apparent phenolic concentration (Prior *et al*., 2005). Moreover, various phenolic compounds respond differently in this assay, depend on the number of phenolic groups (Tawaha *et al*., 2007). From our results, it was observed that different scavenging activities of the extracts against the DPPH system could be due to the presence of different compounds in the extracts.

1.2.2 Reducing power

The reducing power assay has been used as one of important antioxidant capacity for medicinal herbs. The presence of reductants (antioxidants) in the extracts would result in the reduction of Fe^{3+}/ferric cyanide complex to the ferrous form by donating an electron. Increasing absorbance at 700 nm indicates an increase in reducing ability. All of Thai herb extracts exhibited reducing power ranging from 0.22±0.01 to 0.43±0.02. Mangosteen exhibited highest reducing power (0.43±0.02) follow by sappan tree (0.41±0.02) and cassod tree (0.40±0.00). The different phenolic compositions of the plants caused for the different reducing activities. Many

Table 9 Total phenolic content, reducing power and DPPH free radical scavenging activity of Thai herbs[a]

Herbs	Total phenolic content (mg GAE/g extract)	Antioxidant capacities	
		Reducing power	DPPH (IC_{50}) (µg/ml)
Betle vine	175.82±4.99f	0.36±0.01def	6.28±0.06b
Malacca tree	223.35±7.87d	0.35±0.01efghi	1.88±0.03a
Siamese neem tree	101.57±1.18g	0.39±0.00c	14.15±0.31de
Lime	40.29±0.60kl	0.24±0.00l	69.90±1.57o
Cassod tree	345.64±6.24a	0.40±0.00b	1.86±0.03a
Pomegranate	169.82±13.59f	0.27±0.01k	2.47±0.02a
Mulberry tree	51.14±0.16j	0.38±0.02cd	16.24±0.26e
Noni Indian mulberry	36.14±0.14lm	0.33±0.01hi	23.90±0.38g
Clove	201.20±5.76e	0.35±0.01efgh	2.74±0.01a
Sappan tree	335.03±1.38b	0.41±0.02b	2.31±0.03a
Kachai	38.54±0.21l	0.23±0.01lm	63.90±1.11n
Wan-Chak-Mot-Luk	34.80±0.52lm	0.26±0.00k	24.29±0.06gh
Java long pepper	29.54±0.21mn	0.33±0.00i	39.62±0.99l
Safflower	46.75±1.48jk	0.36 ± 0.02de	31.96±0.43j

Table 9 (Continued)

Herbs	Total phenolic content (mg GAE/g extract)	Antioxidant capacities	
		Reducing power	DPPH (IC_{50}) (µg/ml)
Senna	67.98±0.91i	0.34±0.01ghi	21.86±0.41g
Fabaceae	87.36±0.63h	0.30±0.00j	29.16±0.56i
Mangosteen	262.58±4.23c	0.43±0.02a	1.46±0.00a
Cinnamon	222.43±5.76d	0.35±0.01efgh	1.69±0.02a
Nutgrass	65.07±0.83i	0.36±0.00ef	13.25±0.09d
Sea holly	35.49±0.98lm	0.30±0.02j	10.34±7.64c
Turmeric	53.86±0.50j	0.31±0.02j	26.83±0.03hi
Ringworm bush	50.21±0.76j	0.34±0.01fghi	27.06±0.01i
Kalmegh	63.73±0.21i	0.34±0.01ghi	19.20±0.90f
Trailing eclipta	21.88±0.73n	0.22±0.01m	35.54±0.38k
The creat	22.39±0.21n	0.23±0.01lm	31.95±0.96j

[a] Values are the mean ± standard deviation (*n*=3)

researchers have been reported the reducing power values in other plants. Parsley and cilantro have been shown antioxidant activity with reducing activities values of 25.9-44.5% of 1 mM ascorbic acids (Wong and Kitts, 2006). Likewise, Trabelsi *et al*. (2010) reported the reducing power of acetone/water (8:2) *Limoniastrum monopetalum* extract that showed the high capacity with an EC_{50} of 240 µg/ml.

1.2.3 DPPH free radical scavenging

DPPH free radical scavenging assay is widely used to evaluate the antioxidant capacity of extracts from different plants (Ho *et al*., 2010). The IC_{50} is defined as amount of extract necessary to inhibit DPPH radical concentration by 50%. Thus, a lower IC_{50} value corresponds to a higher antioxidant activity of the plant extracts. In this study, DPPH free radical scavenging activity of Thai herb extracts showed similar trend with the result of total phenolic content and reducing power, indicating that DPPH radical scavenging activity of Thai herb extracts is highly related to the amount of phenolic compounds in the extracts. Among all test extracts, it was observed that seven Thai herb extracts exhibited the strongest antioxidant activity. Mangosteen showed highest antioxidant activity with IC_{50} value of 1.46 ± 0.00 µg/ml followed by cinnamon, cassod tree, malacca tree, sappan tree, pomegranate and clove with IC_{50} values of 1.69 ± 0.02, 1.86 ± 0.03, 1.88 ± 0.03, 2.31 ± 0.03, 2.47 ± 0.02 and 2.74 ± 0.01 µg/ml, respectively. While, lime and kachai showed lowest antioxidant activity with IC_{50} values of 69.90 ± 1.54 and 63.90 ± 1.11 µg/ml, respectively. Our results showed that many plants selected were rich in phenolic constituents and demonstrated good antioxidant capacity. Significant differences between antioxidant capacities were likely due to genotype and environmental differences (namely, climate, location, temperature, fertility, diseases and pest exposure) within species, choice of parts tested and determination methods (Kim and Lee, 2004; Shan *et al*., 2005).

Similar result was reported by Okonogi *et al*. (2007) who found that pomegranate had the highest DPPH scavenging activity (IC_{50} 3 µg/ml). Furthermore,

leaf aqueous extract, flower and stem bark ethanol extracts of Siamese neem tree (*Azadirachta indica* A.) exhibited high antioxidant activity with IC_{50} at 26.5, 27.9 and 30.6 µg/ml, respectively (Sithisarn *et al*., 2005). Moreover, Liu *et al*. (2008) reported the antioxidant activity of emblica fruit (*Phyllanthus emblica* L.) with 81.5-120.9 mg gallic acid equivalents (GAE/g) total phenolic content and 20.3-38.7 mg quecetin equivalents (QE)/g flavonoid content. From antioxidant activity results, it was observed that five ethanol Thai herb extracts contained remarkable high total phenolic content (222.43±5.76 to 345.64±6.24 mg/ml extracts), reducing power (0.35±0.01 to 0.43±0.02) and DPPH free radical scavenging (1.46±0.00 to 2.31±0.03 µg/ml).

The correlation coefficients (*r*) and coefficients of determination (r^2) were shown in Figure 4. Results revealed that values of antioxidant activity determined by two different methods were comparable. There was a positive linear correlation between total phenolic content and antioxidant capacity of DPPH and reducing power assays (r = 0.455 and 0.623, $p < 0.05$, respectively), indicating that the values of antioxidant capacities assayed by the two different methods were highly correlative. These results showed that the three assay methods were all suitable and reliable for assessing total antioxidant capacities of plant extracts, although there were some samples showing differences in total antioxidant capacities between assay methods in the present study. These results were consistant with many researchers who reported positive correlation between total phenolic content and antioxidant activity (Zheng and Wang, 2001; Cai *et al*., 2004; Wong *et al*., 2006; Bouayed *et al*., 2007; Surveswaran *et al*., 2007; Borneo *et al*., 2009).

The Folin-Ciocalteu assay gives a crude estimate of the total phenolic compounds present in an extract. It is not specific to polyphenols, but many interfering compounds may react with the reagent, giving elevated apparent phenolic concentrations (Prior *et al*., 2005). Moreover, various phenolic compounds respond differently in this assay, depending on the number of phenolic groups (Singleton and Rossi, 1965) and total phenolic content does not incorporate necessarily all the antioxidant that may be present in an extract. Hence, this may explain the equivocal

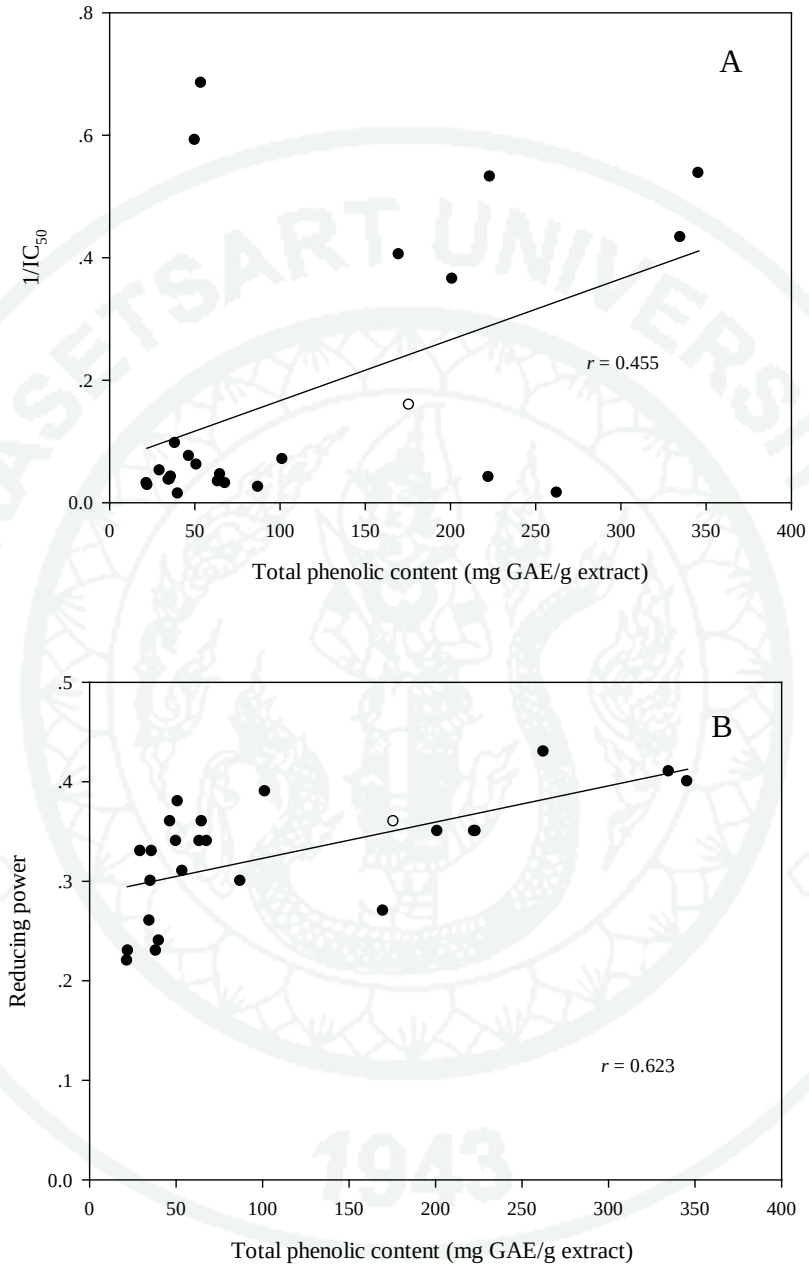


Figure 4 Relationship between the total phenolic content and antioxidant capacity in twenty five Thai herb extracts. (A) linear correlation between total phenolic content and antioxidant capacity measured by DPPH assay and (B) linear correlation between total phenolic content and antioxidant capacity measured by reducing power assay.

correlation between total phenolic content and antioxidant activity of Thai herbs as shown in Table 8.

In this study, it was observed that there were three plants namely cassod tree, malacca tree and pomegranate containing remarkably high antimicrobial and antioxidant activities. Thus, the plants were selected for further investigation.

2. Effect of solvent types on antimicrobial activity

Table 10 showed the effect of the herb extracts (cassod tree, malacca tree and pomegranate) using various solvent types on the growth inhibition of coconut milk spoilage microorganisms. It was found that the ethanol extracts of these herbs showed higher antimicrobial activity than water extracts, methanol and ethyl acetate extracts. On the other hand, chloroform and hexane extracts could not inhibit all test microorganisms. This was probably because phenolic compound in the plants with the medium hydrophilic properties was easily dissolved in polar solvent (Zhang *et al*., 2007). Similarly, the ethanol extracts of artichoke (*Cynara scolymus* L.) leaf exhibited higher antimicrobial activity against 15 microbial species (7 foodborne bacterial pathogens, 4 yeasts and 4 molds (Zhu *et al.,* 2005). The methanol and water extracts from starfish (*Asterina pectinifera*) were found to be the most active against *Aspergillus* spp. and *Cryptococcus reoformans* (Choi *et al.,* 1999). Loizzo *et al*. (2010) found that the water extract of *Artocarpus heterophyllus* L. (jacktree) leaves exhibited the high inhibitory activity against *S. aureus* with inhibition zone diameter of 15$\pm$1 mm. Furthermore, the ethyl acetate and water extracts of the plants were the most efficient antimicrobial compounds (Nostro *et al.,* 2000; Springfield *et al.,* 2003). Burnet *et al*. (2009) also reported that the ethyl acetate and n-butanol extracts of horsetail (*Equisetum arvense* L.) inhibited the growth of *P. aeruginosa*, *S. aureus* and *B. cereus*, while the petroleum ether and chloroform extracts did not show any antimicrobial activity against the test bacteria.

Table 10 Effect of solvent types of Thai herbs extraction against spoilage microorganism using agar well diffusion method

Solvent	Zone of Inhibition (mm)								
	KUB1	KUB2	KUB3	KUB4	KUB5	KUK1	KUK2	*E. cloacae*	*T. mucoides*
Cassod tree									
Ethanol	23.00±1.00a	22.67±0.58a	25.00±1.00a	16.67±0.58a	23.00±1.00a	18.33±0.58	23.00±0.00a	-	16.17±0.29a
Water	12.83±1.15b	11.83±0.58d	12.00±0.00c	12.00±0.00c	16.33±0.58b	-	-	-	11.33±0.58c
Methanol	24.00±0.87a	21.33±0.29b	15.67±0.29b	15.83±0.29b	-	-	15.50±0.00b	12.33±0.29	14.50±0.50b
EA[a]	13.33±0.58b	16.17±0.29c	11.00±0.00d	10.83±0.29d	10.50±0.50c	-	11.17±0.29c	-	-
Chloroform	-[b]	-	-	-	-	-	-	-	-
Hexane	-	-	-	-	-	-	-	-	-
Pomegranate									
Ethanol	20.00±0.00a	16.33±2.89b	25.00±1.73a	15.67±1.53a	22.33±2.08a	-	-	-	16.33±0.58a
Water	18.50±0.50b	19.00±0.00ab	17.33±0.29b	16.33±0.58a	18.00±0.00b	-	20.83±0.29a	-	14.17±0.28b
Methanol	17.67±0.29c	19.83±0.29a	14.67±0.29c	15.33±0.58a	14.50±0.50c	-	15.00±0.00b	-	14.00±0.00b
EA	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-
Hexane	-	-	-	-	-	-	-	-	-

Table 10 (Continued)

Solvent	Zone of Inhibition (mm)								
	KUB1	KUB2	KUB3	KUB4	KUB5	KUK1	KUK2	*E. cloacae*	*T. mucoides*
Malacca Tree									
Ethanol	12.33±0.58c	14.00±1.73b	21.00±2.00a	16.67±2.52a	21.00±1.73a	16.00±0.00b	14.00±0.00b	-	17.50±0.50a
Water	17.00±0.00a	17.67±0.58a	16.83±0.29b	15.00±0.00a	16.00±1.00b	-	-	-	15.83±2.02a
Methanol	15.33±0.29b	17.50±0.87a	16.50±0.00b	16.00±0.00a	15.00±0.50b	11.00±0.00a	15.00±0.00a	11.50±0.50	16.50±0.50a
EA	-	-	11.00±0.00c	11.17±0.29b	10.00±0.00c	-	10.83±0.58c	-	12.17±0.29b
Chloroform	-	-	-	-	-	-	-	-	-
Hexane	-	-	-	-	-	-	-	-	-

[a] EA : Ethyl acetate

[b] - : not detected (diameter of wells were 8 mm).

Values expressed are mean ± SD of three experiments.

Mean values with different letter in a column are significantly different ($P \leq 0.05$).

Previously, the antibacterial activity of cassod tree has been reported by Bukar *et al*. (2009). The results also revealed that aqueous extract possesses higher antipseudomonal activity with inhibition zone of 30 mm than chloroform and methanol extracts. This could be attributed to more phytochemical constituents including tannins and saponins which good dissolved in water, ethanol and methanol.

3. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The MIC/MBC for the extracts selected was examined by broth dilution method. Results were shown that the MIC and MBC/MFC of selected herb extracts (cassod tree, malacca tree and pomegranate) on the inhibition of nine test strains (Table 11). It demonstrated that a wide range of MIC and MBC/MFC values depended on different microbial strains. The ethanol extract of cassod tree showed the highest antimicrobial activity with MIC of 0.3-1.2 mg/ml. MIC of malacca tree and pomegranate ranged from 1.2 to 2.4 mg/ml. It was observed that MBC values of the plants were similar to MIC values except for *B. licheniformis* KUB3. Previously, MIC values of malacca tree and pomegranate has been reported (Mayachiew and Devahastin 2008; Zoreky, 2009). It had great antimicrobial activity against *S. aureus* (MIC = 1.397% (w/v)) and *S.* Enteritidis (MIC = 4% (w/v)). In other study, the garlic extract and nutmeg extract at concentration of 25% (v/v) could inhibit *Salmonella* spp. (Indu *et al.,* 2006). Ponce *et al.* (2003) reported that the clove oil concentration of 0.049 ml/100 ml was needed to inhibit the growth of native microflora of organic Swiss chard. The essential oil of clove at 0.125% and 0.25% could inhibit *V. parahaemolyticus* and *E. coli* ATCC 25158 (Moreira *et al.,* 2005; Yano *et al.,* 2006) and galangal extract of 0.325 mg/ml showed the inhibitory effect against *S. aureus* (Oonmetta-aree *et al.,* 2006).

It was observed that MIC/MBC values of the extracts were difference from inhibition zone. It could be explained by the different methods of antimicrobial testing on plant extracts. During incubation, it was assumed that the crude extracts diffuses

Table 11 Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of ethanol extracts of Thai herbs against spoilage microorganism

Microorganisms	Concentrations (mg/ml)	Herb extracts		
		Malacca Tree	Pomegranate	Cassod Tree
Bacteria				
Bacillus licheniformis KUB1	MIC	1.2	1.2	0.6
	MBC	1.2	1.2	0.6
Bacillus licheniformis KUB2	MIC	2.4	2.4	0.3
	MBC	2.4	2.4	0.3
Bacillus licheniformis KUB3	MIC	2.4	2.4	1.2
	MBC	>3	>3	3
Bacillus licheniformis KUB4	MIC	1.2	2.4	0.6
	MBC	3	2.4	0.6
Bacillus licheniformis KUB5	MIC	1.2	2.4	0.6
	MBC	1.2	2.4	0.6
Klebsiella pneumoniae KUK1	MIC	2.4	-[a]	-
	MBC	2.4	-	-
Klebsiella pneumoniae KUK2	MIC	2.4	-	0.6
	MBC	2.4	-	0.6
Enterobacter cloacae	MIC	2.4	-	-
	MBC	2.4	-	-
Yeast				
Trichosporon mucoides	MIC	1.2	2.4	1.2
	MFC	1.2	2.4	1.2

[a] - : not detected

out from the well into the agar medium, creating a circular concentration gradient that decreases logarithmically with increasing distance from the well. As the crude extracts diffuses out from the well, the bacteria multiply creating a lawn of visible growth on the agar except in area (zones) around the well where diffused molecules possessed properties to inhibit bacterial growth (Othman *et al*., 2011). In addition, minimum inhibitory concentration (MIC) is a quantitative endpoint measurement most commonly used for evaluating the antimicrobial effect of antibiotics. However, MIC provides little information on the kinetic changes in bacterial growth when an antimicrobial agent is present (Li *et al*., 1993). MIC values provide information on the concentration of antimicrobial agents that halts bacterial growth under visible threshold after 24 h. Monitoring bacterial growth in the presence of antimicrobial agents over a specified time period shows changes in bacterial growth rate as early as 2 h since the start of incubation. This can be explained by the intrinsic activity of antimicrobial agents and the interaction between antibacterial effect and bacterial response. Even under controlled experimental conditions, factors controlling the ultimate endpoint, as in the MIC assay, vary markedly between different animicrobials, therefore causing variations in results. From these reasons, effect of Thai herb extracts at MIC concentrations on growth inhibition in nutrient broth was investigated for further study.

4. Effect of Thai herb extracts on growth inhibition in nutrient broth medium

Among all Thai herbs, based on the antimicrobial activity, malacca tree, cassod tree and pomegranate were selected for further study in growth inhibition of all test strains (*B. licheniformis* KUB1, KUB2, KUB3, KUB4 and KUB5, *K. pneumoniae* KUK1 and KUK2 and *E. cloacae*) in nutrient broth medium. Results were shown in Figure 6-13.

In the absence of the extracts (control), the initial bacterial count of all test strains was 6.11 log CFU/ml and steadily increased, reaching 10.22 log CFU/ml after 20 h of incubation (Figure 5). In the presence of the extracts, viable cell counts of *B.*

licheniformis KUB1 immediately decreased from 6.11 to 4.46-5.05 log CFU/ml after 15 min of incubation and remained constant at 3.16-4.76 log CFU/ml until the end of incubation (20 h). Among three extracts, cassod tree extract showed the greatest bacterial inhibition followed by pomegranate and malacca tree extracts, respectively. Similar trend obtained for *B. licheniformis* KUB4 and KUB5 (Figure 8-9). In addition, all three extracts showed similar antimicrobial effect against *B. licheniformis* KUB2 and KUB3 (Figure 6-7). The viable cell counts immediately decreased from 6.30-6.45 to 4.98-5.98 log CFU/ml after 15 min and remained constant at ca. 4 log CFU/ml until the end of incubation (20 h).

As shown in Figure 10 and 12, *K. pneumonia*e KUK1 and *E. cloacae,* Gram-negative bacterial cells were very sensitive to malacca tree extract with immediately reduction of viable cell counts below the level of detection (<1 log CFU/ml) after 15 min of incubation. It was obviously seen that the extract of malacca tree displayed the bactericidal effect with concentration of 2.4 mg/ml. It could be explained that bacterial cells were totally killed and unable to recover on subsequent incubation (Phillips and Duggan, 2002). While, it appeared that all test extracts have bacteriostatic effects that had suppresses the growth and propagation of *B. licheniformis* KUB1, KUB2, KUB3, KUB4 and KUB5 and *K. pneumoniae* KUK2.

Based on these results, the crude ethanol extract of cassod tree were selected for further study in factor (coconut oil concentration and pH) affecting on antimicrobial efficiency against *B. licheniformis* KUB1 which this strain was the most sensitive.

5. Factors affecting on antimicrobial efficiency of Thai herb extracts

5.1 Coconut oil

Effect of coconut oil on the antimicrobial efficiency of cassod tree extract was examined by comparing cell growth in nutrient broth containing 10%, 20%, 30%

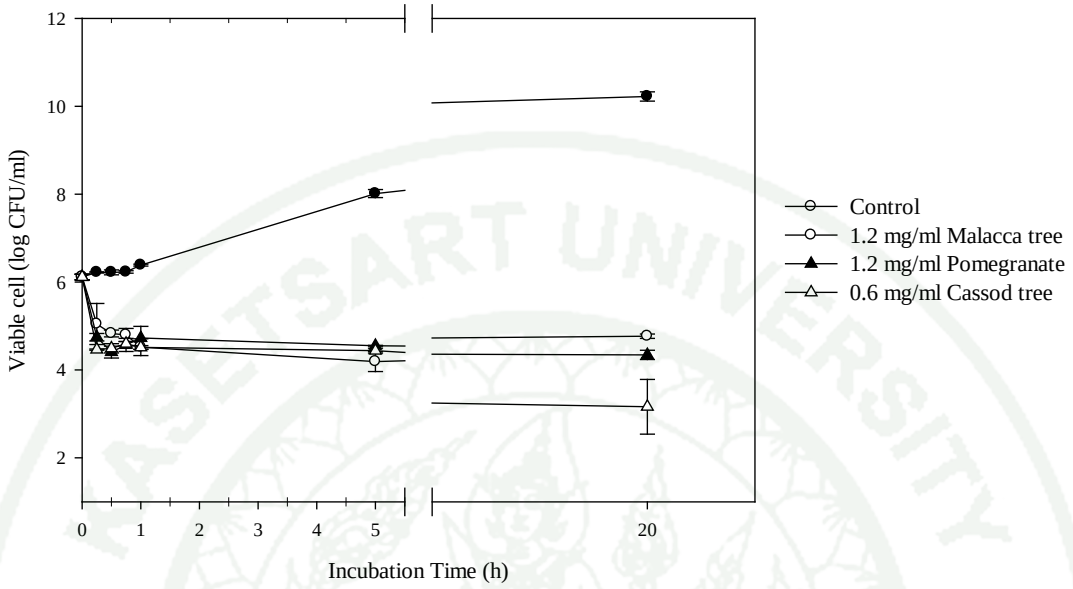


Figure 5 Growth inhibition of *B. licheniformis* KUB1 in nutrient broth by the extracts of malacca tree, pomegranate and cassod tree

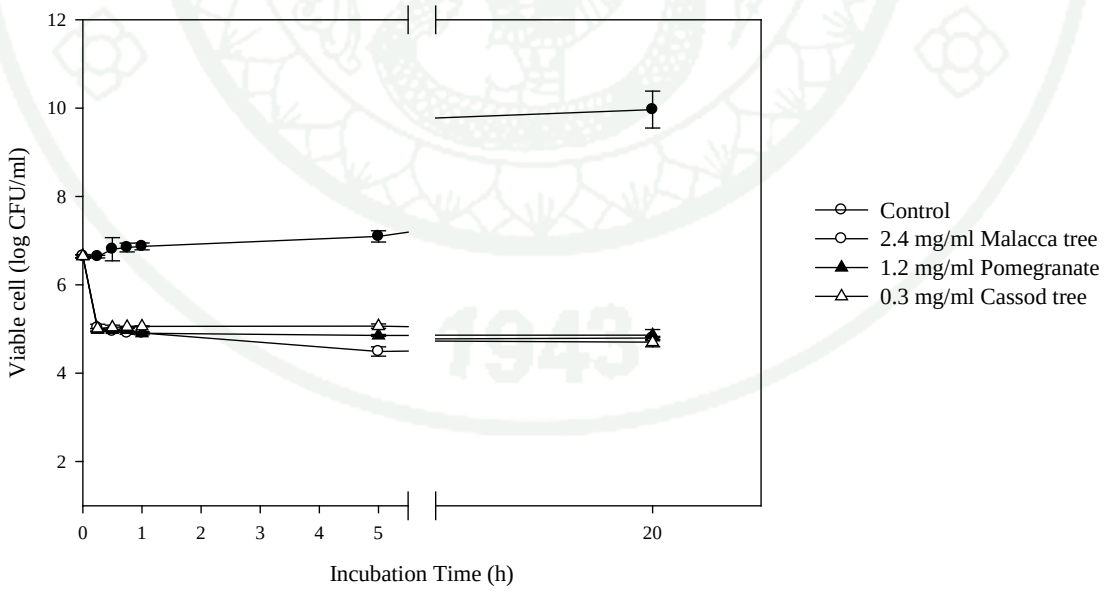


Figure 6 Growth inhibition of *B. licheniformis* KUB2 in nutrient broth by the extracts of malacca tree, pomegranate and cassod tree

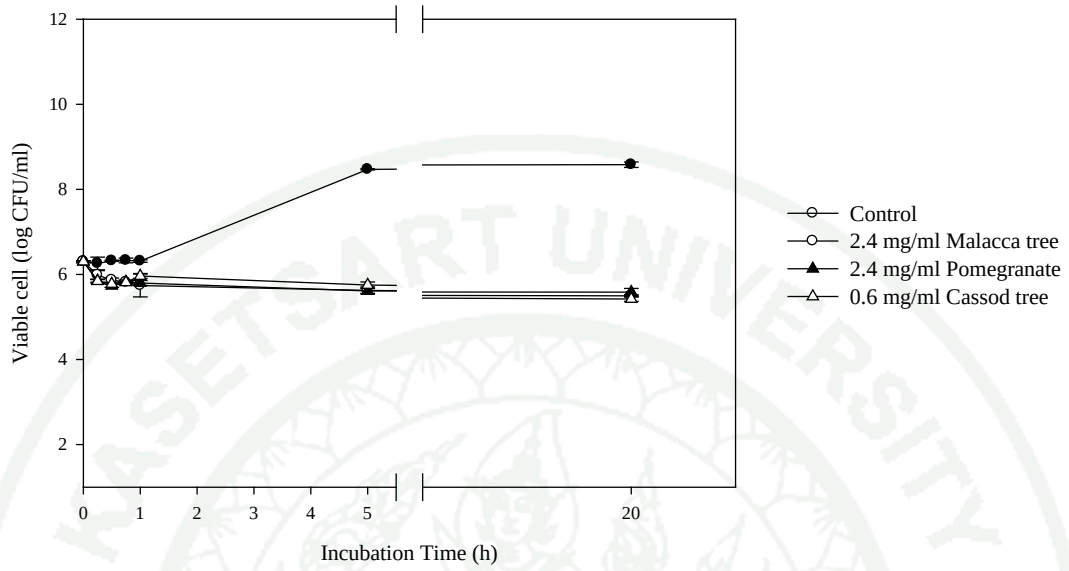


Figure 7 Growth inhibition of *B. licheniformis* KUB3 in nutrient broth by the extracts of malacca tree, pomegranate and cassod tree

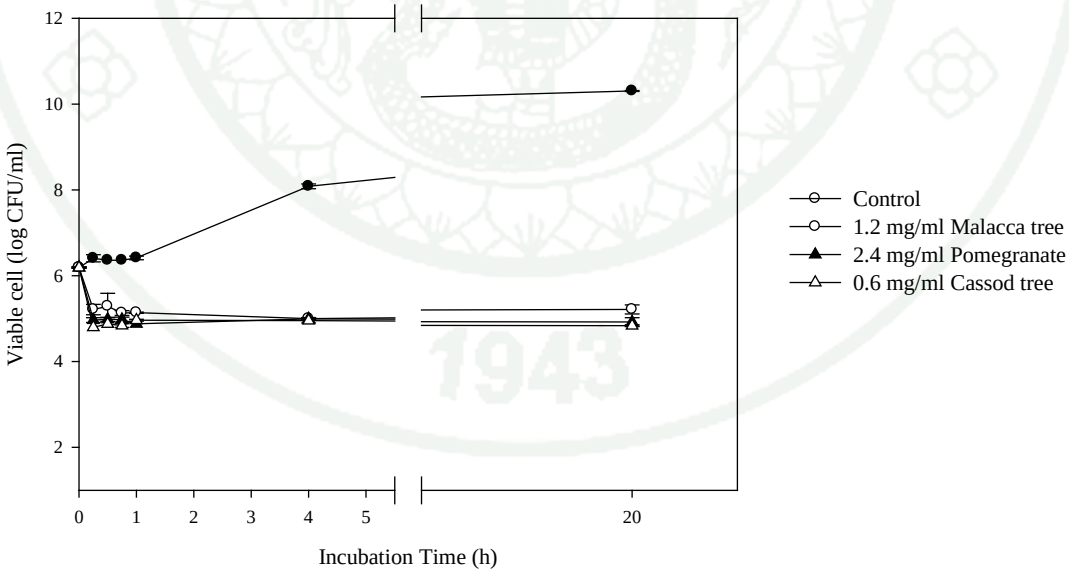


Figure 8 Growth inhibition of *B. licheniformis* KUB4 in nutrient broth by the extracts of malacca tree, pomegranate and cassod tree

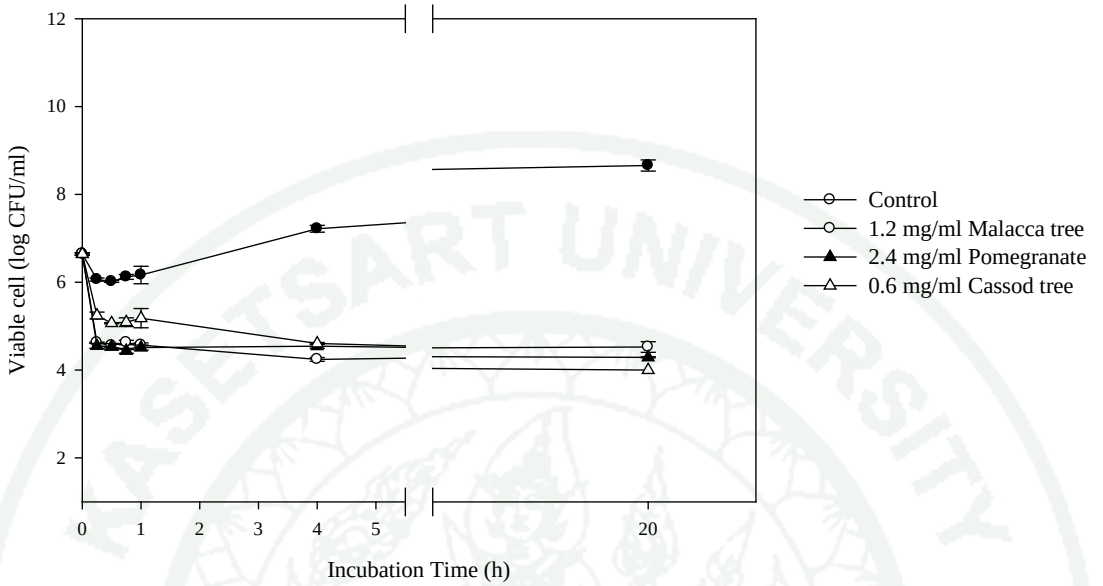


Figure 9 Growth inhibition of *B. licheniformis* KUB5 in nutrient broth by the extracts of malacca tree, pomegranate and cassod tree

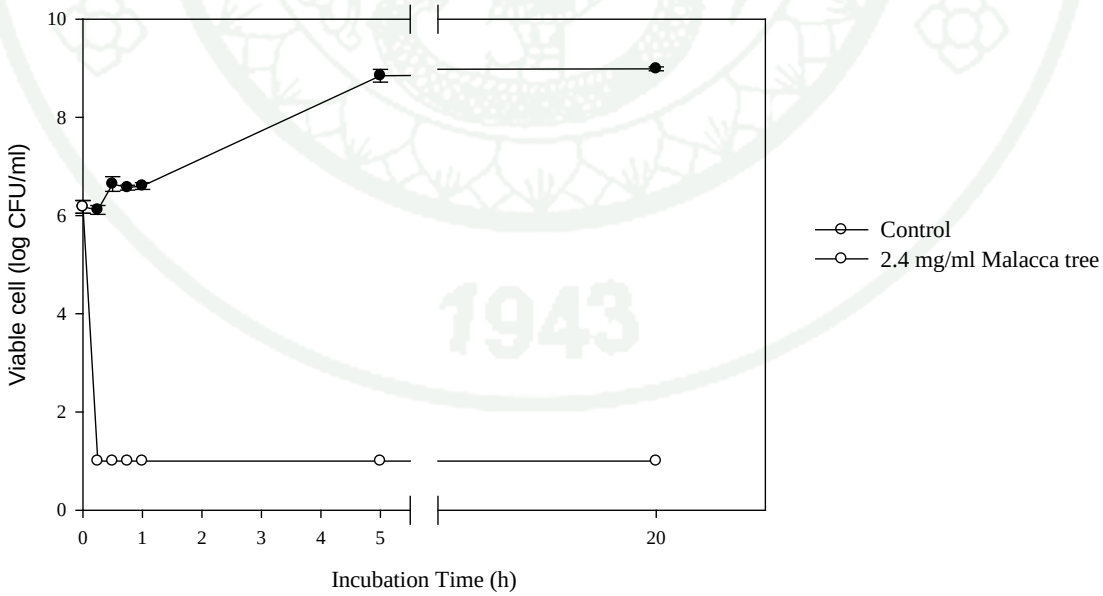


Figure 10 Growth inhibition of *K. pneumoniae* KUK1 in nutrient broth by the extract of malacca tree

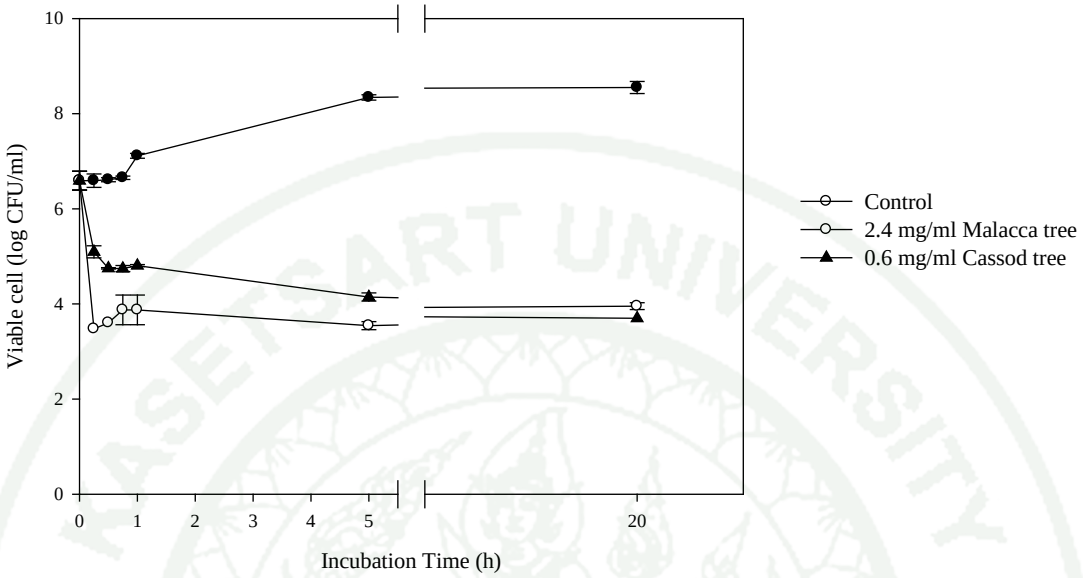


Figure 11 Growth inhibition of *K. pneumoniae* KUK2 in nutrient broth by the extracts of malacca tree and cassod tree

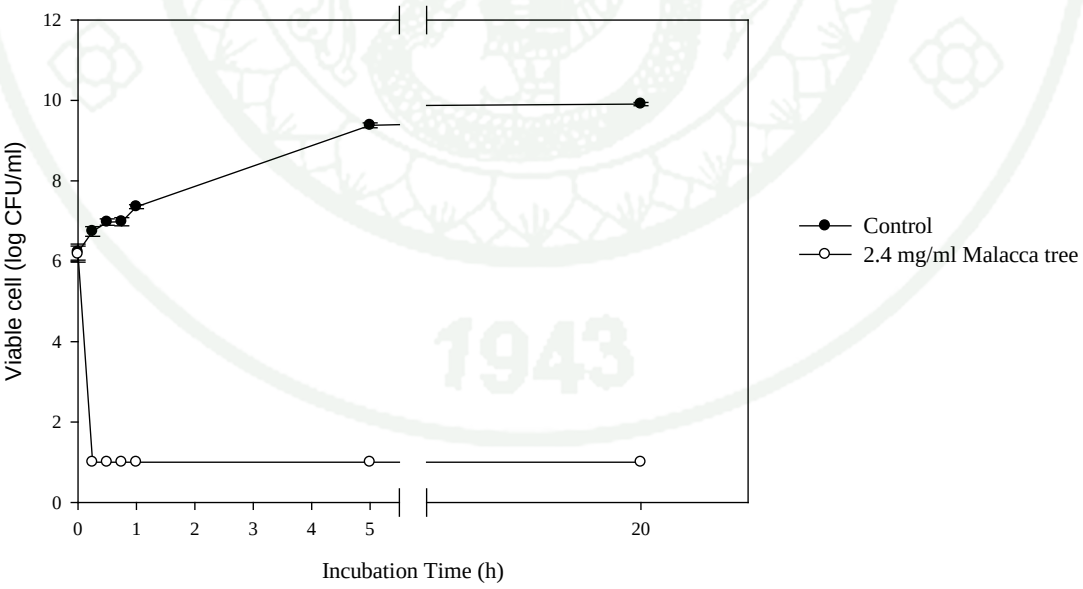


Figure 12 Growth inhibition of *E. cloacae* in nutrient broth by the extract of malacca tree

and 40% coconut oil. Results were shown in Figure 13. In the absence of herb extract and coconut oil (negative control), *B. licheniformis* KUB1 with the initial cell counts of 6.25 log CFU/ml grew exponentially during 3 h of incubation and reached the stationary phase within 9 h of incubation at 37°C. Maximum of viable cell counts was 8.85 log CFU/ml. Comparing to positive control (without coconut oil), cassod tree extract exhibited a great antibacterial efficiency and reduced below the level of detection (<1 log) after 24 h of incubation. Viable cell reduction of 1.6 log CFU/ml was observed after 1 h of incubation and viable cell counts continouosly decreased to below the detection (< 1 log CFU/ml) after 24 h of incubation.

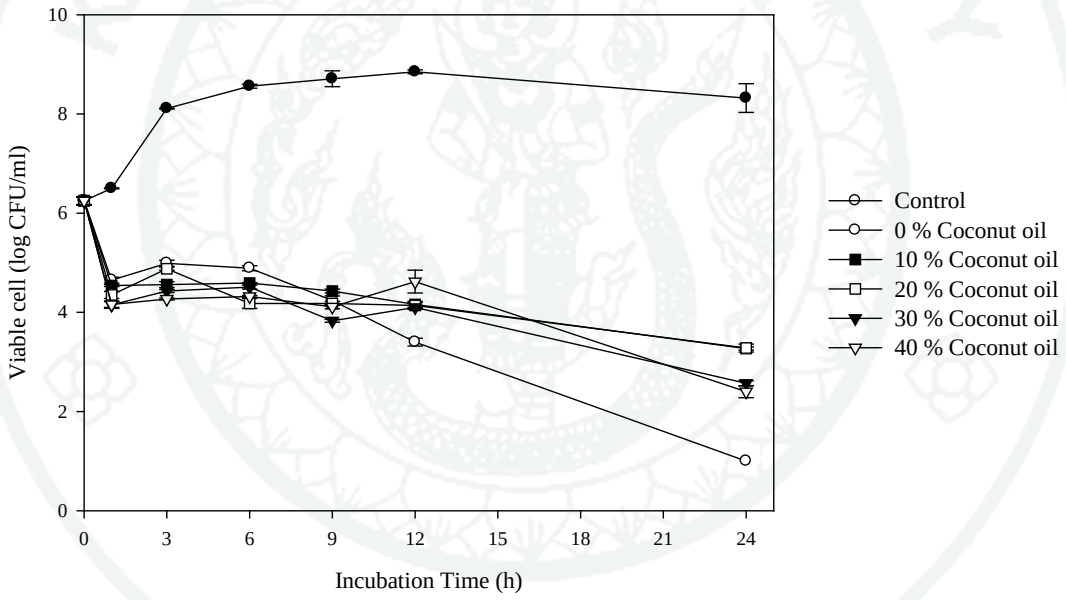


Figure 13 Effect of coconut oil on growth inhibition of *B. licheniformis* KUB1 in nutrient broth by the extract of cassod tree

Similar trend was noticed in treatment of cassod tree extract and coconut oil but higher survival of the test strain was observed in all coconut oil concentrations. *B. licheniformis* KUB1 with the initial bacterial count of 6.25 log CFU/ml rapidly decreased to 1.7, 1.9, 2.1 and 2.0 log CFU/ml at 1 h of incubation in 10%, 20%, 30% and 40% coconut oils, respectively. After that, viable cells remained constant at ca. 4 log CFU/ml for further 12 h of incubation. It was indicated that antimicrobial

efficiency of cassod tree extract was reduced by coconut oil concentration. The coconut oil in medium could form a protective coat around bacteria, thereby protecting them from the cassod tree extract. Smith-Palmer *et al*. (2001) also suggested that the lipid fraction of the food absorbs the antimicrobial agent, thus decreasing the concentration in the aqueous phase and bactericidal action. Singh *et al*. (2003) reported that thyme essential oil reduced bacterial populations significantly in zero- and low-fat hotdogs, but not in full-fat hotdogs. Cava *et al*. (2007) found that the antimicrobial activity of cinnamon and clove essential oils against *L. monocytogenes* was reduced in milk samples with higher fat content. Similarly, Canillac and Mourey (2004) also observed that the addition of dairy fat into a test medium reduced the antilisterial efficiency of *Picea excelsa* essential oil. Glass and Johnson (2004) reported that the antibotulinal effects of nisin and fatty acids were reduced by 20% milk fat or soybean oil.

5.2 pH

Effect of pH on the antimicrobial efficiency of cassod tree extract was examined by comparing cell growth in nutrient broth adjusted pH 5, 6 and 7. Results showed in Figure 14. In control, growth of *B. licheniformis* KUB1 at pH 6 and 7 with the initial cell counts of 6.44 log CFU/ml was similar during 3 h of incubation and reached a level of 8.23-8.37 log CFU/ml within 6 h of incubation. While, bacterial strain at pH 5 could not growth by decreasing from 6.44 log CFU/ml to 4.28 log CFU/ml and below to detection limit (<1 log CFU/ml) at 12 h of incubation.

In the addition of cassod tree extract, viable cells at pH 7 rapidly decreased to ca. 3 log CFU/ml after 1 h and below 1 log CFU/ml after 12 h of incubation. While, viable cells at pH 5 and 6 showed a sharp drop in the bacterial cell after 1 h and then maintained at ca. 3 log CFU/ml until the end of incubation that indicated the bacteriostatic effect. From this result, it was observed that antimicrobial efficiency of the extract was reduced at low pH. This was probably because stability of the extract was loss in low pH. Generally, antimicrobial activity of extract increases with

decreasing pH. However, the relationship was not linear for cassod tree extract and the antimicrobial effect was greater at pH 7 than 5. Corresponding to Wen *et al*. (2003), the antilisterial effect of chlorogenic acid was greater at pH 6.5 than pH 5.5. Furthermore, the pH stability of extract affects to growth inhibition efficiency. Puttarak *et al*. (2010) found that rhiacanthins-D isolated from *Rhinacanthus nasutus* extract were not stable in pH 5.5. In general, low pH condition could decreased the internal pH of the microbial cell by ionization of undissociated acid molecules that effect to disruption of substrate transport by altering cell membrane permeability or reduction of proton motive force and chelation of metal ions essential for microbial growth (Massilia *et al*., 2009).

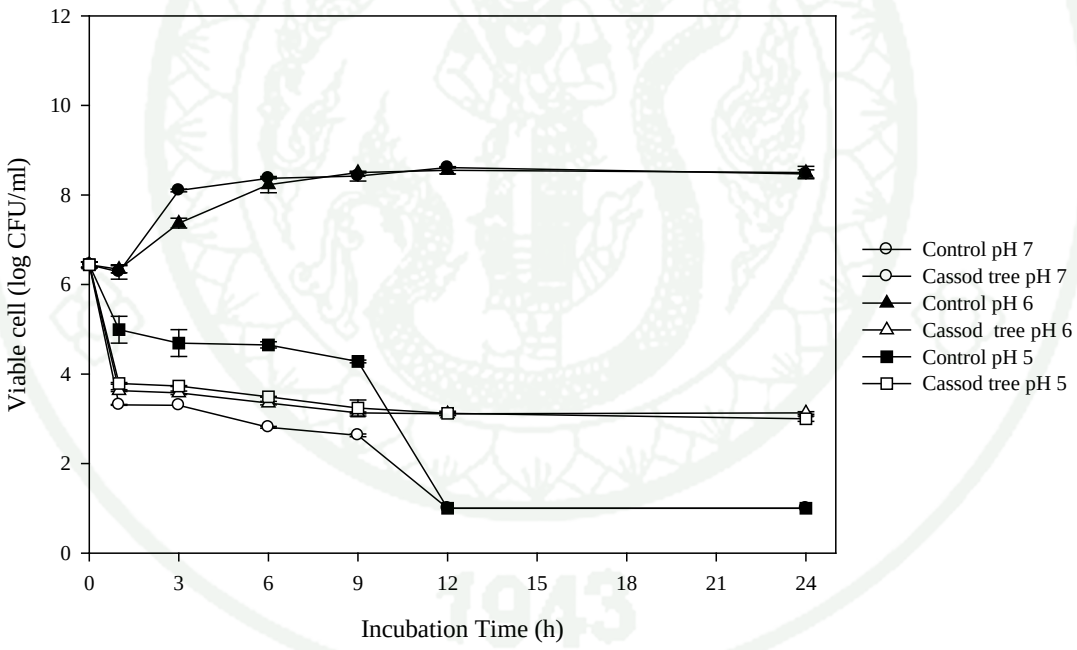


Figure 14 Effect of pH on growth inhibition of *B. licheniformis* KUB1 in nutrient broth by the extract of cassod tree

Other researchers reported that acid environment or medium enhanced the antimicrobial activity (Ji *et al*., 2008). Apostolidis *et al*. (2008) found that low pH values could reduce the bacterial growth of *L. monocytogenes* in Tryptic Soy Broth (TSB). Corresponding to Tassou and Nychas (1995), the growth of *S.* Enteritidis was

higher at pH 7 and 7.8 than at pH 5.5 and 6 in samples with the same inoculum and with the same concentration of oleuropein. Juven *et al*. (1994) mentioned that pH of meat was an important factor affecting the activity of essential oils. At low pH, the hydrophobicity of some essential oils increases and while they may tend to partition in the lipid phase of food, they could also dissolve more easily in the lipid phase of the bacterial membrane and have enhanced antimicrobial action.

6. Antimicrobial efficiency of Thai herb extracts in model food

6.1 Antimicrobial efficiency of cassod tree extract in coconut milk

Antimicrobial efficiency of cassod tree extract in coconut milk against *B. licheniformis* KUB1 was investigated. Results were shown in Figure 15. In coconut milk without cassod tree extract (control), the strain with initial bacterial counts of 6.51 log CFU/ml grew exponentially during 6 h incubation and reached the stationary phase with viable cell count of 8.99 log CFU/ml within 24 h of incubation at 37°C. With the addition of cassod tree extract at concentration of 0.3, 0.6 and 1.2 mg/ml, viable cells could not grow but remained viable at ca. 6 log CFU/ml until 24 h of incubation excepted for concentration of 1.2 mg/ml cassod tree extract, which viable cell counts decreased after 12 h and reduced to 4.56 log CFU/ml after 24 h of incubation.

In comparison between broth medium and coconut milk, the inhibition efficiency of cassod tree extract in broth medium was higher than in coconut milk (cell reduction bacterial cell of 3 log CFU/ml while, coconut milk reduced 2 log CFU/ml. It could suggest that efficiency of cassod tree in broth media was higher than in coconut milk. It has been suggested that the efficiency of natural antimicrobials may be reduced by certain food components. A similar behavior was observed by other studies. Owen and Palombo (2007) reported that the components in cream milk, skim milk, salami pâté and brie cheese appeared to inhibit anti-listerial activity of the native Australian traditional medicinal plants, necessitating higher concentrations to

control microbial growth. Nasar-Abbas and Halkman (2004) also reported that concentration of sumac extract exerts a desirable antibacterial effect may be higher in foods than *in vitro*. Moreira *et al*. (2007) reported the difference between beef and spinach in structure and composition, and especially in the fat, protein and water contents, could explain the differences in the inhibitory effects of clove oil. Intrinsic factors (fat, starch, proteins, salts and temperature) affect the behavior of microorganisms and effect to antimicrobial activity (Tassou and Nychas, 1995). Pomegranate peel extracts have been reported a potential to use in food products. The extract concentrations of 0.1% and 0.5% were able to extend the shelf life of commercially prepared chicken chilly and chicken lollipop by 2 weeks (Kanatt *et al*., 2010).

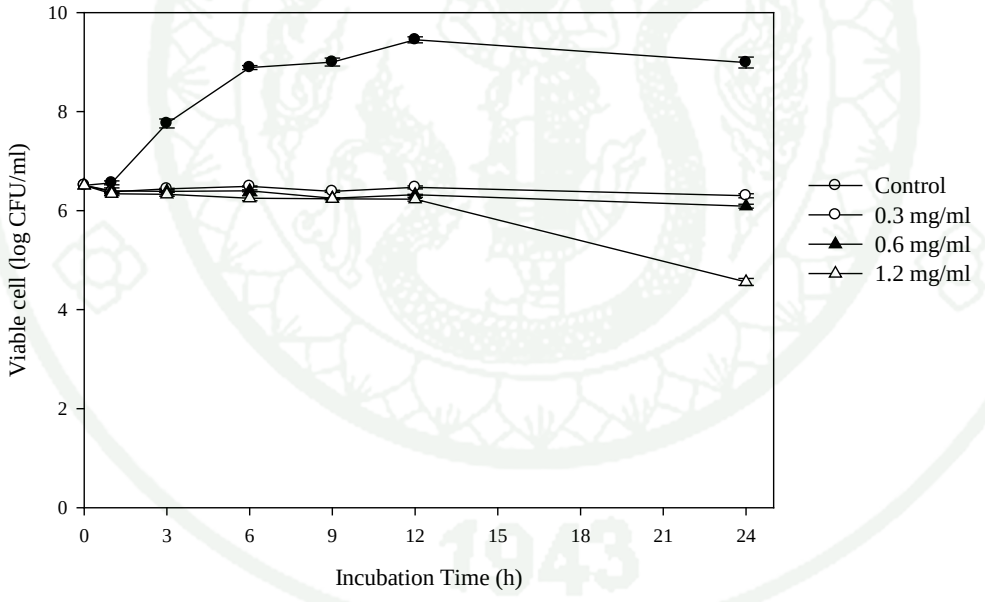


Figure 15 Growth inhibition of *B. licheniformis* KUB1 in coconut milk by the extract of cassod tree at 37°C

6.2 Antimicrobial efficiency of cassod tree extract in coconut milk cream

Results from figure 16 showed antimicrobial efficiency of cassod tree extract for growth inhibition of *B. licheniformis* KUB1 in sterile coconut milk cream.

In control, exponential growth of *B. licheniformis* KUB1 was found during 6 h of incubation and remained constant until the end of incubation with viable cell counts of 9.19 log CFU/ml. While, 0.6 and 1.2 mg/ml cassod tree extract could reduce the viable cells of 2.90-2.93 log CFU/ml after 6 h of time exposure and remained constant at ca. 6 log CFU/ml until the end of incubation (24 h). However, concentration of 0.3 mg/ml cassod tree extract could inhibit bacterial growth in period of 9 h, after that strain continued to grow with viable cell counts of 8.56 log CFU/ml at 24 h of incubation. From these results, it could be explained that the ingredients of coconut milk cream have affected to antimicrobial efficiency of cassod tree and improved microbial resistance. It was agreed with Nasar-Abbas and Halkman (2004) that high concentrations of the extract were necessary to inhibit growth of bacteria in food product because the fat, protein and salt contents of food improve microbial resistance. From these results, bacterial cells also were able to survive until the end of incubation. It was indicated that the extracts primarily kills those cells that were sublethally injured. However, sublethally injured cell had repaired their damages, becoming capable of forming colonies in the medium (Phillips and Duggan, 2002)

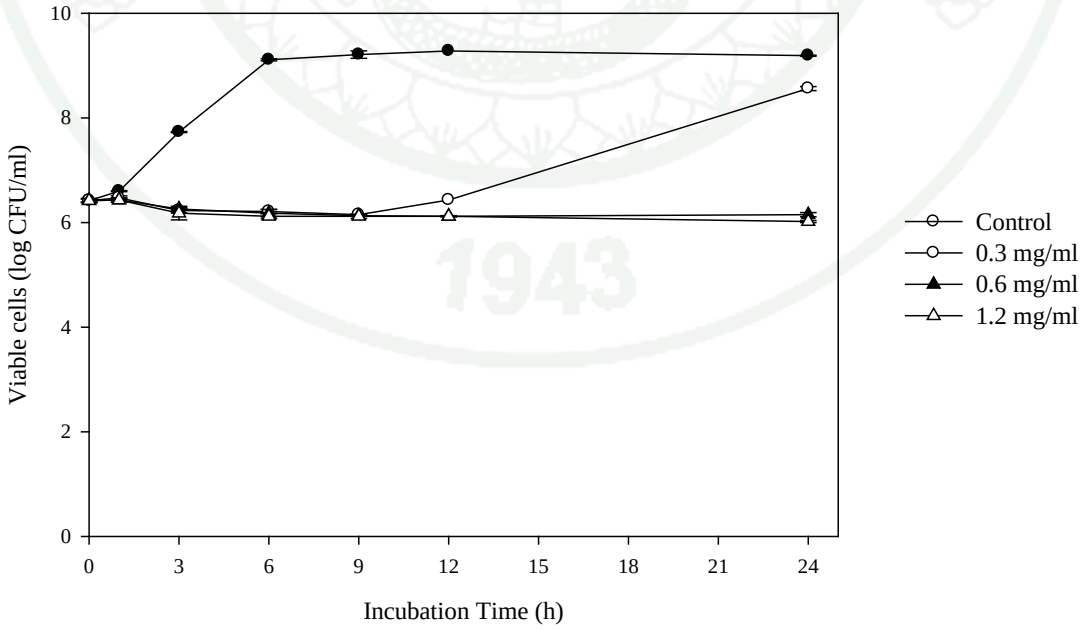


Figure 16 Growth inhibition of *B. licheniformis* KUB1 in coconut milk cream by the extract of cassod tree at 37°C

For unsterile coconut milk cream, difference results of antimicrobial efficiency of cassod tree extract to inhibition bacterial growth was shown in Figure 17. In the addition of 0.3 and 0.6 mg/ml cassod tree extract, the strain with initial viable cell counts of 6.13 log CFU/ml grew exponentially to 7.32-7.37 log CFU/ml during 6 h and reached the stationary phase within 9 h of incubation at 37°C. Similar result was obtained in the control. It was suggested that concentration of 0.3 and 0.6 mg/ml cassod tree extract could not inhibit bacteria cells in unsterile coconut milk cream. When the concentration increasing to 1.2 mg/ml, viable cells decreased ca. 1 log CFU/ml during the first 12 h first of incubation time but increased to the same levels (8.95 log CFU/ml) at 24 h of incubation. This concentration of the extracts could extend the shelf-life of coconut milk cream for a period of 12 h.

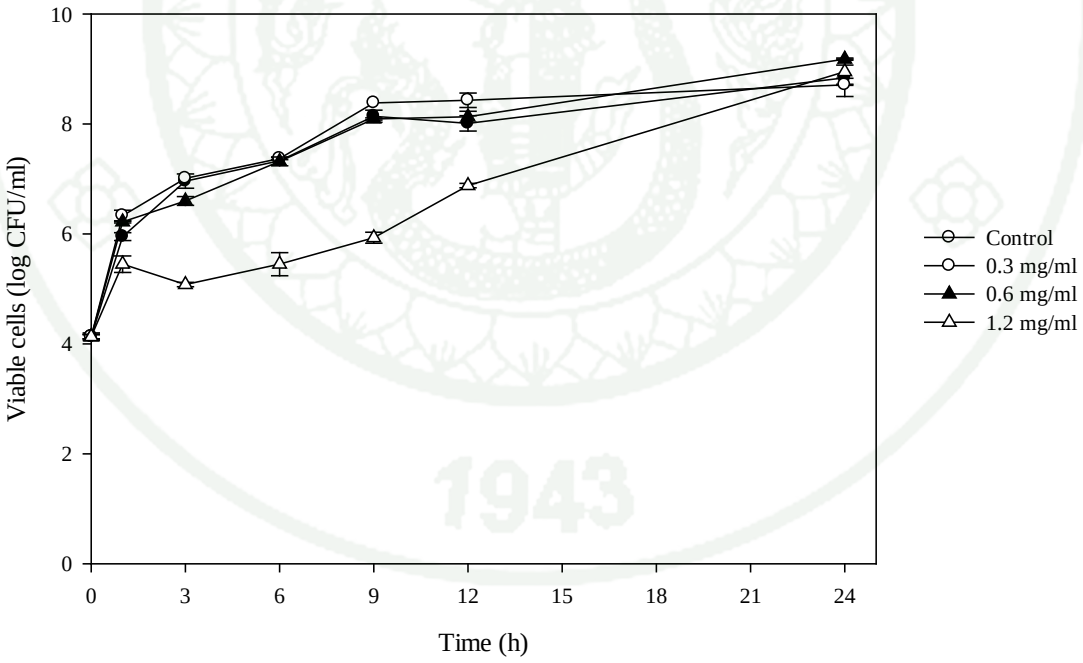


Figure 17 Growth inhibition of bacteria in unsterile coconut milk cream by the extract of cassod tree at 37°C

It was observed that the extracts could not inhibit the bacterial growth in unsterile coconut milk. This might be because the native microorganisms in coconut milk cream such as *E. cloacae*, *C. lusitaniae* and *C. tropicalis* known as predominant microorganism in coconut milk was resistant to cassod tree extract (Table 7).

7. Transmission electron microscopy analysis

The electron micrographs of *B. licheniformis* KUB1 and *K. pneumoniae* KUK2, both untreated cells and cells treated with 1 mg/ml cassod tree extract, are presented in Figure 18-19. In the control, the cells had a typical structure showing a regular shape including clear and smooth cell walls (Figure 18i, ii, 19i, ii). The untreated cells clearly exhibited a dark outer peptidoglycan layer and a densely-stained cytoplasmic membrane bilayer. The electron micrographs of *K. pneumoniae* KUK2 treated with cassod tree extract showed morphological changes of cell wall. It could be observed that outer membrane appeared be much more diffuse and lighter in appearance than the control cells and, in some cases, it almost disappeared (Figure 19iii, iv). Cassod tree extract might attack the phospholipid cell membrane, causing increased permeability and leakage of cytoplasm (Mayachiew *et al*., 2010).

For *B. licheniformis* KUB1 treated with cassod tree extract, bacterial cells showed localized disintegration of peptidoglycan, leaking of cytoplasm and irregular aggregation in the cytoplasm (Figure 18iii, iv). The partial hydrophobicity of phenolic compounds in cassod tree extract would allow them to bind the outer membrane causing changes in membrane fluidity. The irregularity in the cell shape supports the theory of localized chemical interactions at the cell surface. Smaller phenolic compounds can enter the cell and disrupt metabolism (Lacombe *et al*., 2010). In addition, the mode of action of antimicrobial agent depended on the type of microorganisms, physiological state, cell wall structure and membrane arrangement (Wu *et al*., 2008). Moreover, Ultee *et al*. (2002) found hydroxyl group of some phenolic compounds (carvacrol, thymol, cymene and methanol) are important for the

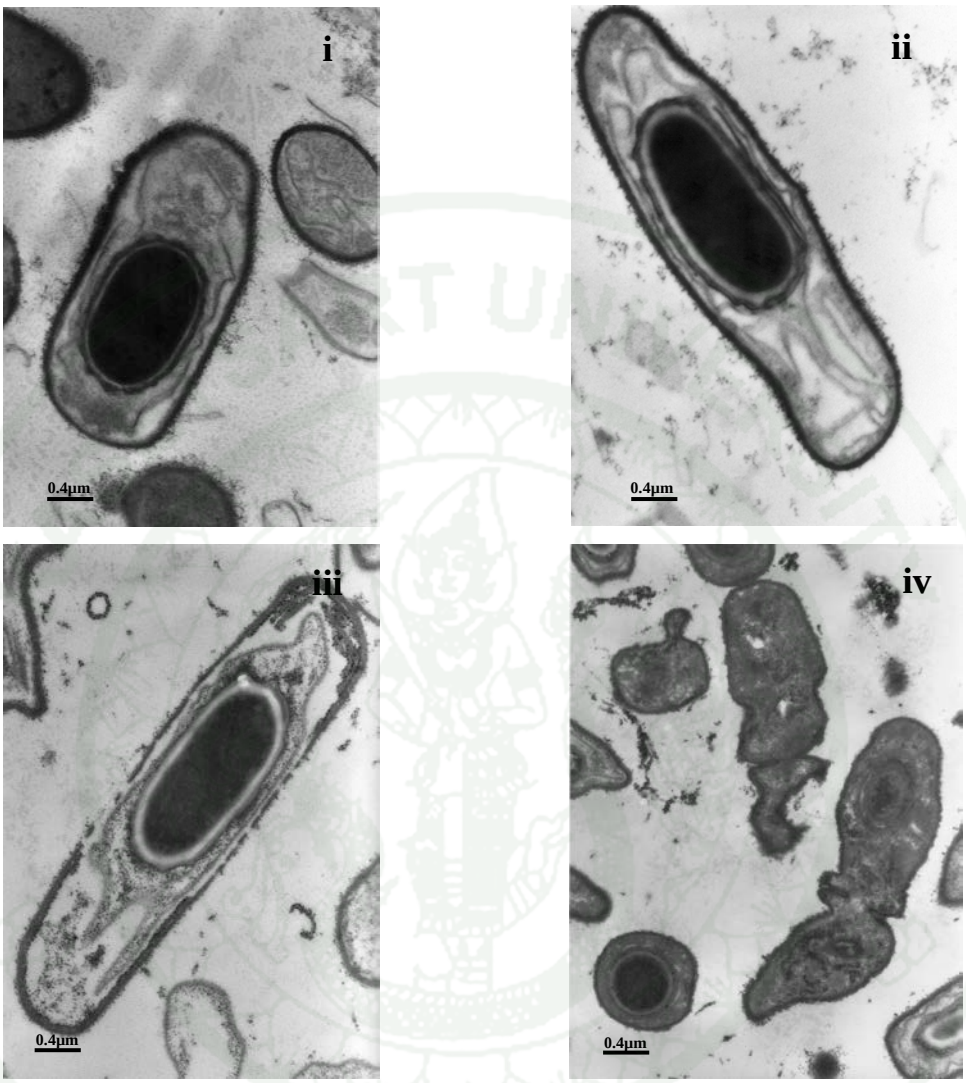


Figure 18 Transmission electron microscopy micrographs of *B. licheniformis* KUB1 (x 20,000) cells in (i,ii) sterile 8.5 µg/ml saline as control and (iii, iv) 1 mg/ml cassod tree extract as treatment

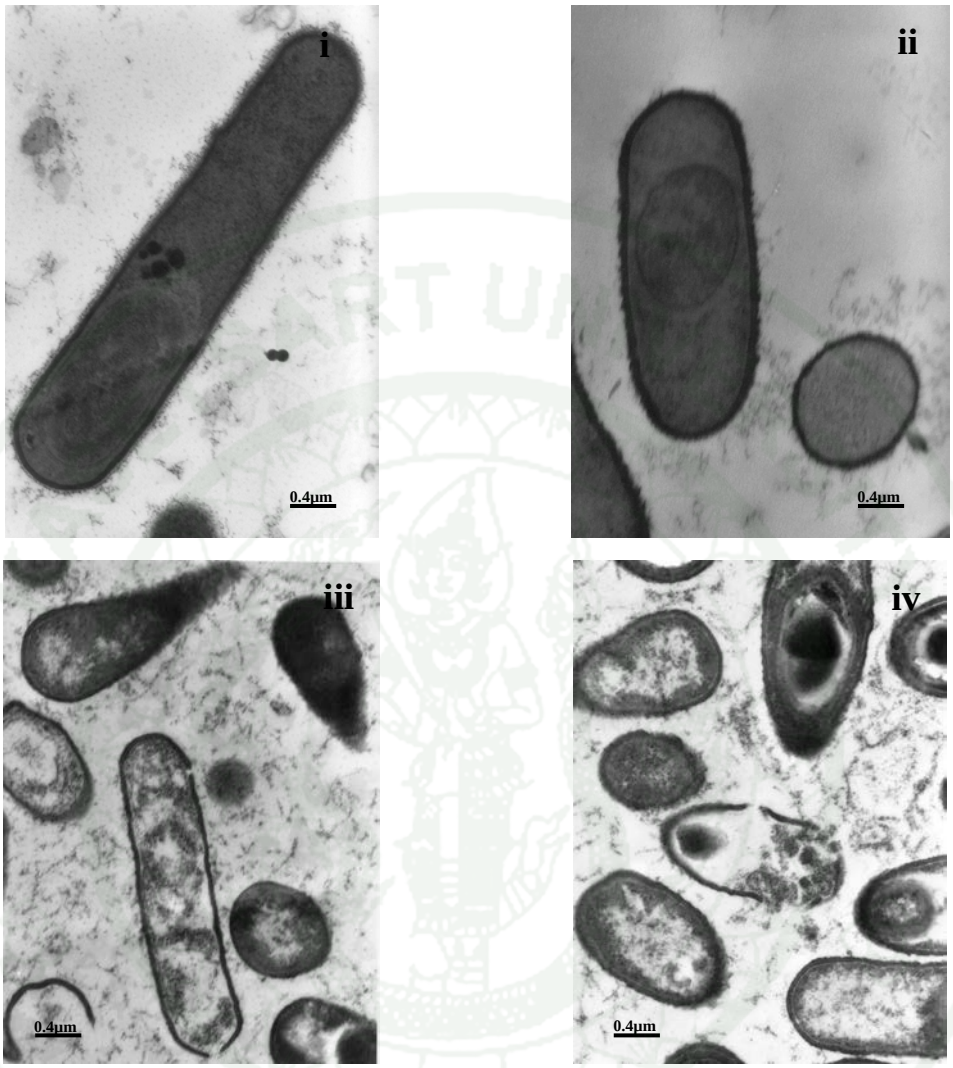


Figure 19 Transmission electron microscopy micrographs of *K. pneumoniae* KUK2 (x 20,000) cells in (i,ii) sterile 8.5 µg/ml saline as control and (iii, iv) 1 mg/ml cassod tree extract as treatment

antimicrobial activity that acts as a proton exchanger, thereby reducing the pH gradient across the cytoplasmic membrane and lead to cell death.

8. Identification of Thai herb extracts compounds

In preliminary study, ethanol crude extract of cassod tree was analyzed by HPLC with photodiode-array detector (PDA) at different wavelengths ranging from 210 to 400 nm. The HPLC-PDA chromatogram recorded at 320 nm showed the best profile of four sharp peaks at the retention time of 26.6 min (C1), 27.8 min (C2), 28.2 min (C3) and 32.8 min (C4) with yield of 25.1, 1.3, 14.2 and 4.2 mg/g of ethanol extract, respectively (Figure 20). Furthermore, LC-TOF-MS was employed to analyze the compounds separated by HPLC. In ESI-TOF-MS experiment, accurate molecular mass of the compounds can be obtained. The mass spectrum and tentatively identified compound of the peaks were given in Figure 21and Table 12. The ESI-TOF-MS mass spectrum in negative mode of C1 exhibited a base peak $[M-H]^-$ at m/z 243, an fragment ion at m/z 201, 159, 109 and its molecular formula was found to be $C_{14}H_{12}O_4$. Based on their MS data, C1 was tentatively identified as 3,3',4',5-tetrahydroxystilbene (piceatannol). For C3, the analysis of mass spectrometry indicated that the m/z of $[M-H]^-$ ion was 487 and the fragment ions were 361, 159, 123, 109. Formula molecule of C3 was $C_{28}H_{24}O_8$. Based on literature data, C3 was tentatively identified as 3-prenyl-4-(dihydrocinamoyoxy)-cinnamic acid. Furthermore, C2 and C4 gave $[M-H]^-$ at m/z 727 and 377, respectively. Unfortunately, there were no available data in LC/MS library. Therefore, C2 and C4 were unknown.

The structure of all purified compounds was also confirmed by NMR spectroscopy. Results showed that C2, C3 and C4 could not be identified by NMR spectroscopy due to their low concentration present in the extract. Therefore, only C1 was identified and the NMR spectroscopic data of the compound was summarized in Table 13. The ^{1}H NMR spectrum displayed a proton signal located at δ6.15 (1H, t, J = 2.2 Hz), 6.42 (2H, d, J = 2.2 Hz), 6.74 (2H, d, J = 6.6, 14.7 Hz), 6.83 (1H, dd, J = 1.8, 8.0 Hz), 6.88 (1H, d, J = 16.5 Hz), 6.97 (1H, d, J = 1.8 Hz). The ^{13}C NMR

spectrum displayed fourteen carbon signals at δ 102.68 (C-4´), 105.79 (C-2´, C-6´), 113.87(C-2), 116.47(C-5), 120.22 (C-6), 127.06 (C-α, C-β), 129.74 (C-1), 131.11(C1´), 141.35 (C-4), 46.58 (C-3), 159.71 (C-3´, C-5´). Based on the literature data (Brinker and Seigler, 1991), C1 was identified as piceatannol and the structure was shown in Figure 22.

Piceatannol is a tetrahydric polyphenol and an analogue form of resveratrol. The content of piceatannol in cassod tree was 722.88 µg/g of crude extract. In general, the amount of piceatannol in other plants was very low in the nanogram range. Lin *et al*. (2007) reported amount of piceatannol in Chinese plants that the piceatannol contents in roots of *Ampelopsis brevipedunculaata*, *Arachis hypogaea* and *Vitis thunbergii* were 412±20, 2,945±4,126 and 130±4,970 ng/g. Furthermore, piceatannol was isolated from *Euphorbia lagascae* (Ferrigni *et al*., 1984), grape (Ha *et al*., 2009; Yim *et al*., 2010) and *Vaccinium* berries (Rimando *et al*., 2004). Other plants, such as *Rheum* spp. (Matsuda *et al*., 2001), *Machura pomifera* (Wang *et al*., 1983) and *Senna* spp. (Arrieta-Baez *et al*., 1999), have also been reported to contain piceatannol in very low concentrations. This result showed that cassod tree contains a high content of piceatannol when compared to other plants suach as grape (0.052 µg/g) and berries (138-422 ng/g) (Bavaresco *et al*., 2002; Rimando *et al*., 2004).

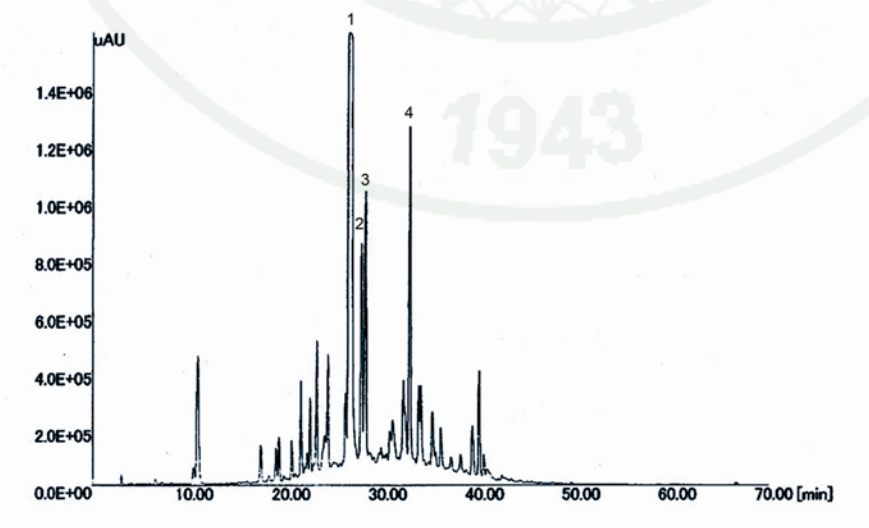


Figure 20 HPLC–PDA chromatogram recorded at 320 nm of cassod tree

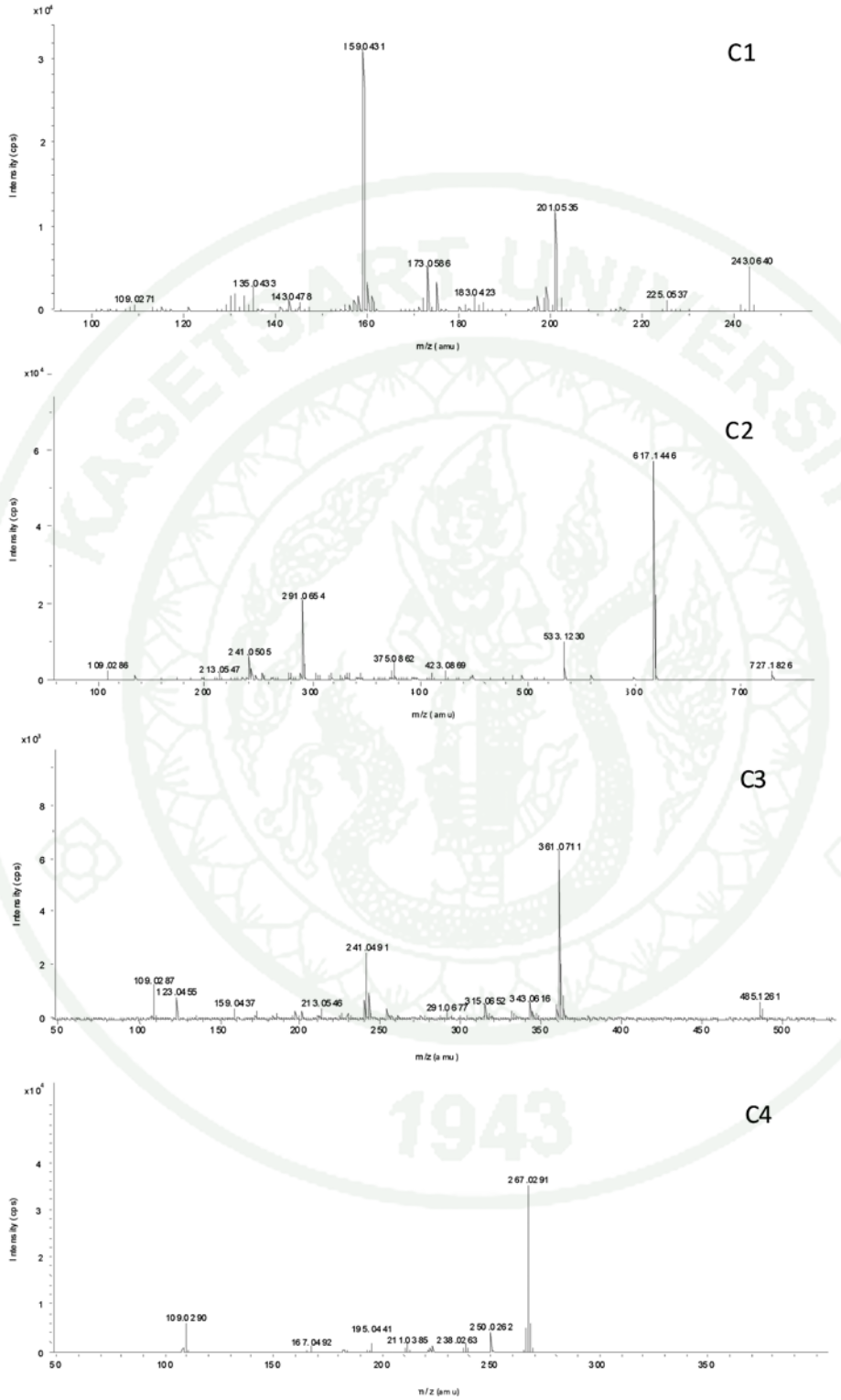


Figure 21 Mass spectrum of purified compounds in cassod tree

Table 12 Chromatographic and LC-TOF-MS data for the tentative identification of bioactive compound in the ethanol extract of cassod tree

Compounds	Retention time (min)	LC-TOF-MS				Tentative identification
		MW	$[M-H]^-$ m/z	MS^2 m/z	Formula	
C1	26.6	244	243	201, 159, 109	$C_{14}H_{12}O_4$	Piceatannol (3,3',4',5-Tetrahydroxystilbene)
C2	27.8	728	727	617, 533.1, 291, 109	$C_{42}H_{32}O_{12}$	unknown
C3	28.2	488	487	361, 159, 123, 109	$C_{28}H_{24}O_8$	3-prenyl-4-(dihydrocinnamoyloxy)-cinnamic acid
C4	32.8	378	377	267, 250, 195, 109	$C_{21}H_{14}O_7$	Unknown

Table 13 ^{13}C (100 MHz)- and ^{1}H (400 MHz)-NMR chemical shifts of piceatannol in MeOD-*d4* with tetramethylsilane as an internal standard

Position	Experiment		Literature data	
	$\delta\ ^{13}C$ (ppm)	$\delta\ ^{1}H$ (ppm)	$\delta\ ^{13}C^{a}$ (ppm)	$\delta\ ^{1}H^{a}$ (ppm)
1	129.74			-
2	113.87	6.97 (d, 1.8)		6.97 (d, 2.0)
3	146.58	-		-
4	141.35	-		-
5	116.47	6.74 (d, 6.6)		6.73 (d, 8.0)
6	120.22	6.83 (dd, 1.8,8.0)		6.83 (dd, 2.0,8.0)
α	127.06	6.88 (d, 16.5)		6.89 (d, 16.0)
β	127.06	6.74 (d, 14.7)		6.73 (d, 16.0)
1'	131.11	-		-
2'	105.79	6.42 (d, 2.2)		6.43 (d, 2.0)
3'	159.71	-		-
4'	102.68	6.15 (t, 2.2)		6.15 (t, 2.0)
5'	159.71	-		-
6'	105.79	6.42 (d, 2.2)		6.43 (d, 2.0)

[a] Chemical shift data were referenced by Brinker and Seigler (1991).

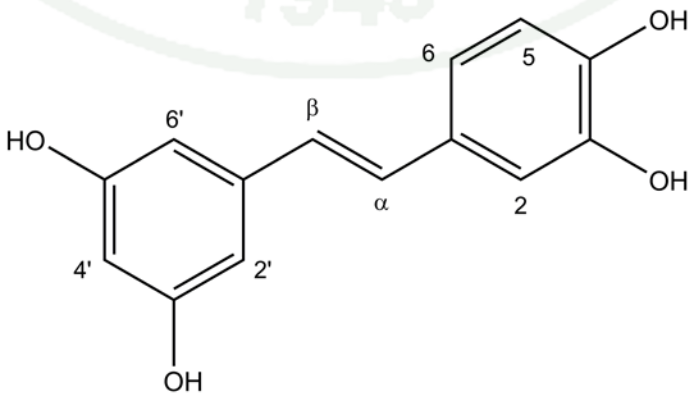


Figure 22 Structure of piceatannol isolated from cassod tree

8.1 Antioxidant activity of piceatannol

Antioxidant activity of piceatannol was determined using DPPH (2, 2-diphenyl-1-picrylhydrazine) assay, widely used to evaluate the free radical scavenging effects of natural antioxidants. This method is an easy, rapid and sensitive way survey the antioxidant activity of a specific compound or plant extracts (Koleva *et al*., 2002; Miceli *et al*., 2009). The concentration for 50% inhibition (IC_{50}) was shown in Table 14. The IC_{50} of piceatannol, ethanol crude extract and trolox were 4.36, 1.86 and 5.60 µg/ml DPPH, respectively. It was observed that antioxidant activity of piceatannol was slightly higher than that of trolox. However, its antioxidant activity was significantly lower than that of the ethanol crude extract (IC_{50} = 1.86 µg/ml DPPH). This might be due to a synergistic effect of several phenolic compounds in the crude extract (Céspedes *et al*., 2010; Jiang *et al*., 2005; Kitzberger *et al*., 2007; Ismail *et al*., 2010). Interestingly, the potency of radical scavenging effect of heartwood extract was about 24 times greater than flower extract (IC_{50} = 45 µg/ml DPPH) (Kaur *et al*., 2006). Moreover, piceatannol isolated from the leaf and stem of *Vitis amurensis* showed significant inhibitory activity with the IC_{50} values of 32.5 (Ha *et al*., 2006). Normally, the number of hydroxyl group and the presence of an olefinic linkage of oilgostilbene structure might play an important role in the DPPH radical reducing (Cai *et al*., 2006). Other reports suggested that piceatannol is able to induce apoptosis in many cancer cell lines (Ku *et al*., 2005).

8.2 Antibacterial activity of piceatannol

MIC/MBC values of piceatannol against coconut milk spoilage bacteria were determined by microtitre plate method. As shown in Table 15, piceatannol showed inhibition effect against the test bacteria with MIC value of 1 and 2 mg/ml and MBC value of 2 and 4 mg/ml for *B. licheniformis* KUB1 and *K. pneumoniae* KUK2, respectively. Results also revealed that ethanol crude extract exhibited the antibacterial activity slightly higher than piceatannol. According to Yim *et al*. (2010), piceatannol from *Vitis amurensis* displayed antimicrobial activity against oral

pathogen (*Streptococcus mutans* and *S. sanguis*) with MIC and MBC values of 50 and 100 µg/ml, respectively.

Table 14 IC_{50} of piceatannol and ethanol crude extract by DPPH assay

Sample	DPPH radical scavenging activity IC_{50} (µg/ml)[a]
Piceatannol (Peak 1)	4.36±0.17
Peak 2	10.68±0.28
Peak 3	6.33±3.86
Peak 4	10.36±0.41
Crude extract	1.86±0.31
Trolox	5.60±0.15

[a] Values expressed are mean ± SD of three experiments.

Table 15 MIC and MBC values of piceatannol and crude extract from cassod tree against coconut milk spoilage bacteria

Bacteria	MIC/MBC values (mg/ml)			
	Piceatannol		Crude extract	
	MIC	MBC	MIC	MBC
B. licheniformis KUB1	1	2	0.5	2
K. pneumoniae KUK2	2	4	1	4

Furthermore, it was observed that MIC/MBC values depended on type of microbial strains. *B. licheniformis* KUB1 (Gram-positive) was found to be more sensitive to the plant extract than *K. pneumoniae* KUK2 (Gram-negative). This result was in agreement with many other studies reported on other plants (Gilles *et al*., 2010; Jayaprakasha *et al*., 2003; Okoh *et al*., 2010; Oroojalian *et al*., 2010). It may be related to a difference in the structure of their cell wall. Gram-positive bacteria do not

have an outer membrane and their cell walls are made up of twenty times as much peptidoglycan than that of Gram-negative bacteria. In addition, antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane resulting in a leakage of the cytoplasm (Shan *et al*., 2007).

In this study, Thai herb extracts showed the potential as the antimicrobial and antioxidant agents and cassod tree were selected to determine the major chemical compositions using HPLC-MS and NMR spectroscopy. Major compound was piceatannol which exhibited the antibacterial and antioxidant activities.

CONCLUSION

This study supports the idea that some Thai herbs could be a good source of natural antioxidants and antimicrobials to be used by food industry. A wide range of antioxidant capacities and antimicrobial activities was observed in twenty five Thai herb extracts. Among them, the ethanol extract of cassod tree was found to have excellent antioxidant capacity with high total phenolic content, reducing power and DPPH free radical scavenging values and antimicrobial activity against spoilage microorganisms of coconut milk with MIC of 0.6 mg/ml. Also, it could inhibit the growth of the test strain of *B. licheniformis* KUB1, KUB2, KUB3, KUB4 and KUB5, *K. pneumoniae* KUK1 and KUK2 and *E. cloacae* in broth medium. However, coconut oil and pH were found to decrease the antimicrobial efficiency of cassod tree extract. In food model, the extract was able to inhibit the growth of spoilage bacteria in coconut milk and coconut milk cream and extend shelf-life of unsterile coconut milk cream. In addition, the mechanism of cell destruction of cassod tree extract observed by TEM involved cell wall and cell membrane disruption, leaking of cytoplasm and irregular aggregation in the cytoplasm.

Finally, major bioactive compound with antibacterial and antioxidant activities was purified from the extract of cassod tree and characterized using LC-MS and NMR as piceatannol. This finding was reported for the first time that piceatannol is present in cassod tree in high content (722.88 µg/g of crude extract), much higher than other plants such as grape and berries. Therefore, the results of this study suggest that cassod tree would be a potential new source of natural food additives and antioxidant food supplements. However, further study might want to focus on consumer's acceptance of the extract in coconut milk and *in vivo* antioxidant activity.

LITERATURE CITED

Abdalla, A.E.M., S.M. Darwish, E.H.E. Ayad and E.M. El-Hamahmy. 2007. Egyptian mango by product 2: Antioxidant and antimicrobial activities of extract and oil from mango seed kernel. **Food Chem**. 103: 1141-1152.

Al-Zoreky, N.S. 2009. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. **Int. J. Food Microbiol.** 134: 244-248.

Apostolidis, E., Y.I. Kwon and K. Shetty. 2008. Inhibition of *Listeria monocytogenes* by oregano, cranberry and sodium lactate combination in broth and cooked ground beef systems and likely mode of action through proline metabolism. **Int. J. Food Microbiol**. 128: 317-324.

Arrieta-Baez, D., L.G. Zepeda-Vallejo and M. Jimenez-Estrada. 1999. Phytochemical studies on *Senna skinneri* and *Senna wislizeni*. **Nat. Prod. Lett.** 13: 223-228.

Assimopoulou, A.N., D. Boskou and V.P. Papageorgiou. 2004. Antioxidant activities of alkannin, shikonin and *Alkanna tinctoria* root extracts in oil substrates. **Food Chem**. 87: 433-438.

Basaga, H., C. Tekkaya and F. Acikel. 1997. Antioxidative and free radical scavenging properties of rosemary extract. **LWT**. 30: 105-108.

Bavaresco, L., M. Fregoni, M. Trecisan, F. Mattivif, U. Vrhovsk and R. Falchetti. 2002. The occurrence of the stilbene piceatannol in grapes. **Vitis** 41: 133-136.

Baylis, C.L., S. MacPhee, A.J. Robinson, R. Griffiths, K. Lilley and R.P. Betts. 2004. Survival of *Escherichia coli* O157:H7, O111:H- and O26:H11 in artificially contaminated chocolate and confectionary products. **Int. J. Food Microbiol**. 96: 35-48.

Borneo, R., A.E. León, A. Aguirre, P. Ribotta and J.J. Cantero. 2009. Antioxidant capacity of medicinal plants from the province of Córdoba (Argentina) and their *in vitro* testing in a model food system. **Food Chem**. 112: 664-670.

Bouayed, J., K. Piri, H. Rammal, A. Dicko, F. Desor, C. Younos and R. Soulimani. 2007. Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. **Food Chem**. 104: 364-368.

Braga, L.C., J.W. Shupp, C. Cummings, M. Jett, J.A. Takahashi, L.S. Carmo, E. Chartone-Souza and A.M.A. Nascimento. 2005. Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production. **J. Ethnopharmacol.** 96: 335-339.

Brinker, A.M and D.S. Seigler. 1991. Isolation and identification of piceatannol as a phytoalexin from sugarcane. **Phytochemistry** 30: 3229-3232.

Brunet, J.M., G.S. Ćetković, S.M. Djilas, V.T. Tumbas, S.S. savatović, A.I. Mandić, S.L. Markov and D.D. Cvetković. 2009. Radical scavenging and antimicrobial activity of horsetail (*Equisetum arvense* L.) extracts. **Int. J. Food Sci. Technol**. 44: 269-278.

Bukar, A., M.D. Mukhtar and A.S. Hassan. 2009. Phytochemical screening and antibacterial activity of leaf extracts of *Senna siamea* (Lam) on *Pseudomonas aeroginosa*. **Bajopus**. 2: 139-142.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in food. **Int. J. Food Microbiol**. 94: 223-253.

Cai, Y.Z., M. Sun, J. Xing, Q. Luo and H. Corke. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. **Life Sci.** 74: 2157-2184.

_____, _____, _____, _____ and _____. 2006. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. **Life Sci**. 78: 2872-2888.

Canillac, N and A. Mourey. 2004. Effects of several environmental factors on the anti-*Listeria monocytogenes* activity of an essential oil of *Picea excels*. **Int. J. Food Microbiol**. 92: 95-103.

Carson, C.F., B.J. Mee and T.V. Riley. 2002. Mechanism of action of *Melaleuca alternifolia* (Tea Tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. **Antimicrob. Agents Chemother**. 46(6): 1914-1920.

Cava, R., E. Nowak, A. Taboada and F. Marin-Iniesta. 2007. Antimicrobial activity of clove and cinnamon essential oils against *Listeria monocytogenes* in pasteurized milk. **J. Food Prot**. 70: 2757–2763.

Cerrutti, P and S.M. Alzamora. 1996. Inhibitory effects of vanillin on some food spoilage yeasts in laboratory media and fruits purées. **Int. J. Food Microbiol**. 29: 379-386.

Ceylan, E and D.Y.C. Fung. 2004. Antimicrobial activity of spices. **J. Rapid Methods Automation Microbiol**. 12: 1-55.

Céspedes, C. L., M. Valdez-Morales, J.G. Avila, M. El-Hafidi, J. Alarcón and O.P. López. 2010. Phytochemical profile and the antioxidant activity of Chilean wild black-berry fruits, *Aristotelia chilensis* (Mol) Stuntz (Elaeocarpaceae). **Food Chem**. 119: 886-895.

Chanwitheesuk, A., A. Teerawutgulrag and N. Rakariyatham. 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. **Food Chem**. 92: 491-497.

Cheeptham, N and G.H.N. Towers. 2002. Light-mediated activities of some Thai medicinal plant teas. **Fitoterapia** 73: 651-662.

Chirinos, R., D. Campos, N. Costa, C. Arbizu, R. Pedreschi and Y. Larondelle. 2008. Phenolic profiles of Andean mashua (*Tropaeolum tuberosum* Ruíz & Pavón) tubers: Identification by HPLC-DAD and evaluation of their antioxidant activity. **Food Chem**. 106: 1285-1298.

Choi, D.H., S. Shin and I.K. Park. 1999. Characterization of antimicrobial agents extracted from *Asterina pectinifera*. **Int. J. Antimicrob. Agents.** 11: 65-68.

Cowan, M.M. 1999. Plant products as antimicrobial agents. **Clin. Microbiol. Rev**. 12: 564-582.

Davidson, P.M and A.L. Branen. 1993. **Antimicrobial in Foods**. Marcel Dekker, New York.

Davidson, P.M and M.A. Harrison. 2002. Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. **Food Technol**. 56: 69-78.

Davidson, P.M and M.E. Parish. 1989. Methods for testing the efficacy of food antimicrobials. **Food Technol.** 43: 148-155.

Department of business economics with cooperation of the customes department. 2003. **The percentage of exports markets for Thai coconut**. Available Source: http://www.foodmarketexchange.com/datacenter/product/fruit/coconut/detail/dc_pi_ft_coconut0701.htm, April 15, 2011.

Devatkal, S., K. Narsaiah and A. Borah. 2010. Antioxidant effect of extracts of kinnow rind, pomegranate rind and seed powders in cooked goat meat patties. **Meat Sci**. 85: 155-159.

Dorman, H.J.D and S.G. Deans. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. **J. Appl. Microbiol**. 88: 308-316.

Duh, P.D. 1999. Antioxidant activity of water extract of four Harng Jyur (*Chrysanthemum morifolium* Ramat) varities in soybean oil emulsion. **Food Chem**. 66: 471-476.

Dupont, S., N. Caffin, B. Bhandari and G.A. Dykes. 2006. *In vitro* antibacterial activity of Australian native herb extracts against food-related bacteria. **Food Control** 17:929-932.

Ebrahimabadi, A.H., Z. Bidgali, A. Mazoochi, F.J. Kashi and H. Batooli. 2010. Essential oils composition, antioxidant and antimicrobial activity of the leaves and flowers of *Chaerophyllum macropodum* Boiss. **Food Control** 21: 1173-1178.

El-Kamali, H.H and E.M.A. El-Karim. 2009. Evaluation of antibacterial activity of some medicinal plants used in Sudanese traditional medicine for treatment of wound infections. **Acad. J. Plant Sci.** 2: 246-251.

Emiroğlu, Z.K., G.P. Yemiş, B.K. Coşkun and K. Candoğan. 2010. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. **Meat Sci**. 86: 283-288.

Farbood M.I., J.H. MacNeil and K. Ostovar. 1976. Effects of rosemary spice extractive on growth of microorganisms in meats. **J. Milk Food Technol**. 39: 675-679.

Fasseas, M.K., K.C. Mountzouris, P.A. Tarantilis, M. Polissiou and G. Zervas. 2007. Antioxidant activity in meat treated with oregano and sage essential oils. **Food Chem**. 106: 1188-1194.

Fazeli, M.R., G.A. Amin, M.M.A. Attari, H. Ashtiani, H. Jamalifar and N. Samadi. 2007. Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria. **Food Control** 18: 646-649.

Fernandez, W.L., Ignacio, E.V., Linga, C.K and Malapitan, A.H. 1970. Bihourly bacterial plate count of gata (coconut milk) stored for 24h at 30º, 20º and 10º. **Philippine Agriculturist** 54: 202-209.

Ferrigni, N.R., J.L. McLaughlin, R.G. Powell and C.R. Smith. 1984. Use of potato disc and brine shrimp bioassays to detect activity and isolation of piceatannol as the antileukemic principle from the seeds of *Euphorbia lagascae*. **J. Nat. Prod.** 47: 347-352.

Fitzgerald, D.J., M. Stratford, M.J. Gasson, J. Ueckert, A. Bos and A. Narbad. 2004. Mode of antimicrobial action of vanillin against *Escherichia coli*, *Lactobacillus plantarum* and *Listeria innocua*. **J. Appl. Microbiol**. 97: 104-113.

Georgantelis, D., I. Ambrosiadis, P. Katikou, G. Blekas, S.A. Georgakis. 2007. Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4°C. **Meat Sci**. 76: 172-181.

Ghafar, F.M.A., K.N. Prasad, K.K. Weng and A. Ismail. 2010. Flavonoid, hesperidine, total phenolic contents and antioxidant activities from *Citrus* species. **Afr. J. Biotechnol.** 9: 326-330.

Gilles, M., J. Zhao, M. An and S. Agboola. 2010. Chemical composition and antimicrobial properties of essential oils of three Australian *Eucalyptus* species. **Food Chem**. 119: 731-737.

Glass, K.A. and E.A. Johnson. 2004. Antagonistic effect of fat on the antibotulinal activity of food preservatives and fatty acids. **Food Microbiol**. 21: 675-682.

Gutierrez. J. and C.B. Ryan. 2009. Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. **Food Microbiol**. 26: 142-150.

_____, _____ and P. Bourke. 2008. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. **Int. J. Food Microbiol**. 124: 91-97.

Ha, D. T., H. Kim, P.T. Thuong, T.M. Ngoc, I. Lee, N.D. Hung and K. Bae. 2009. Antioxidant and lipoxygenase inhibitory activity of oligostilbenes from the leaf and stem of *Vitis amurensis*. **J. Ethnopharmacol**. 125: 304-309.

Halliwell, B.B. 1974. Superoxide dismutase, catalase and glutathione peroxidase: Solution to the problems of living with oxygen. **New Phytologist**. 105: 1075-1086.

Han, J and K.S. Rhee. 2005. Antioxidant properties of selected oriental non-culinary/nutraceutical herb extracts as evaluated in raw and cooked meat. **Meat Sci**. 70: 25-33.

Hili, P., E.S. Evans and R.G. Veness. 1997. Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. **Lett. Appl. Microbiol.** 24: 264-275.

Ho, C.H., I. Noryati, S.F. Sulaiman and A. Rosma. 2010. In vitro antibacterial and antioxidant activities of *Orthosiphon stamineus* Benth. extracts against foodborne bacteria. **Food Chem**. 122: 1168-1172.

Holley, R.A and D. Patel. 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. **Food Microbiol**. 22: 273-292.

Indu, M.N., A.A.M. Hatha, C. Abirosh, U. Harsha and G. Vivekanandan. 2006. Antimicrobial activity of some of the south indian spices against serotypes of *Escherichia coli, Salmonella, Listeria monocytogenes* and *Aeromones hydrophila*. **Braz. J. Microbiol.** 37: 153-158.

Iqbal, S., S. Haleem, M. Akhtar, M. Zia-ul-Haq and J. Akbar. 2008. Efficiency of pomegranate peel extracts in stabilization of sunflower oil under accelerated conditions. **Food Res. Int.** 41: 194-200.

Ismail, H.I., K.W. Chan, A.A. Mariod and M. Ismail. 2010. Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts. **Food Chem**. 119: 643-647.

Jaturapronchai, W. 2003. **Extraction and antimicrobial effect of Thai herbs and spices extracts**. M.S. Thesis, Kasetsart University.

Jayana, B.L., T. Prasai, A. Singh and K.D. Yami. 2010. Study of antimicrobial activity of lime juice against *Vibrio cholera*. **Scientific World.** 8: 44-46.

Jayaprakasha, G. K., T. Selvi and K.K. Sakariah. 2003. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. **Food Res. Int**. 36: 117-122.

Jayaraman, S.K., M.S. Manaharan and S. Illanchezian. 2008. Antibacterial, antifungal and tumor cell suppression potential of *Morida citrifolia* fruit extracts. **J. IJTB.** 3: 44-49.

Jenie, B.S.L., N. Andarwulan, N.L. Puspitasari-Nienaber and L. Nuraida. 2001. Antimicrobial activity of *Piper betle* Linn. extract towards foodborne pathogens and spoilage microorganisms. Institute of Food Technologists Annual Meeting. Available Source: http://ift.confex.com/ift/2001/techprogram/paper_9068.htm, March 31, 2011.

Ji, L.L., Y.M. Luo and G.L. Yan. 2008. Studies on the antimicrobial activities of extracts from *Eupatorium lindleyanum* DC against food spoilage and food-borne pathogens. **Food Control** 19: 995-1001.

Jiang, R.W., K. M. Lau, H.M. Lam, W.S. Yam, L.K. Leung, K.L. Choi, M.M.Y. Waye, T.C.W. Mak, K.S. Woo and K.P. Fung. 2005. A comparative study on aqueous root extracts of *Pueraria thomsonii* and *Pueraria lobata* by antioxidant assay and HPLC fingerprint analysis. **J. Ethnopharm**. 96: 133-138.

Jung, D.S., F.W. Bodyfelt and M.A. Dawschel. 1992. Influence of fat and emulsifiers on the efficacy of nisin inhibiting *Listeria monocytogenes* in fluid milk. **J. Dairy Sci**. 75: 387-393.

Juven, B.J., J. Kanner, F. Schved and H. Weislowicz. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. **J. Appl. Bacteriol**. 76: 626-631.

Kanatt, S.R and R. Chander. 2010. Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. **Int. J. Food Sci. Technol**. 45: 216-222.

_____, _____ and A. Sharma. 2007. Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. **Food Chem**. 100: 451-458.

Kapperud, G., S. Gustavsen, I. Hellesnes, A.H. Hansen, J. Lassen, J. Hirn, M. Jahkola, M.A. Montenegro and R. Helmuth. 1990. Outbreak of *Salmonella* Typhimurium infection traced to contaminated chocolate and caused by a strain lacking the 60-megadaltran virulence plasmid. **J. Clin. Microbiol**. 28: 2595-2601.

Kasem, N., Y.H. Han and P. Moongkarndi. 2007. Antioxidant and cytoprotective activities of methanolic extract from *Garcinia mangostana* Hulls. **Sci. Asia.** 33: 283-292.

Kaur, G., M. Alam, Z Jabbar, K. Javed and M. Athar. 2006. Evaluation of antioxidant activity of *Cassia siamea* flowers. **J. Ethnopharmacol**. 108: 340-348.

Kim, D.O and C.Y. Lee. 2004. Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. **Crit. Rev. Food Sci. Nutr**. 44: 253-273.

Kitzberger, C.S.G., A. Smânia, R.C. Pedrosa and S.R.S. Ferreira. 2007. Antioxidant and antimicrobial activities of shiitake (*Lentinula edodes*) extracts obtained by organic solvents and supercritical fluids. **J. Food Eng**. 80: 631-638.

Kotzekidou, P., P. Giannakidis and A. Boulamatsis. 2008. Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens *in vitro* and on the fate of inoculated pathogens in chocolate. **LWT**. 41: 119-127.

Krasaekoopt, W and A. Kongkarnchanatip. 2005. Antimicrobial properties of Thai traditional flower vegetable extracts. **AU. J. T**. 9: 71-74.

Kreydiyyeh, S.I., J. Usta and R. Copti. 2000. Effect of cinnamon, clove and some of their constituents on the Na^+-K^+-ATPase activity and alanine absorption in the rat jejunum. **Food Chem. Toxicol**. 38: 755-762.

Kruawan, K and K. Kangsadalampai. 2006. Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs. **Thai J. Pharm. Sci.** 30: 28-35.

Ku, K.L., P.S. Chang, Y.C. Cheng and C.Y. Lien. 2005. Production of stilbenoids from the callus of *Arachis hypogaea*: a novel source of the anticancer compound piceatannol. **J. Agric. Food Chem.** 53: 3877-3881.

Kubola, J., S. Siriamornpun and N. Meeso. 2011. Phytochemicals, vitamin C and sugar content of Thai wild fruits. **Food Chem**. 126: 972-981.

Lachowicz, K.J., G.P. Jones, D.R. Briggs, F.E. Bienvenu, J. Wan, A. Wilcock and M.J. Coventry. 1998. The synergistic preservative effects of the essential oils of sweet basil (*Ocimm basilicum* L.) against acid-tolerant food microflora. **Lett. Appl. Microbiol**. 26: 209-214.

Lacombe, A., V.C.H. Wu, S. Tyler and K. Edwards. 2010. Antimicrobial action of the American cranberry constituents; phenolics, anthocyanins, and organic acids, against *Escherichia coli* O157:H7. **Int. J. Food Microbiol**. 139: 102-107.

Laguerre, M., J. Lecomte and P. Villeneuve. 2007. Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. **Prog. Lipid Res**. 46: 244-282.

Lahijani, M.S.S., H.R.R. Kateb, R. Heady and D. Yazdani. 2006. The effect of German chamomile (*Marticaria recutita* L.) extract and tea tree (*Melaleuca alternifolia* L.) oil used as irrigants on removal of smear layer: a scanning electron microscopy study. **Int. Endodontic J**. 39: 190-195.

Lemay, M.J., J. Choquette, P.J. Delaquis, C. Gariépy, N. Rodrigue and L. Saucier. 2002. Antimicrobial effect of natural preservatives in a cooked and acidified chicken meat model. **Int. J. Food Microbiol**. 78: 217-226.

Li, R.C., D.E. Nix and J.J. Schentag. 1993. New turbidimetric assay for quantitation of viable bacterial densities. **Antimicrobial Agents Chemother**. 37: 371-374.

Lin, Y.T., R.G. Labbe and K. Shetty. 2004. Inhibition of *Listeria monocytogenes* in fish and meat systems by use of oregano and cranberry phytochemical synergies. **Appl. Environ. Microbiol**. 70: 5672-5678.

Lin, L.L., C.Y. Lien, Y.C. Cheng and K.L. Ku. 2007. An effective sample preparation approach for screening the anticancer compound piceatannol using HPLC coupled with UV and fluorescence detection. **J. Chromatogr. B.** 853: 175-182.

Lipipun, V., M. Kurokawa, R. Suttisri, P. Taweechotipatr, P. Pramyothin, M. Hattori and K. Shiraki. 2003. Efficacy of Thai medicinal plant extracts against herpes simplex virus type1 infection *in vitro* and *in vivo*. **Antiviral Res**. 60: 175-180.

Liu, X., M. Zhao, J. Wang, B. Yang and Y. Jiang. 2008. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. **J. Food Com. Anal**. 21: 219-228.

Loizzo, M.R., R. Tundis, U.G. Chandrika, A.M. Abeysekera, F. Menichini and N.G. Frega. 2010. Antioxidant and antibacterial activities on foodborne pathogens of *Artocarpus heterophyllus* Lam. (Moraceae) leaves extracts. **J. Food Sci**. 75: M291-M295.

López, J., N. Zhi., L.A. Carbonell., J.A. Alvarez and V. Kuri. 2005. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. **Meat Sci.** 69: 371-380.

López, A.S., E. Palou and A.L. Malo. 2007. Susceptibility of foodborne bacteria to binary combinations of antimicrobials at selected a_w and pH. **J. Appl. Microbiol**. 102: 486-497.

Luo, W., M. Zhao, B. Yang, J. Ren, G. Shen and G. Rao. 2011. Antioxidant and antiproliferative capacities of phenolics purified from *Phyllanthus emblica* L. fruit. **Food Chem**. 126: 277-282.

Lück, E and M. Jager. 1997. **Antimicrobial food additives: Characteristics, uses, effects**. 2nd. Springer-Verlag, New York.

Machado, T.B., A.V. Pinto, M.C.F.R. Pinto, I.C.R. Leal, M.G. Silva, A.C.F. Amaral, R.M. Kuster and K.R. Netto-dosSantos. In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analoques, against methicillin-resistant *Staphylococcus aureus*. **Int. J. Antimicrobial Agents** 21: 279-284.

Maisuthisakul, P., M. Suttajit and R. Pongsawatmanit. 2007a. Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. **Food Chem**. 100: 1409-1418.

Maisuthisakul, P., R. Pongsawatmanit and M.H. Gordon. 2007b. Characterization of the phytochemicals and antioxidant properties of extracts from Teaw (*Cratoxylum formosum* Dyer). **Food Chem**. 100: 1620-1629.

Mann, C.M., S.D. Cox and J.L. Markham. 2000. The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). **Lett. Appl. Microbiol**. 30: 294-297.

Massilia, R.M.R., J.M. Melgar and O.M. Belloso. 2009. Antimicrobial activity of malic acid against *Listeria monocytogenes, Salmonella* Enteritidis and *Escherihia coli* O157:H7 in apple, pear and melon juices. **Food Control** 20: 105-112.

Matsuda, H., N. Tomohiro, K. Hiraba, S. Harima, S. Ko, K. Matsuo, M. Yoshikawa and M. Kubo. 2011. Study on anti-oketsu activity of rhubarb II. Anti-allergic effects of stilbene components from *Rhei undulati* rhizome (dried rhizome of *Rheum undulatum* cultivated in Korea). **Biol. Pharm. Bull.** 24: 264-267.

Mayachiew, P and S. Devahastin. 2008. Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts. **LWT**. 41: 1153-1159.

McCarthy, T.L., J.P. Kerry, J.F. Kerry, P.B. Lynch and D.J. Buckley. 2001. Assessment of the antioxidant potential of natural food and plant extracts in fresh and previously frozen pork patties. **Meat Sci**. 57: 177-184.

Miceli, N., A. Trovato, P. Dugo, F. Cacciola, P. Donato, A. Marino,V. Bellinghieri, T.M.L. Barbera, A. Guvenc and M.F. Taviano. 2009. Comparative analysis of flavonoid profile, antioxidant and antimicrobial activity of the berries of *Juniperus communis* L. var. *communis* and *Juniperus communis* L. var. *saxatilis* Pall. from Turkey. **J. Agric. Food Chem.** 57: 6570-6577.

Michiels, J., J. Missotten, D. Fremaut, S.D. Smet and N. Dierick. 2007. *In vitro* dose-response of carvacrol, thymol, eugenol and trans-cinnamaldehyde and interaction of combinations for the antimicrobial activity against the pig gut flora. **Livestock Sci**. 109: 157-160.

Mielnik, M.B., E. Olsen, G.Vogt, D. Adeline and G. Skrede. 2006. Grape seed extract as antioxidant in cooked, cold stored turkey meat. **LWT**. 39: 191-198.

Moleyar, V and P. Narasimham. 1992. Antibacterial activity of essential oil components. **Int. J. Food Microbiol**. 16: 337-342.

Moreira, M.R., A.G. Ponce, C.E. del Valle and S.I. Roura. 2005. Inhibitory parameters of essential oils to reduce a foodborne pathogen. **LWT.** 38: 565-570.

_____, _____, _____ and _____. 2007. Effects of clove and tea tree oils on *Escherichia coli* O157:H7 in blanched spinach and minced cooked beef. **J. Food Process. Preserve**. 31: 379-391.

Munoz, R.E. E.E. Bargiota and P.M. Davidson. 1987. Effect of selected phenolic compounds on the membrane-bound adenosine triphosphatase of *Staphylococcus aureus*. **Food Microbiol**. 4: 239-249.

Muñoz, M., L. Guevara, A. Palop, J. Tabera and P.S. Fernández. 2009. Determination of the effect of plant essential oils obtained by supercritical fluid extraction on the growth and viability of *Listeria monocytogenes* in broth and food systems using flow cytometry. **LWT**. 42: 220-227.

Mytle, N., G.L. Anderson, M.P. Doyle and M.A. Smith. 2006. Antimicrobial activity of clove (*Syzgium aromaticum*) oil in inhibiting *Listeria monocytogenes* on chicken frankfurters. **Food Control** 17: 102-107.

Nagulendran, K.R., S. Velavan, R. Mahesh and V.H. Begum. 2007. In vitro antioxidant activity and total polyphenolic content of *Cyperus rotundus* Rhizomes. **E-J. Chem.** 4: 440-449.

Nahak, G and P.K. Sahu. 2010. *In vitro* antioxidative activity of *Azadirachta indica* and *Melia azedavach* leaves by DPPH scavenging assay. **J. Am. Sci.** 6: 123-128.

Nalina, T and Z.H.A. Rahim. 2007. The crude aqueous extract of *Piper betle* L. and its antibacterial effect towards *Streptococcus mutans*. **Am. J. Biotechnol. Biochem.** 3: 10-15.

Nanasombat, S and P. Lohasupthawee. 2005. Antibacterial activity of crude ethanolic extracts and essential oils of spices against salmonellae and other enterobacteria. **KMITL. Sci. Tech. J.** 5: 527-538.

_____ and N. Teckchuen. 2009. Antimicrobial, antioxidant and anticancer activities of Thai local vegetables. **J. Med. Plants Res**. 3: 443-449.

Nasar-Abbas, S.M and A.K. Halkman. 2004. Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some foodborne bacteria including pathogens. **Int. J. Food Microbiol**. 97: 63-69.

Natta, L., K. Orapin, N. Krittika and B. Pantip. 2008. Essential oil from Zingiberaceae for anti foodborne bacteria. **Int. Food Res. J.** 15: 337-346.

Naz, S., S. Jabeen, S. Ilyas, F. Manzoor, F. Aslam and A. Ali. 2010. Antibacterial activity of *Curcuma longa* varieties against different strains of bacteria. **Pak. J. Bot.** 42: 455-462.

Negi, P.S and Jayaprakasha, G.K. 2003. Antioxidant and antibacterial activities of *Punica granatum* peel extracts. **J. Food Sci**. 68: 1473-1477.

Nes, I.F and T. Eklind. 1983. The effect of parabens on DNA, RNA and protein synthesis in *Escherichia coli* and *Bacillus subtilis*. **J. Appl. Bacteriol**. 54: 237-242.

Ngarmsak, M., P. Delaquis, P. Toivonen, T. Ngarmsak, B. Ooraikui and G. Mazza. 2006. Antimicrobial activity of vanillin against spoilage microorganisms in stored fresh-cut mangoes. **J. Food Prot**. 69: 1724-1727.

Nikkhah, E., M. Khayami and R. Heidari. 2009. *In vitro* antioxidant activity of berry (*Morus alba* var. *nigra*). **Int. J. Plant Prod.** 3: 15-18.

Nostro, A., M.P. Germanò, V. Angelo, A. Marino and M.A. Cannatelli. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. **Lett. Appl. Microbiol.** 30: 379-384.

Oliveira, C.E.V., T.L.M. Stamford, N.J.G. Neto and E.L. Souza. 2010. Inhibition of *Staphylococcus aureus* in broth and meat broth using synergies of phenolics and organic acids. **Int. J. Food Microbiol**. 137: 312-316.

Okoh, O.O., A.P. Sadimenko and A.J. Afolayan. 2010. Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. **Food Chem**. 120: 308-312.

Okonogi, S., C Duangrat, S. Anuchpreeda, S. Tachakitirungrod and S. Chowwanapoonpohn. 2007. Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. **Food Chem**. 103: 839-846.

Okoro, I.O., A. Osagie and E.O. Asibor. 2010. Antioxidant and antimicrobial activities of polyphenols from ethnomedicinal plants Nigeria. **Afr. J. Biotechnol.** 9: 2989-2993.

Oonmetta-aree, J., T. Suzuki, P. Gasaluck and G. Eumkeb. 2006. Antimicrobial properties and action of galangal (*Alpinia galangal* Linn.) on *Staphylococcus aureus*. **LWT.** 39: 1214-1220.

Oroojalian, F., R. Kermanshahi, M. Azizi and M.R. Bassami. 2010. Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. **Food Chem**. 120: 765-770.

Othman, M., H.S. Loh, C. Wiart, T.J. Khoo, K.H. Lim and K.N. Ting. 2011. Optimal methods for evaluating antimicrobial activities from plant extracts. **J. Microbiol Methods** 84: 161-166.

Owen, R.J and E.A. Palombo. 2007. Anti-listerial activity of ethanolic extracts of medicinal plants, *Eremophila alternifolia* and *Eremophila duttonii*, in food homogenates and milk. **Food Control** 18: 387-390.

Owoyale, J.A., G.A. Olatunji and S.O. Oguntoye. 2005. Antifungal and antibacterial activities of an alcoholic extract of *Senna alata* leaves. **J. Appl. Sci. Environ. Mgt.** 9: 105-107.

Ozen, T., I. Demirtas and H. Aksit. 2011. Determination of antioxidant activities of various extracts and essential oil compositions of *Thymus praecox* subsp. *Skorpilii* var. *skorpilii*. **Food Chem**. 124: 58-64.

Palakawong, C., P. Sophanodora, S. Pisuchpen and S. Phongpaichit. 2010. Antioxidant and antimicrobial activities of crude extracts from mangosteen (*Garcinia mangostana* L.) parts and some essential oils. **Int. Food Res. J.** 17: 583-589.

Pan, Y., J. Zhu, H. Wang, X. Zhang, Y. Zhang, C. He, X. Ji and H. Li. 2007. Antioxidant activity of ethanolic extract of *Cortex fraxini* and use in peanut oil. **Food Chem**. 103: 913-918.

Pandit, V.A and L.A. Shelef. 1994. Sensitivity of *Listeria monocytogenes* to rosemary (*Rosmarinus officinalis* L.). **Food Microbiol**. 11: 57-63.

Panichayupakaranant, P., S. Tewtrakul and S. Yuenyongsawad. 2010. Antibacterial, anti-inflammatory and anti-allergic activities of standardized pomegranate rind extract. **Food Chem**. 123: 400-403.

Parekh, J and S. Chanda. 2008. *In vitro* antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. **Afr. J. Biotechnol.** 7: 4349-4353.

Patel, J.D., A.K. Shrivastava and V. Kumar. 2009. Evaluation of some medicinal plants used in traditional wound healing preparations for antibacterial property against some pathogenic bacteria. **J. Clin. Immunol. Immunopathol. Res.** 1: 7-12.

Pearson, L.J. and E.H. Marth. 1990. Behavior of *Listeria monocytogenes* in the presence of cocoa, carrageenan and sugar in a milk medium incubated with and without agitation. **J. Food Prot**. 53: 30-37.

Peter, K.V. 2004. **Handbook of herbs and spices**. CRC Press, Cambridge.

Phattayakorn, K and P. Wanchaitanawong. 2009. Antimicrobial activity of Thai herb extracts against coconut milk spoilage microorganisms. **Kasetsart J**. 43: 752-759.

Phillips, C.A and J. Duggan. 2002. The effect of temperature and citric acid, alone, and in combination with nisin, on the growth of *Arcobacter butzleri* in culture. **Food Control** 13: 463-468.

Phomkaivon, N and V. Areekul. 2009. Screening for antioxidant activity in selected Thai wild plants. **As. J. Food Ag-Ind**. 2: 433-440.

Pokorny, J., N. Yanishlieva and M. Gordon. 2001. **Antioxidants in food**. CRC Press, Cambridge.

Pol, I.E. and E.J. Smid. 1999. Combined action of nisin and carvacrol on *Bacillus cereus* and *Listeria monocytogenes*. **Lett. Appl. Microbiol**. 29: 166-170.

Politeo, O., M. Jukic and M. Milos. 2007. Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil. **Food Chem**. 101: 379-385.

Ponce, A.G., R. Fritz, C. Valle and S.I. Roura. 2003. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. **LWT**. 36: 679-684.

Prior, R.L., X. Wu and K. Schaich. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. **J. Agric. Food Chem**. 53: 4290-4302.

Proestos, C., N. Chorianopoulos, G.J.E. Nychas and M. Komaitis. 2005. RP-HPLC Analysis of the phenolic compounds of plant extract. Investigation of their antioxidant capacity and antimicrobial activity. **J. Agric. Food Chem**. 53: 1190-1195.

Pundir, R.K and P. Jain. 2010. Comparative studies on the antimicrobial activity of black pepper (*Piper nigrum*) and turmeric (*Curcuma longa*) extracts. **Int. J. appl. Bio. Pharm. Technol.** 1: 492-501.

Radhika, P and K.R. Lakshmi. 2010. Antimicrobial activity of the chloroform extracts of the root and the stem of *Andrographis paniculata* Nees. **Int. Res. J. Microbiol.** 1: 037-039.

Rauha, J.P., S. Remes, M. Heinonen, A. Hopia, M. Kähkönen, T. Kujala, K. Pihlaja, H. Vuorela and P. Vuorela. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. **Int. J. Food Microbiol**. 56: 3-12.

Rehman, Z. 2006. Citrus peel extract – A natural source of antioxidant. **Food Chem**. 99: 450-454.

Rimando, A.M., W. Kalt, J.B. Magee, J. Dewey and J.R. Ballington. 2004. Resveratrol, pterostilbene, and piceatannol in *Vaccinium* berries. **J. Agric. Food Chem.** 52: 4713-4719.

Sallam, Kh.I., M. Ishioroshi and K. Samejima. 2004. Antioxidant and antimicrobial effects of garlic in chicken sausage. **LWT.** 37: 849-855.

Sarikurkcu, C., K. Arisoy, B. Tepe, A. Cakir, G. Abali and E. Mete. 2009. Studies on the antioxidant activity of essential oil and different solvent extracts of *Vitex agnus castus* L. fruits from Turkey. **Food Chem. Toxicol**. 47: 2479-2483.

Sawangjaroen, N and K. Sawangjaroen. 2005. The effects of extracts from anti-diarrheic Thai medicinal plants on the in vitro growth of the intestinal protozoa parasite: *Blastocytis hominis*. **J. Ethnopharm**. 98: 67-72.

Scifò, G.O., C.L. Randazzo, C. Restuccia, G. Fava and C. Caggia. 2009. *Listeria innocua* growth in fresh cut mixed leafy salads packaged in modified atmosphere. **Food Control** 20: 611-617.

Sebranek, J.G., V.J.H. Sewalt, K.L. Robbins and T.A. Houser. 2005. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. **Meat Sci**. 69: 289-296.

Seow, C.C. and C.N. Gwee. 1997. Coconut milk: chemistry and technology. **Int. J. Food Sci. Technol**. 32: 189-201.

Sestili, P., A. Guidarelli, M. Dacha and O. Cantoni. 1998. Quercetin prevents DNA single strand breakage and cytotoxicity caused by tertbutylhydroperoxide: free radical scavenging versus iron chelating mechanism. **Free Radic. Biol. Med**. 25: 196–200.

Shan, B., Y. Cai, J.D. Brooks and H. Corke. 2007a. The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. **Int. J. Food Microbiol**. 117: 112-119.

_____, _____, M. Sun and H. Corke. 2005. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. **J. Agric. Food Chem**. 53: 7749-7759.

Shan, B., Y. Cai, J.D. Brooks and H. Corke. 2009. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. **J. Sci. Food Agric**. 89: 1879-1885.

Shin, I.S., H. Masuda and K. Naohide. 2004. Bactericidal activity of wasabi (*Wasabia japonica*) against *Helicobacter pylori*. **Int. J. Food Microbiol**. 94: 255-261.

Si, W., J. Gong, R. Tsao, M. Kalab, R. Yang and Y. Yin. 2006. Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. **J. Chromatogr. A** 1125: 204-210.

Sikkema, J., J.A.M. Bont and B. Poolman. 1994. Interactions of cyclic hydrocarbons with biological membranes. **J. Biol. Chem**. 269: 8022-8028.

Simuang, J., N. Chiewchan and A. Tansakul. 2004. Effects of fat content and temperature on the apparent viscosity of coconut milk. **J. Food Eng**. 64: 193-197.

Singh, R., R. Chandra, M. Bose and P.M. Luthra. 2002. Antibacterial activity of *Curcuma longa* rhizome extract on pathogenic bacteria. **Current Sci.** 83: 737-740.

Singh, A., R.K. Singh, A.K. Bhunia and N. Singh. 2003. Efficacy of plant essential oils as antimicrobial agents against *Listeria monocytogenes* in hotdogs. **LWT**. 36: 787-794.

Singh, R., B. Singh, S. Singh, N. Kumar, S. Kumar and S. Arora. 2010. Umbelliferone – An antioxidant isolated from *Acacia nilotica* (L.) Willd. Ex. Del. **Food Chem**. 120: 825-830.

Singleton, V.L and J.A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **Am. J. Enol. Vitic.** 16: 144-158.

Siripongvutikorn, S., P. Thummaratwasik and Y. Huang. 2005. Antimicrobial and antioxidation effects of Thai seasoning, *Tom-Yum*. **LWT**. 38: 347-352.

Siriwatanametanon, N., B.L. Fiebich, T. Efferth, J.M. Prieto and M. Heinvich. 2010. Traditionally used Thai medicinal plants: In vitro anti-inflammatory, anticancer and antioxidant activities. **J. Ethnopharm**. 130: 196-207.

Sithisarn, P., R. Supabphol and W. Gritsanapan. 2005. Antioxidant activity of Siamese neem tree (VP1209). **J. Ethnolpharm**. 99: 109-112.

_____, _____ and _____. 2006. Comparison of free radical scavenging activity of Siamese neem tree (*Azadirachta indica* A. Juss var. *siamensis* Valeton) leaf extracts prepared by different methods of extraction. **Med. Prinpc. Pract.** 15: 219-222.

Smith-Palmer, A., J. Stewart and L. Fyfe. 2001. The potential application of plant essential oils as natural food preservatives in soft cheese. **Food Microbiol**. 18: 463-470.

Souza, E.L., T.L.M. Stamford, E.O. Lima, V.N. Trajano and J.M.B. Filho. 2005. Antimicrobial effectiveness of spices: an approach for use in food conservation systems. **Braz. Arc. Bio. Technol**. 48: 549-558.

Springfield, E.P., G. Amabeoku, F. Weitz, W. Mabusela and Q. Johnson. 2003. An assessment of two Carpobrotus species extracts as potential antimicrobial agents. **Phytomedicine.** 10: 434-439.

Sukanya, S.L., J. Sudisha, P. Hariprasad, S.R. Niranjana, H.S. Prakash and S.K. Fathima. 2009. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. **Afr. J. Biotechnol.** 8: 6677-6682.

Surveswaran, S., Y.Z. Cai, H. Corke and M. Sun. 2007. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. **Food Chem.** 102: 938-953.

Tachakittirungrod, S., S. Okonogi and S. Chowwanpoonpohn. 2007. Study on antioxidant activity of certain plants in Thailand: mechanism of antioxidant action of guava leaf extract. **Food Chem**. 103: 381-388.

Tajkarimi, M.M., S.A. Ibrahim and D.O. Cliver. 2010. Antimicrobial herb and spice compounds in food. **Food Control** 21: 1199-1218.

Tangsuphoom, N and J.N. Coupland. 2009. Effect of surface active stabilizers on the surface properties of coconut milk emulsions. **Food Hydrocolloids** 23: 1801-1809.

Tassou, C.C and G.J.E Nychas. 1995. Inhibition of *Salmonella* Enteritidis by oleuropein in broth and in a model food system. **Lett. Appl. Microbiol**. 20: 120-124.

Tassou, C.C., E.H. Drosinos and G.J.E. Nychas. 1995. Effects of essential oil from mint (*Mentha piperita*) on *Salmonella* Enteritidis and *Listeria monocytogenes* in model food systems at 4°C and 10°C. **J. Appl. Bacteriol**. 78: 593-600.

Tawaha, K., F.Q. Alali, M. Gharaibeh, M. Mohammad and T. El-Elimat. 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. **Food Chem**. 104: 1372-1378.

Taweechaisupapong, S., S. Singhara, P. Lertsatitthanakorn and W. Khunkitti. 2010. Antimicrobial effects of *Boesenbergia pandurata* and *Piper sarmentosum* leaf extracts on Planktonic cells and biofilm of oral pathogens. **Pak. J. Pharm. Sci.** 23: 224-231.

Thani, W., O. Vallisuta, P. Siripong and N. Ruangwises. 2010. Anti-proliferative and antioxidative activities of Thai noni/Yor (*Morinda citrifolia* Linn.) leaf extract. **Southeast Asian J. Trop. Med. Public Health.** 41: 482-489.

Trabelsi, N., W. Megdiche, R. Ksouri, H. Falleh, S. Oueslati, B. Soumaya, H. Hajlaoui and C. Abdelly. 2010. Solvent effects on phenolic contents and biological activities of the halophyte *Limoniastrum monopetalum* leaves. **LWT**. 43: 632-639.

Ultee, A., M.H.J. Bennink and R. Moezelaar. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the foodborne pathogen *Bacillus cereus*. **Appl. Environ. Microbiol**. 68(4): 1561-1568.

Wang, S.C and J.H. Hart. 1983. Heartwood extractives of *Machura pomifera* and their role in decay resistance. **Wood Fiber Sci.** 15: 290-301.

Wang, M., J.E. Simon, I.F. Aviles, K. He, Q.Y. Zheng and Y. Tadmor. 2003. Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). **J. Agric. Food Chem**. 51: 601-608.

Wang, A.Y., M.Y. Zhou and W.C. Lin. 2011. Antioxidative and anti-inflammatory properties of *Citrus sulcata* extracts. **Food Chem**. 124: 958-963.

Wannes, W.A., B. Mhamdi, J. Sriti, M.B. Jemia, O. Ouchikh, G. Hamdaoui, M.E. Kchouk and B. Marzouk. 2010. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italic* L.) leaf, stem and flower. **Food Chem. Toxicol**. 48: 1362-1370.

Wannissorn, B., S. Jarikaseam, T. Siriwangchai and S. Thubthimthed. 2005. Antibacterial properties of essential oils from Thai medicinal plants. **Fitoterapia**. 76: 233-236.

Wen, A., P. Delaquis, K. Stanich and P. Toivonen. 2003. Antilisterial activity of selected phenolic acids. **Food Microbiol**. 20: 305-311.

Wojdylo, A., J. Oszmiański and R. Czemerys. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. **Food Chem**. 105: 940-949.

Wong, P.Y.Y and D.D. Kitts. 2006. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts. **Food Chem**. 97: 505-515.

Wong, S.P., L.P. Leong and J.H.W. Koh. 2006. Antioxidant activities of aqueous extracts of selected plants. **Food Chem**. 99: 775-783.

Wong, T.L., L. Hollis, A. Cornelius, C. Nicol, R. Cook and J.A. Hudson. 2007. Prevalence, numbers, and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail meat samples. **J. Food Prot**. 70: 566-573.

Wu, V.C.H., X. Qiu, A. Bushway and L. Harper. 2008. Antibacterial effects of American cranberry (*Vaccinium macrocarpon*) concentrate on foodborne pathogens. **LWT**. 41: 1834-1841.

Yano, Y., M. Satomi and H. Oikawa. 2006. Antimicrobial effect of spices and herbs on *Vibrio parahaemolyticus*. **Int. J. Food Microbiol.** 111: 6-11.

Ye, X.Q., J.C. Chen, D.H. Liu, P. Jiang, J. Shi, S. Xue, D. Wu, J.G. Xu and Y. Kakuda. 2011. Identification of bioactive composition and antioxidant activity in young mandarin fruits. **Food Chem**. 124: 1561-1566.

Yen, G.C and H.Y. Chen. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. **J. Agric. Food Chem**. 43: 27-32.

Yilmaz, Y and R.T. Toledo. 2004. Health aspects of functional grape seed constituents. **Trend Food Sci. Technol**. 15: 422-433.

Yim, N., D.T. Ha, T.N. Trung, J.P. Kim, S. Lee, M. Na, H. Jung, H.S. Kim, Y.H. Kim and K. Bae. 2010. The antimicrobial activity of compounds from the leaf and stem of *Vitis amurensis* against two oral pathogens. **Bioorg. Med. Chem. Lett**. 20: 1165-1168.

Yin, M.C and C.Y. Chao. 2008. Anti-Campylobacter, anti-aerobic and anti-oxidative effects of roselle calyx extract and protocatechuic acid in ground beef. **Int. J. Food Microbiol**. 127: 73-77.

_____ and W.S. Cheng. 2003. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. **Meat Sci**. 63: 23-28.

Yogisha, S and K.A. Raveesha. 2009. *In vitro* antibacterial effect of selected medicinal plant extracts. **J. Nat. Prod.** 2: 64-69.

Yoshiki, M., K. Tsuge, Y. Tsuruta, T. Yoshimaru, K. Koganemaru, T. Sumi, T. Matsui and K. Matsumoto. 2009. Production of new antioxidant compound from mycosporine-like amino acid, prophyra-334 by heat treatment. **Food Chem**. 113: 1127-1132.

Zhang, S., H. Bi and C. Liu. 2007. Extraction of bioactive components from *Rhodiola sachalinensis* under ultra high hydrostatic pressure. **Separation Purification Tech.** 57: 277-282.

Zhang, H., B. Kong, Y.L. Xiong and X. Sun. 2009. Antimicrobial activities of spice extracts against pathogenic and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4°C. **Meat Sci**. 81: 686-692.

Zheng, W and S.Y. Wang. 2001. Antioxidant activity and phenolic compounds in selected herbs. **J. Agric. Food Chem**. 49: 5165-5170.

Zhu, X., H. Zhang, R. Lo and Y. Lu. 2005. Antimicrobial activites of *Cynara scolymus* L. leaf, head, and stem extracts. **J. Food Sci.** 70: M149-M152.

APPENDICES

Appendix A

Media composition and preparation

Ingredients of each medium were dissolved in distilled water and made up to 1000 ml. pH was adjusted to required pH with 1.0 N HCl or 1.0 N NaOH. The media were autoclaved at 121ºC (15 psi) for 15 min.

1. Nutrient broth (NB)

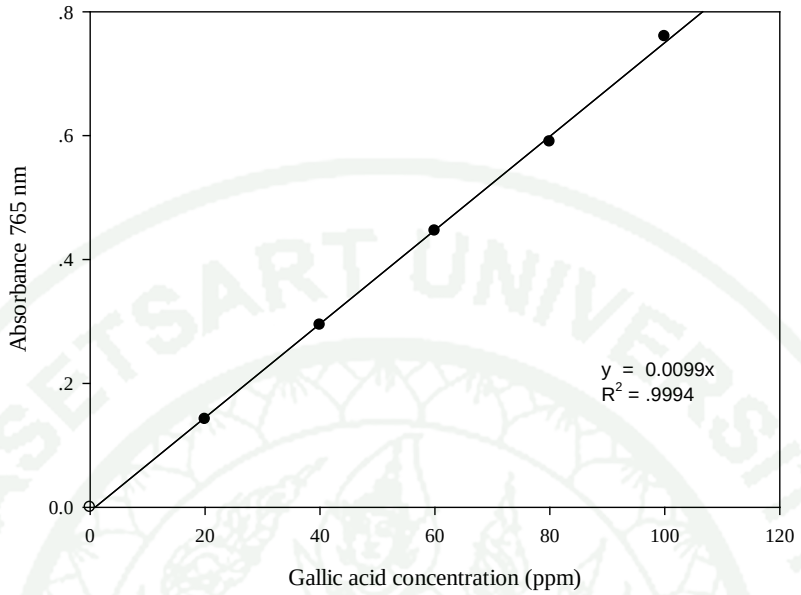
Protease peptone	5.0 g
Beef extract	3.0 g

2. YM medium

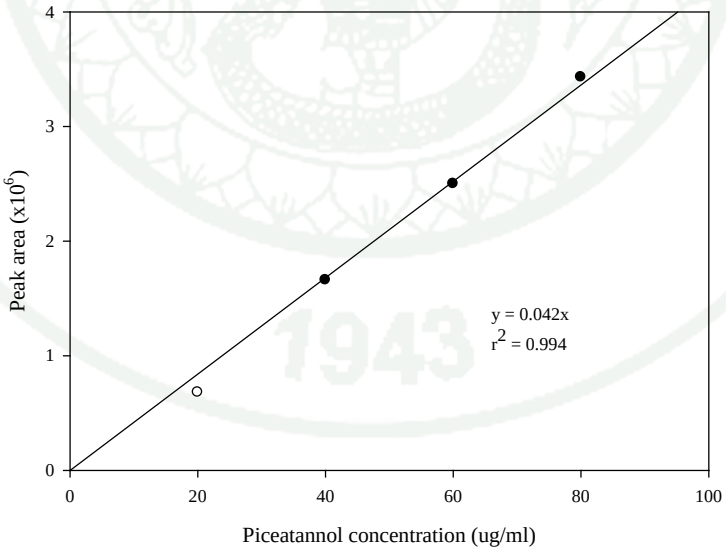
Malt extract	3.0 g
Yeast extract	3.0 g
Peptone	5.0 g
Glucose	10.0 g

Appendix B

Standard curve



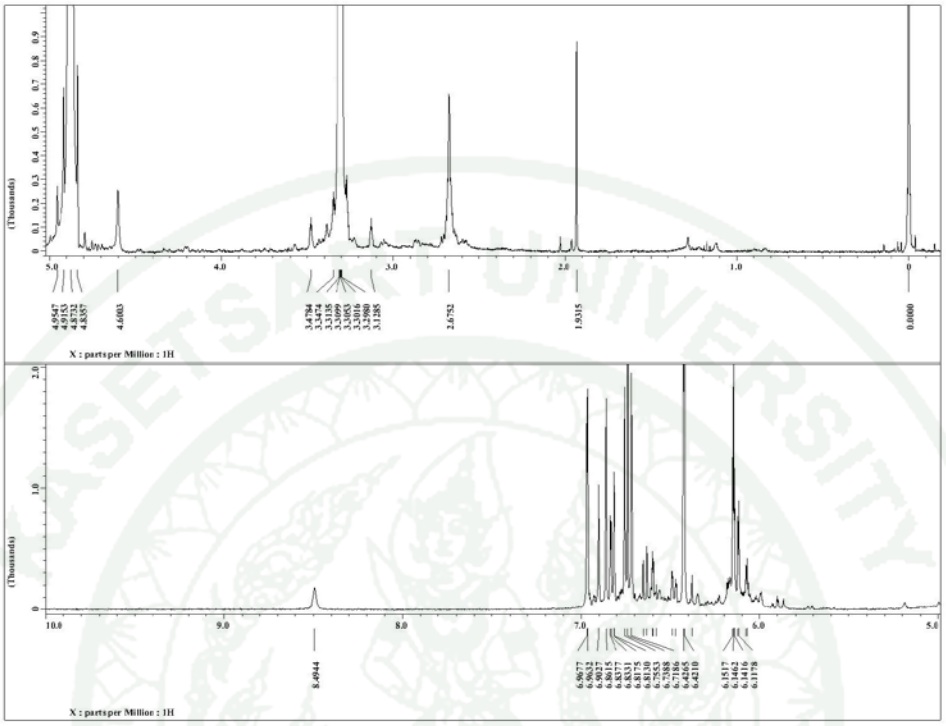
Appendix Figure B1 Standard curve of gallic acid at the absorbance of 765 nm



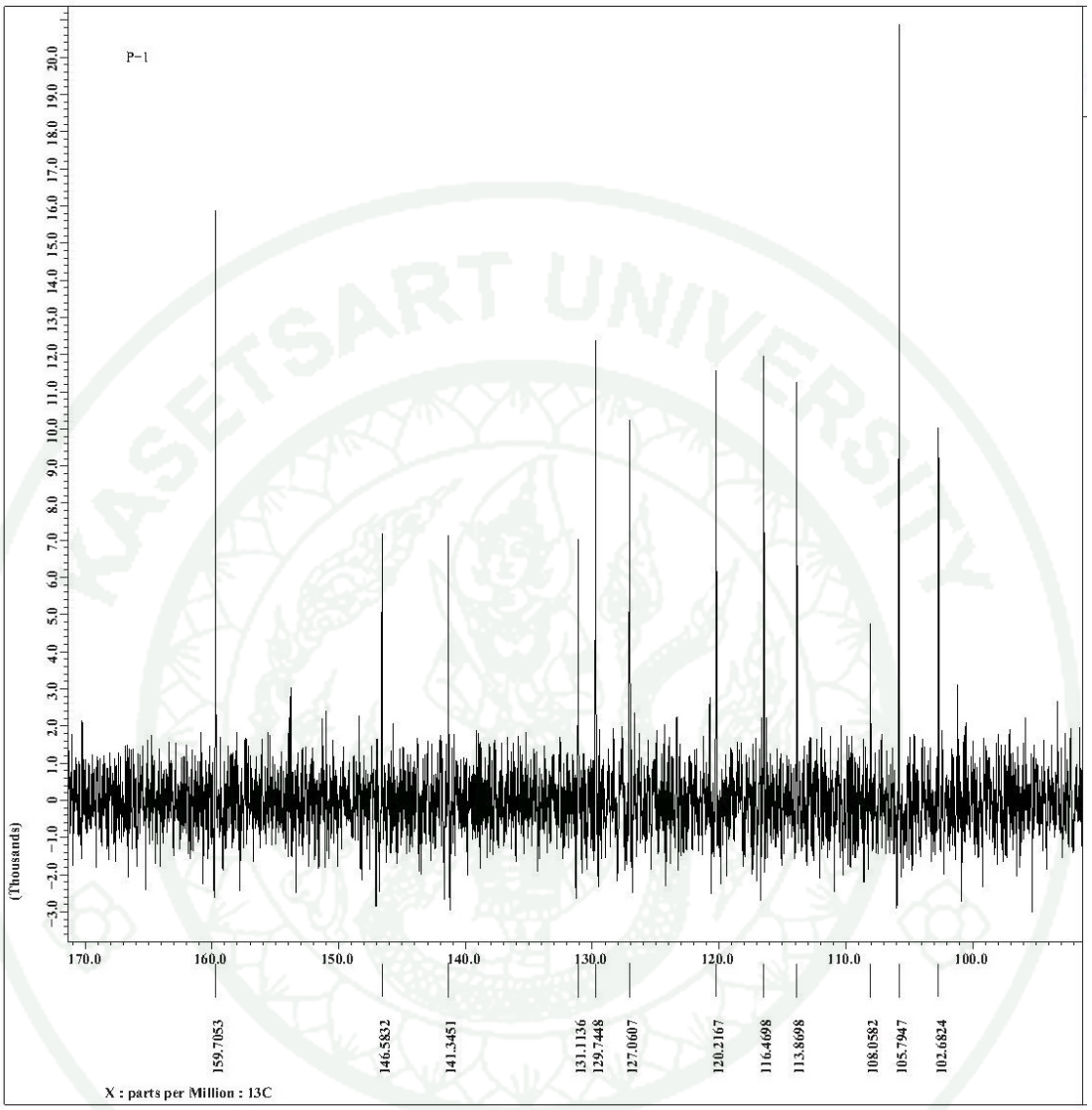
Appendix Figure B2 Standard curve of piceatannol

Appendix C

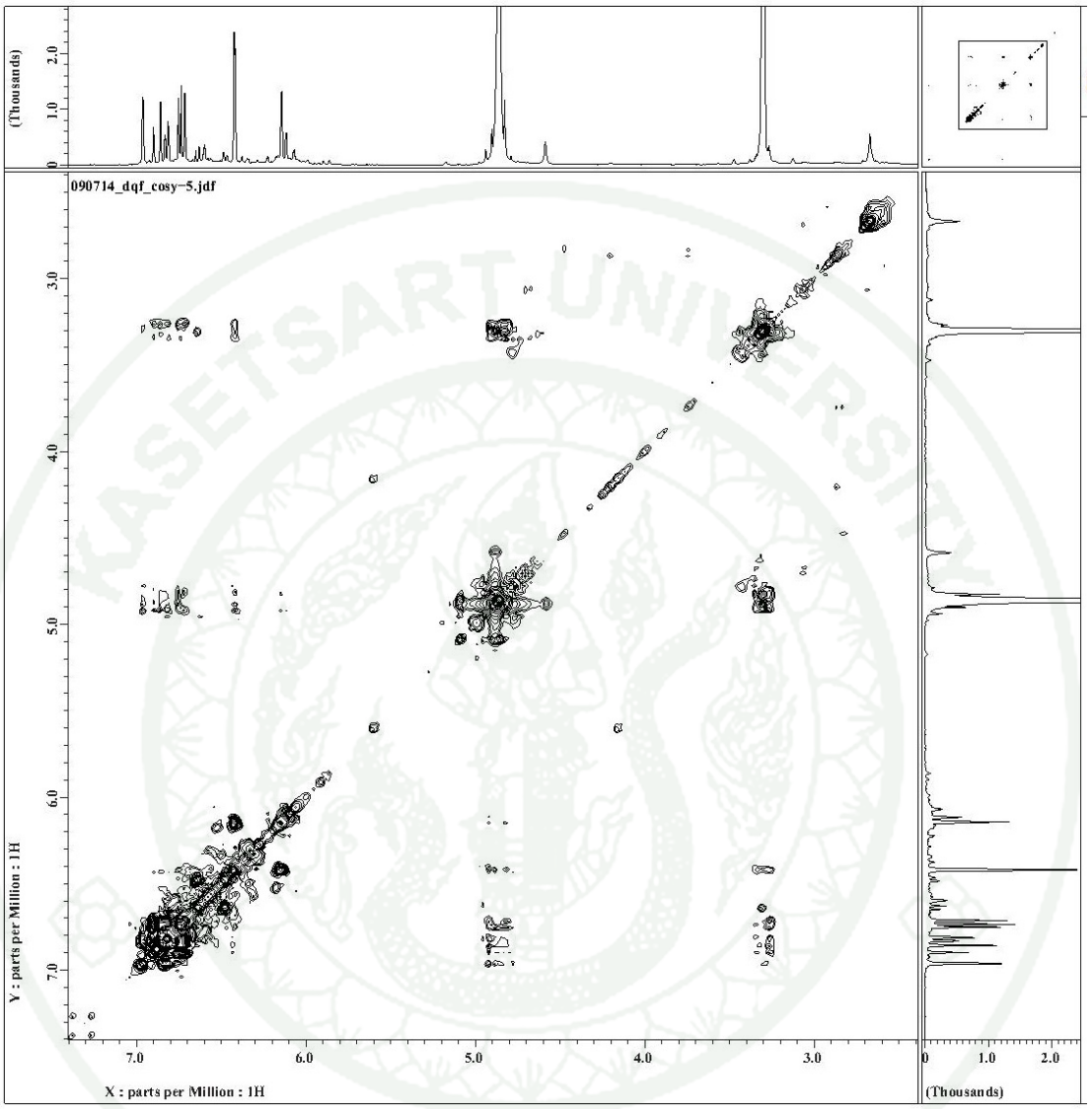
NMR spectrum



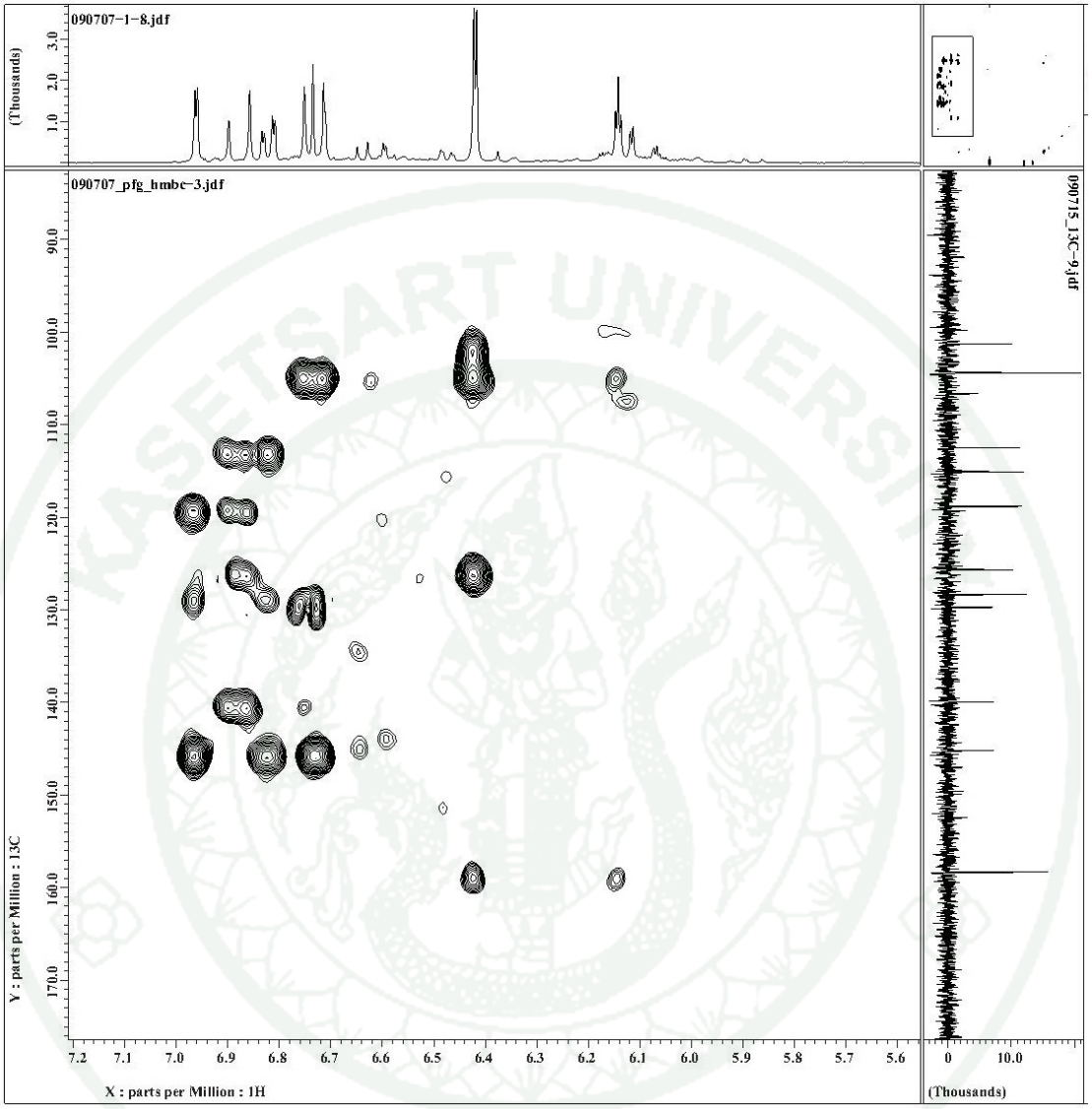
Appendix Figure C1 ^{13}C (100 MHz)- and ^{1}H (400 MHz) spectrum of compound 1 in MeOD-*d4* with tetramenthylsilane as an internal standard



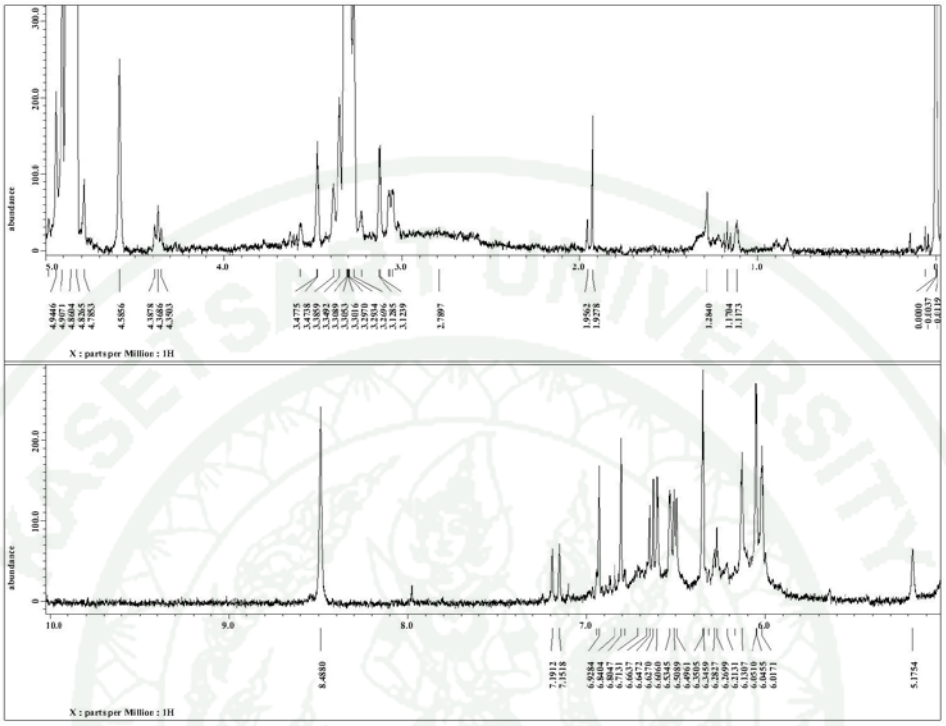
Appendix Figure C2 ^{13}C (100 MHz) spectrum of compound 1 in MeOD-*d4* with tetramenthylsilane as an internal standard



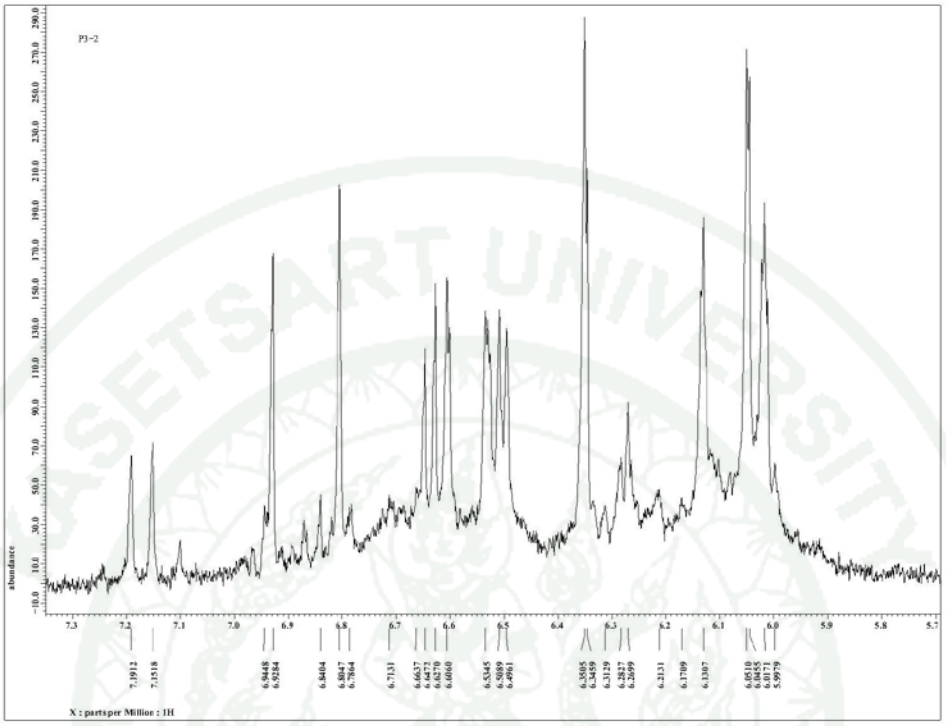
Appendix Figure C3 DQF-COSY spectrum of compound 1 in MeOD-*d4* with tetramenthylsilane as an internal standard



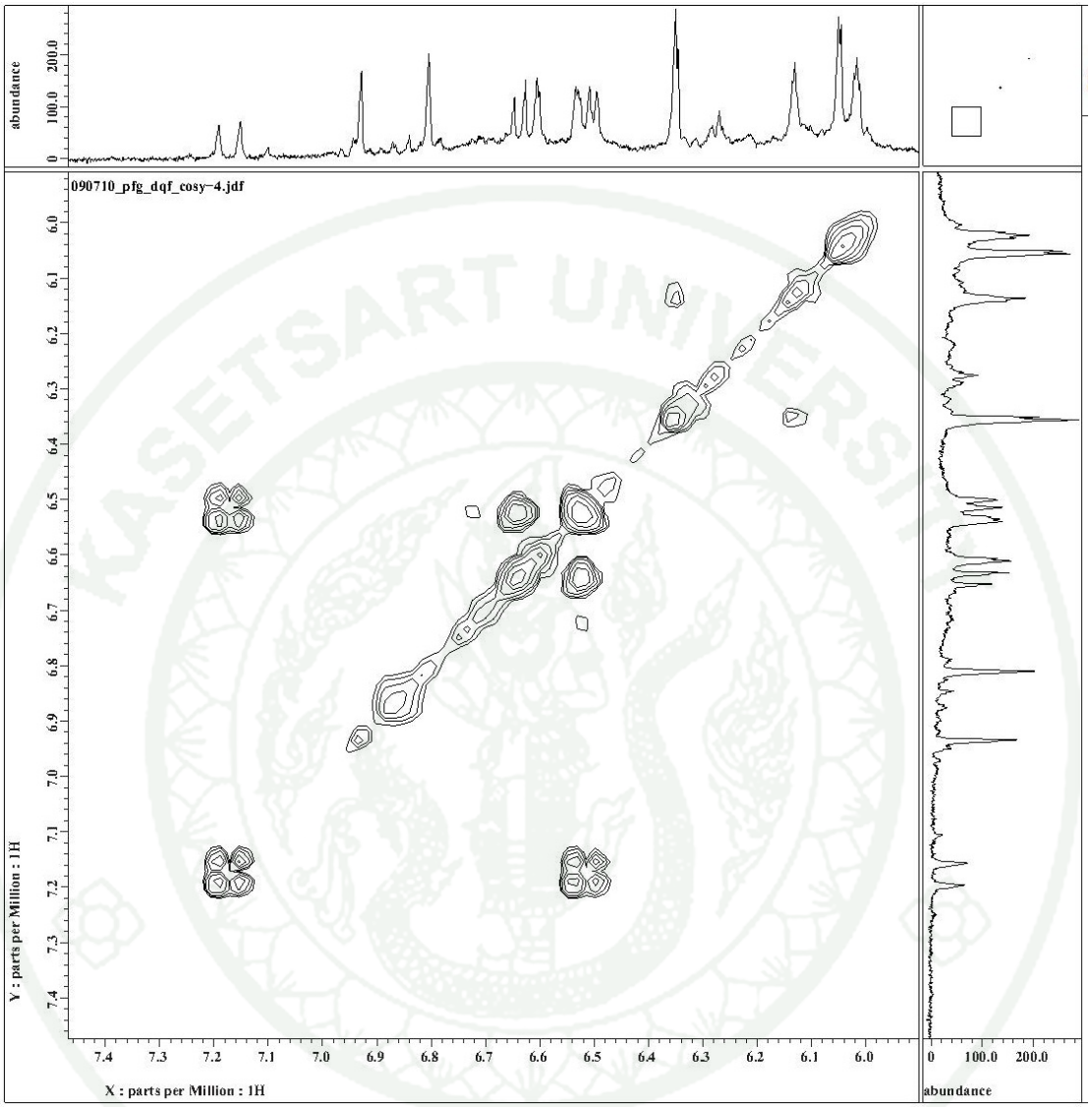
Appendix Figure C4 HMBC spectrum of compound 1 in MeOD-*d4* with tetramenthylsilane as an internal standard



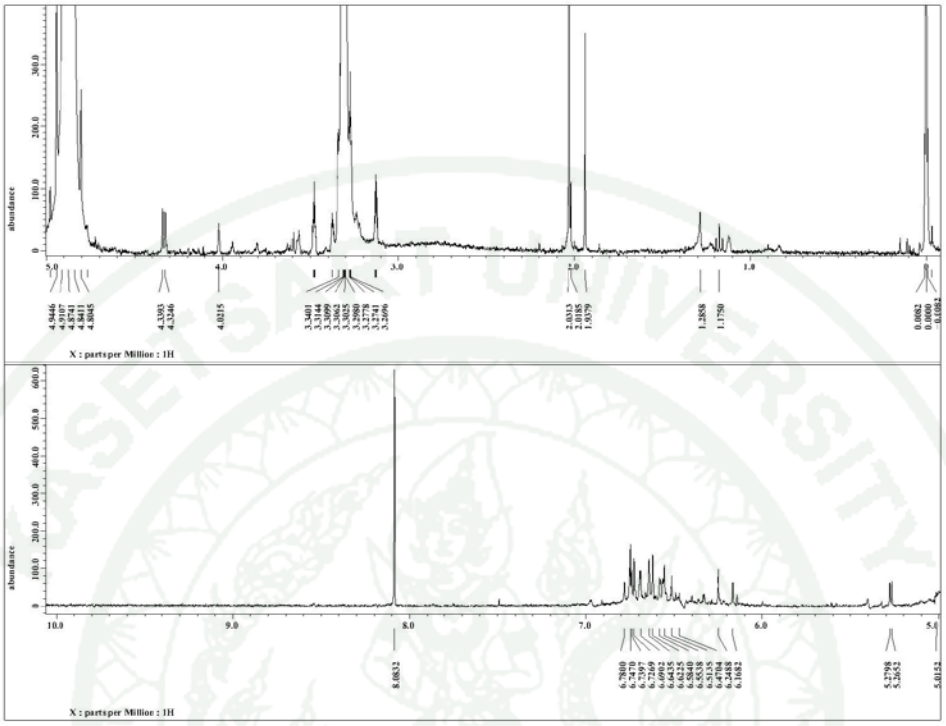
Appendix Figure C5 ^{13}C (100 MHz)- and ^{1}H (400 MHz) spectrum of compound 3 in MeOD-*d4* with tetramenthylsilane as an internal standard



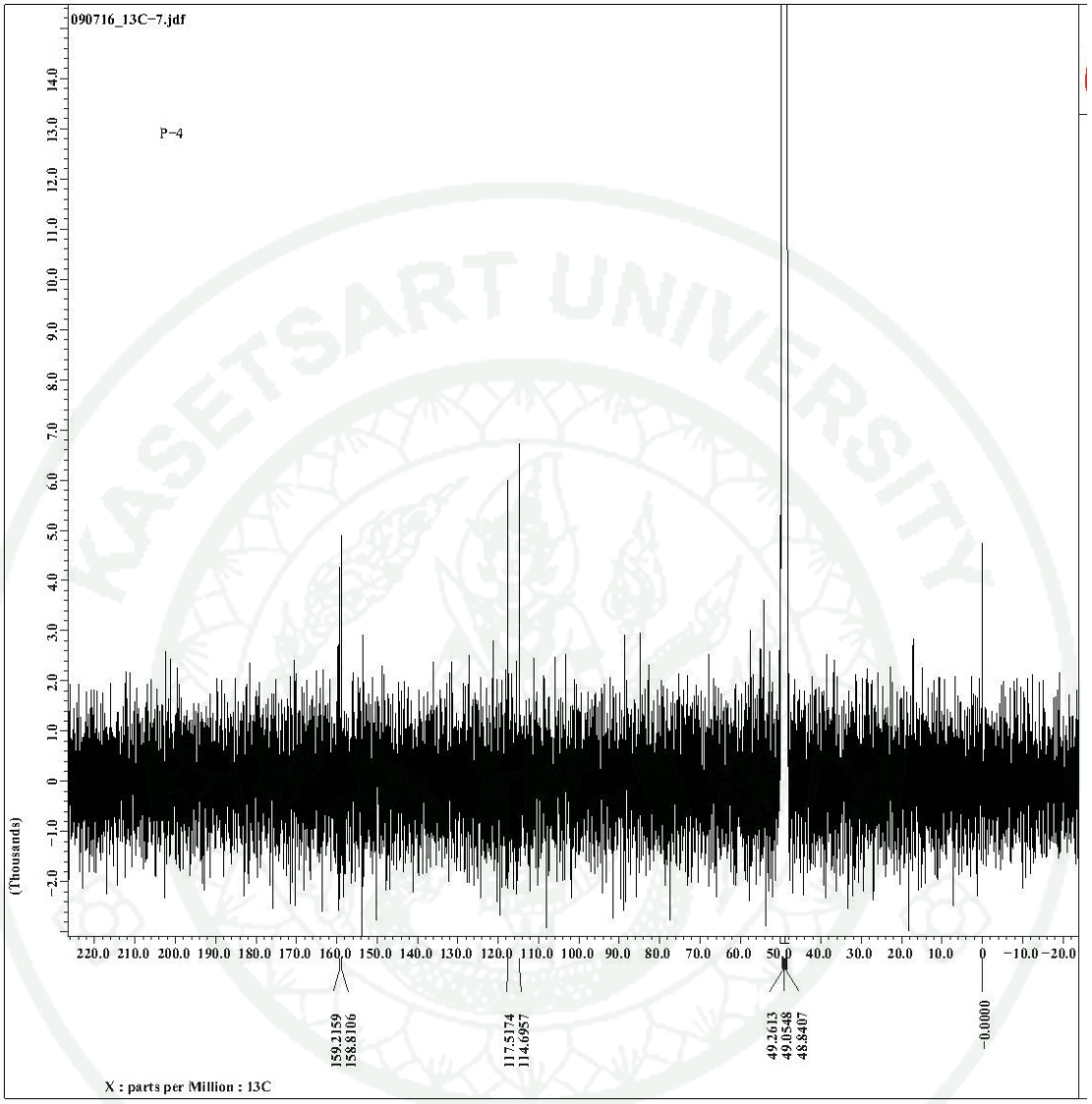
Appendix Figure C6 ^{13}C (100 MHz) spectrum of compound 3 in MeOD-*d4* with tetramenthylsilane as an internal standard



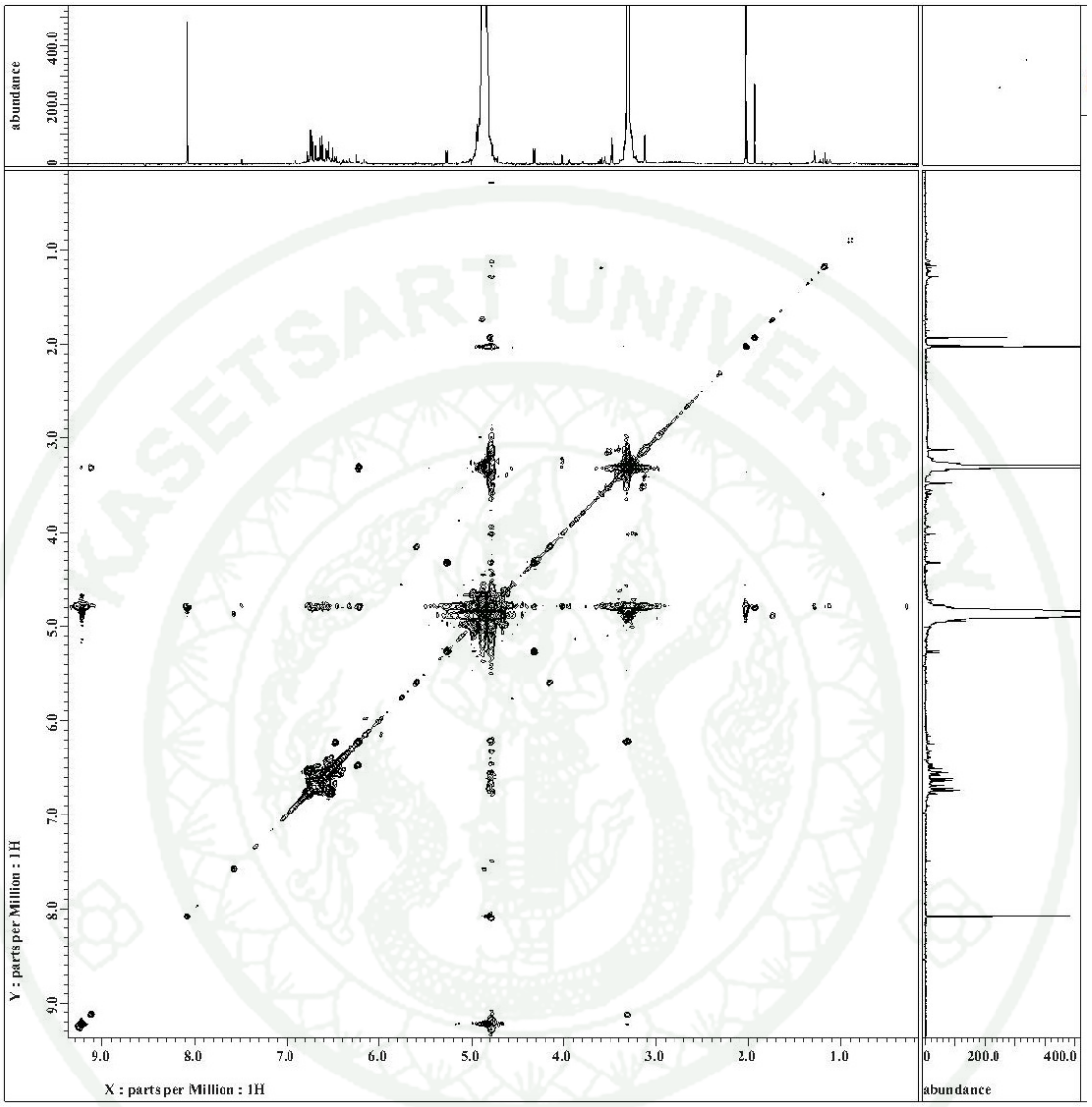
Appendix Figure C7 DQF-COSY spectrum of compound 3 in MeOD-*d4* with tetramenthylsilane as an internal standard



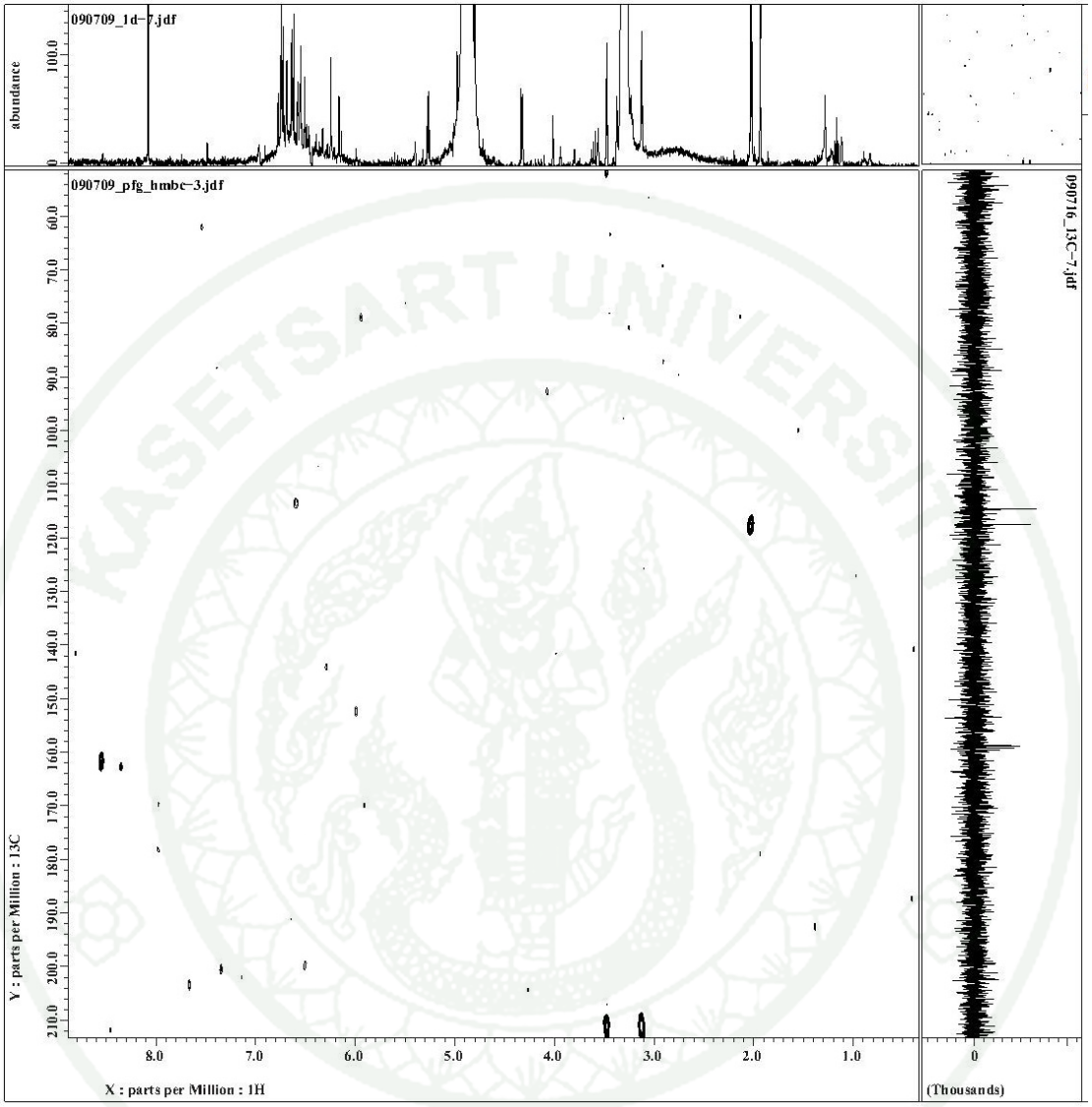
Appendix Figure C8 ^{13}C (100 MHz)- and ^{1}H (400 MHz) spectrum of compound 4 in MeOD-*d4* with tetramenthylsilane as an internal standard



Appendix Figure C9 ^{13}C (100 MHz) spectrum of compound 4 in MeOD-*d4* with tetramenthylsilane as an internal standard



Appendix Figure C10 DQF-COSY spectrum of compound 4 in MeOD-*d4* with tetramenthylsilane as an internal standard



Appendix Figure C11 HMBC spectrum of compound 4 in MeOD-*d4* with tetramenthylsilane as an internal standard

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