

CHAPTER 1

INTRODUCTION

1.1 STATEMENT AND SIGNIFICANCE OF THE PROBLEM

Nowadays most consumers are concerned about using chemical preservatives in foods to prevent lipid oxidation and the growth of spoiling microbes since some of these additives trend to be carcinogenic or can promote the growth of tumors, so that the decrease in chemical preservatives usage is of interest. Lipid oxidation and bacterial contamination are the main factors to determine food quality loss and shelf-life reduction; therefore, delaying lipid oxidation and preventing bacterial cross-contamination are highly important to food processors (Fernandez-Lopez *et al.*, 2005). To inhibit lipid oxidation and microbial growth, the synthetic antioxidants have been widely used, the most common are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylated hydroxyquinone (TBHQ) as potential inhibitors for lipid peroxidation and thereby stabilize fat containing food-stuffs (Löliker, 1991). However, BHA and BHT were found to be anticarcinogenic as well as carcinogenic in experiments or animals and it has been established that they are involved in tumor formation (Botterweck *et al.*, 2000). In recent years, the global demand of natural foods and natural preservatives being consumed for health are preferable. Using plant essential oils and their extracts, the new approach to prevent the proliferation of microorganism or protect food from oxidation, as alternative natural preservative agents is becoming popular due to their medicinal, antimicrobial and antioxidant properties (Pattnaik *et al.*, 1997; Moreno *et al.*, 2002; Sharififar *et al.*, 2007; Al-Fatimi *et al.*, 2007; Yang and Clausen, 2007). The antimicrobial and antioxidant activities of plant essential oils and their extracts are particularly beneficial for utilizations in the food industry such as in raw and processed food preservation, besides those herbal plants and dietary spices are widely cultivated and approved for consumption. Spices are the most popular source of flavor, taste and

aroma. They have been added to food in ancient times, not only as flavoring agents, but also as folk medicine and as food preservatives (Nakatani, 1994). In recent years, spices have been increasingly reported on for their antioxidant and antimicrobial activities (Cantore *et al.*, 2004; Oussalah *et al.*, 2006; Shan *et al.*, 2007; Gulluce *et al.*, 2007; Nedorostova *et al.*, 2009). However, plant essential oils and extracts can perform to both enhance flavor and help preserve food due to their rapid diffusion which has become limited in food industry applications. Besides that, the extracts from spices can be used as antioxidant and antimicrobial agents. Chitosan, the biopolymer obtained by deacetylation of chitin has attracted attention and interest as a potential food preservative of natural origin due to its antibacterial and antifungal activity (Shahidi *et al.*, 1999). Chitosan has also been reported to maintain the quality of fruit and vegetables (Zhao *et al.*, 2004), as a coating agent and an antifungal agent (Jiang *et al.*, 2005). Moreover, for its biopolymer property, chitosan might be used as a matrix to maintain high concentrations of active substances by slowing the migration of the agents away from the food surface such as in the antimicrobial films which were prepared by incorporating bacterial inhibitors into a chitosan matrix functioning as antimicrobial agents (Ouattara *et al.*, 2000). According to both commercial and the public health incentive it is necessary to develop novel natural antioxidant and antimicrobial systems for use in foods and food-related applications. Using the combination of plants extracts and the biopolymer chitosan, it would slowly and in a controlled way, release the active compounds in the plant extracts to prolong their rapid diffusion.

1.2 SPICES

The word “spices” come from the Latin word “species” meaning “specific kind” and in each part of the world spices are called by various names; kruen tet (Thai), rempah (Malaysian and Indonesian), specie (Italian), especerias (Spanish), beharat (Arabic), krooder (Norwegian) or kimem (Ethiopian) e.g. (Uhl, 2000). Spices have been source of flavors in many food industries for a long time, which have use them to stimulate the appetite, add flavor and food texture and create visual appeal in

meals. In some case, spices are even used to mask spoilage or off-flavors in products. Besides that, they have been cultivated for their aromatic, fragrant, pungent or any other desirable properties. The flavor of spices is a combination between taste (mainly due to non-volatile components) and aroma (part of overall volatile components) of which the sensations can be sweet, piney, sour, bitter, spicy, sulfury, earthy and pungent derived from all parts of the plant. The volatile portions of spice extractives are also referred to as essential oils (the particular aroma of spices) while the non-volatile portions include fixed oils, gum, resin, antioxidants and hydrophilic compounds and are usually give the taste. The ratio of volatile to non-volatile portions varies among spices causing similar or different flavors within a genus or even within varieties. Although, spices have two main functions in food products, the first one is the primary function that spices play the role in flavoring food by providing aroma, texture and color and the second one is that spices also act as preservatives, or have nutritional and health functions. Common spices that exhibit antioxidant and antimicrobial activities can provide source of natural preservatives which have become more popular among people who are interested in natural or organic foods and natural healing.

1.2.1 Spices as antioxidants

Lipids are the food molecules that are most susceptible to oxidative free radical reactions due to their polyunsaturated fatty acid, ester of glycerol with fatty acids, triacylglycerols and phospholipids contents. The oxidative reactions of lipids and fat generate peroxides (free radicals) when exposed to air or oxygen and finally become aldehydes and alcohols that give a rancid taste (Fig. 1.1) leading to a decrease quality in processed foods which relates to the number of fatty acid double bonds so that the food processor requires more antioxidants to prevent oxidation and rancidity of various components in foods.

Spices have many components that act as antioxidants to protect cells from the free radicals (Table 1.1). Many studies have reported that phenolic compounds in spices and herbs exhibit antioxidant and pharmaceutical properties (Shan *et al.*, 2005;

Wu *et al.*, 2006). The antioxidative activity of spices and their extracts could be raised from their phenolic compounds which had been found to react with in multiple steps including the initiation, propagation, branching and termination of free radicals. It can inhibit the formation of free radicals from their unstable precursors (initiation) or retard free radical by interrupting the radical chain reaction (propagation and branching) (Ou *et al.*, 2001).

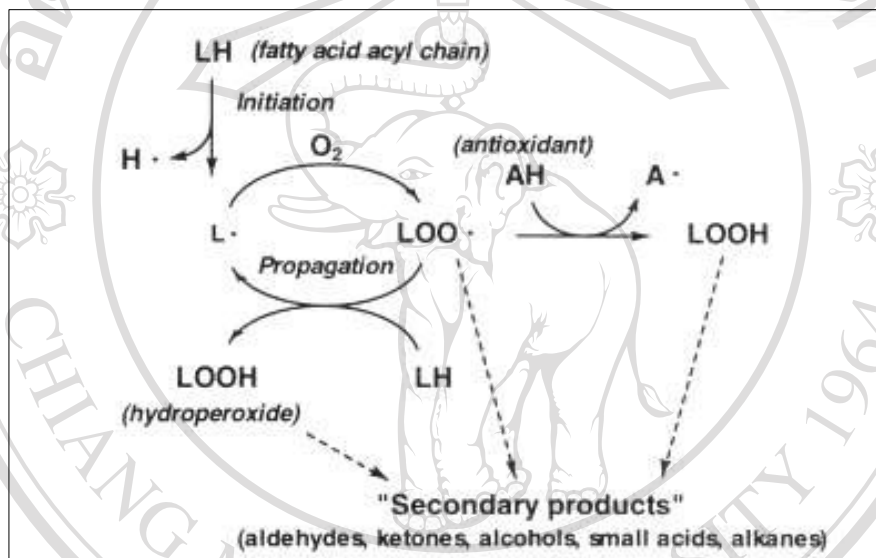


Figure 1.1 Autoxidation of polyunsaturated lipid

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Table 1.1 Antioxidant active chemicals isolated from some of the most common and used spices (USDA, 2003)

Spices (vernacular and scientific names)	Antioxidant compounds
black pepper (<i>Piper nigrum</i>)	ascorbic acid, beta-carotene, camphene, carvacrol, eugenol, myrcene, palmitic acid
caraway (<i>Carum carvi</i>)	beta-carotene, camphene, carvacrol, gamma terpinene, myristicin, quercetin, tannin
chilli pepper (<i>Capsicum frutescens</i>)	alanine, ascorbic acid, beta-carotene, caffeic acid, capsaicin, capsanthin, kaempferol
coriander (<i>Coriandrum sativum</i> L.)	apigenin, ascorbic acid, camphene, chlorogenic acid, myristic acid, trans-anethole
dill (<i>Anethum graveolens</i>)	alpha-tocopherol, anethole, ascorbic acid, caffeic acid, camphene, chlorogenic acid, kaempferol
marjoram (<i>Origanum majorana</i>)	ascorbic acid, beta-carotene, caffeic acid, carvacrol, eugenol, hydroquinone, tran-anethole
oregano (<i>Origanum vulgare</i>)	camphene, carvacrol, gamma terpinene, isoeugenol, myrcene, thymol, linalyl acetate,
rosemary (<i>Rosemarinus officinalis</i>)	apigenin, ascorbic acid, beta carotene, caffeic acid, camphene, chlorogenic acid, terpinen-4-ol
thyme (<i>Thymus vulgaris</i>)	anethole, apigenin, carvacrol, gallic acid, eugenol, gamma terpinene, myrcene, camphene, naringenin, thymol, lauric acid

1.2.2 Spices as antimicrobial agents

The investigation of new and unique spices as antimicrobial agents for food preservation has received increasing attention due to the increased awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives. Certain spices can act as broad-spectrum antimicrobial agents or might also inhibit very specific microbes. The inhibitory effects against specific bacteria are shown in Table 1.2.

Table 1.2 Certain spices and their antimicrobial properties (Kenji and Mitsuo, 1998)

Spice	Effective component	Microorganism
mustard	allyl isothiocyanate	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i>
garlic	allicin	<i>Salmonella typhi</i> , <i>Shigella dysenteriae</i> , molds, yeasts
chile pepper	capsaicin	Molds, bacteria
clove	eugenol	<i>E. coli</i> 0157: H7, <i>S. aureus</i> , <i>Aspergillus</i> , yeast, <i>Acinetobacter</i>
thyme	thymol, isoborneol, carvacrol	<i>Vibrio parahaemolyticus</i> , <i>S. aureus</i> , <i>Aspergillus</i>
ginger	gingerone, gingerol	<i>E. coli</i> , <i>B. subtilis</i>
sage	borneol	<i>S. aureus</i> , <i>B. cereus</i>
rosemary	thymol, borneol	<i>S. aureus</i> , <i>B. cereus</i>

Many spices and herbs possess antimicrobial activity due to their essential oils fractions. As in the previous study, the essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic and onion exhibited antimicrobial activities against both bacteria and fungi (Nychas, 1995). Moreover, the essential oils of dill, coriander and eucalyptus have been reported to inhibit a broad spectrum of microorganisms (Nakatani *et al.*, 1994). The combination spices could be more effective than one spice, for example in Delaquis *et al.* study, they suggested that the mixed fractions of dill, cilantro, coriander, and eucalyptus essential oils resulted in additive, synergistic or antagonistic effects against gram-positive bacteria, gram-negative bacteria and *Saccharomyces cerevisiae* (Delaquis *et al.*, 2002).

It seems likely that phenolic compounds play the main role in antimicrobial activity in the spices and culinary herbs essential oils while the other constituents are believed to contribute little to the antimicrobial effect (Shelef, 1983; Hara-Kudo *et al.*, 2004). Some phenolic compounds that are purified from plant essential oils such as carvacrol, eugenol, linalool, cinnamic aldehyde and thymol can inhibit extensive microorganisms (Hulin *et al.*, 1998).

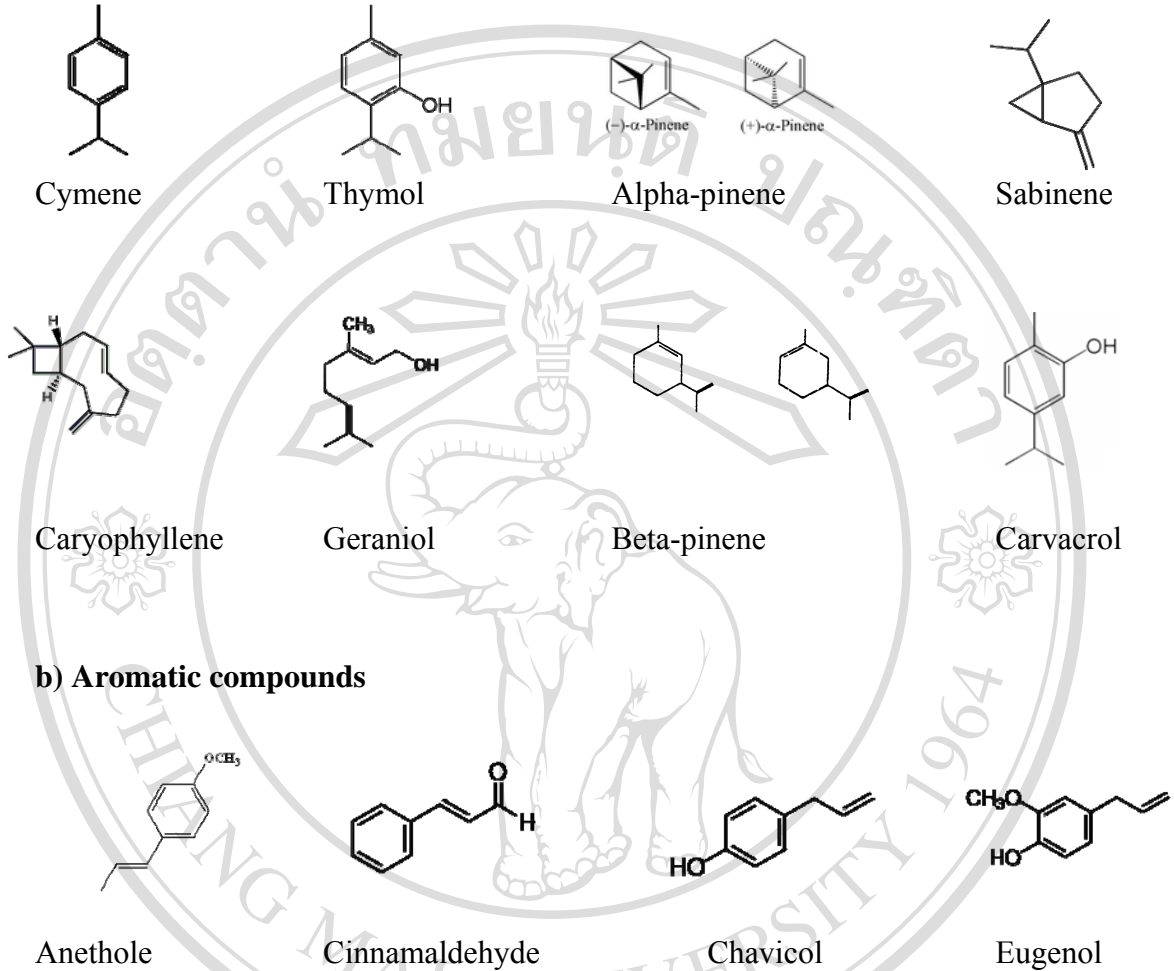
1.3 ESSENTIAL OIL (VOLATILE OIL)

Essential oils have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic applications since the middle ages. Nowadays, essential oils have become more popular in pharmaceutical, sanitary, cosmetic, agricultural and food industries (Bakkali *et al.*, 2008). Essential oils, the natural compounds that are formed as secondary metabolites in aromatic plants, contain several chemical components in different proportions which give the spices' their characteristic aromas as anywhere from 60 to 80% of the total oil (Table 1.3). The major chemical components are terpene (monoterpenes, diterpenes, triterpenes and sesquiterpenes), terpenoid and the other of aromatic and aliphatic constituents (Fig. 1.2). Essential oils in finishing products must be added at a very low level (0.01 to 0.05%) since they can be irritating to the skin, toxic to the nervous system if taken internally and can cause allergic reactions and even miscarriages.

Table 1.3 Example of characterizing essential oil components in some popular spices (Uhl, 2000)

Spice	Components in essential oils
Allspice seed	eugenol; 1,8-cineol; humulene; α -phellandrene
Basil, sweet	linalool; 1,8-cineol; methyl chavicol; eugenol
Cardamom	1,8-cineol; linalool; limonene; α -terpineol acetate
Dill leaf	carvone; limonene; dihydrocarvone; α -phellandrene
Epazote	ascaridol; limonene; ρ -cymene; myrcene; α -pinene
Fennel	anethole; fenchone; limonene; α -phellandrene
Ginger	zingiberene; curcumene; farnescene; linalool; borneol
Juniper	α -pinene; β -pinene; thujene; sabinene; borneol
Kari leaf	sabinene; α -pinene; β -caryophyllene
Lemongrass	citral; myrcene; geranyl acetate; linalool
Marjoram	<i>cis</i> -sabinene; α -terpinene; terpinene 4-ol; linalool
Nutmeg	sabinene; α -pinene; limonene; 1,8-cineol
Oregano	terpinene 4-ol; α -terpinene; <i>cis</i> -sabinene
Pepper, black	sabinene; β -pinene; α -pinene; limonene; 1,8-cineol
Rosemary	1,8-cineol; borneol; camphor; bornyl acetate
Star anise	anethole; α -pinene; β -caryophyllene; limonene
Turmeric	turmerone; dihydroturmerone; sabinene; 1,8-cineol
Zeodary	germacrone-4; furanodienone; curzerenone; camphor

a) Terpenes



c) Terpenoides



Figure 1.2 Chemical structures of selected components of essential oils

Terpenes

Terpenes are made from a combination of isoprene units (5-carbon-base, C_5) to monoterpenes (C_{10}) and sesquiterpenes (C_{15}) while hemiterpenes (C_5), diterpenes (C_{20}), triterpenes (C_{30}) and tetraterpenes (C_{40}) also exist. Apart from that, terpenes containing oxygen are called terpenoids. Monoterpenes, are the most volatile of these terpenes (about 90 % of the essential oils) and give out strong aromas when spices, tissues and cells are disintegrated by heating, crushing, slicing or cutting.

The functions consisting in monoterpenes include carbures (myrcene, ocimene, terpinenes, *p*-cimene, phellandrenes, etc.), alcohol (geraniol, linalool, citronellol, lavandulol, nerol, etc.), aldehydes (geranial, neral, citronellal, etc.), ketone (tegetone, menthones, carvone, pulegone, etc.), ester (linalyl acetate or propionate, citronellyl acetate, etc.), and ethers (1,8-cineole, menthofuran, ascaridole, thymol, carvacrol, etc.). The structure and functions of the sesquiterpenes are similar to those of the monoterpenes such as β -caryophyllene, curcumenes, farnesol, germacrone, etc.

Aromatic compounds

The aromatic compounds are derived from phenylpropane and occur less frequently than the terpenes. The aromatic compounds are comprised of aldehyde (cinnamaldehyde, etc.), alcohol (cinnamic alcohol, etc.), phenols (chavicol, eugenol, etc.), methoxy derivatives (anethole, elemicine, estragole, etc.) and methylene dioxy compounds (adipole, myristicine, etc.).

1.4 CHITOSAN AS FOOD PRESERVATIVES

Chitosan is a β -1,4-linked polymer of glucosamine (2-amino-deoxy- β -D-glucose), a polysaccharide obtained by the deacetylation of chitin (Fig. 1.3). Chitin (poly-N-acetyl-glucosamine) is the second most abundant polymer occurring in nature and is the major constituent of the exoskeleton of crustaceous water animals. Chitosan is a collective name given to a group of polymers deacetylated from chitin. The difference between chitin and chitosan depend on the degree of deacetylation (more than 50 per cent constitutes chitosan). Most chitosan in practical and commercial use comes from the production of deacetylated chitin (more than 85% deacetylated, molecular weights between 100 KDa and 1000 KDa) with the shells of crab, shrimp and krill which is the major-by product of the shellfish processing industry being the most available source of chitosan. An important property of chitosan is its positive charge in acidic solution due to the presence of primary amines on the molecule.

The main driving force in the development of new applications for chitosan lies in the fact that the polysaccharide is not only naturally abundant, but it is also nontoxic and biodegradable (Brine, 1984). Chitosan has attracted attention and interest as a potential food preservative of natural origin due to its biological activities, such as its antimicrobial activity against a wide range of food borne filamentous fungi, yeast and bacteria (No *et al.*, 2002; Sagoo *et al.*, 2002) involved in human colonic bacteria (Šimůnek *et al.*, 2006). Chitosan has an increasing role as a biopolymer to maintain high concentrations of antimicrobial agents in the food industry. Ouattara *et al.* (2000) suggested that the antibacterial sprays or dips have been done to overcome those contaminations, however direct surface application of antibacterial substances has some limitations because the active substances could be neutralized, evaporated or diffused. So, chitosan might be a preferred method to avoid these problems, moreover it also showed antimicrobial activities. Many studies have shown that chitosan has great potential to have its antimicrobial property improved by incorporating antimicrobial agents. Pranoto *et al.* (2005) have reported that garlic oil incorporated into chitosan film led to an increase in its antimicrobial efficacy against food pathogenic bacteria (*Staphylococcus aureus*, *Listeria*

monocytogenes and *Bacillus cereus*). Babiker (2002) has reported that gluten peptides have no antimicrobial effects against gram negative bacteria, but after being conjugated with high-molecular weight chitosan, this greatly enhanced its antimicrobial efficiency toward gram-negative bacteria and greatly improved its emulsifying properties at an acidic pH. Moreover, it has been reported that there is no cell (mammalian cell) toxicity in the conjugate.

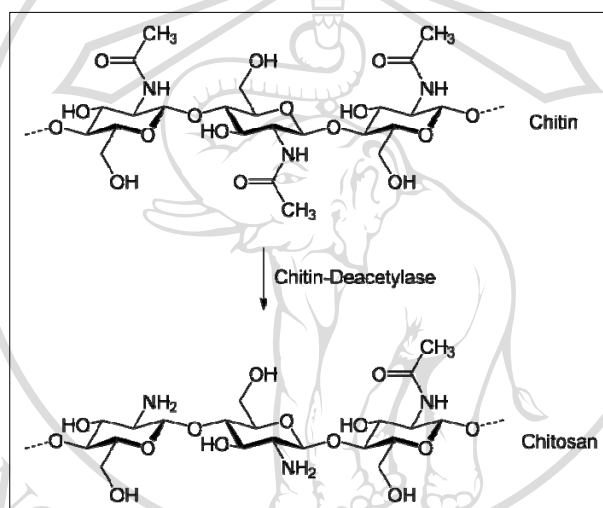


Figure 1.3 Structure of chitin (above) and chitosan (bottom)

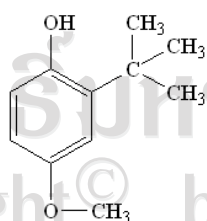
1.5 PHENOLIC ANTIOXIDANTS AS ANTIMICROBIAL AGENTS

Synthetic antioxidants have been evaluated for use in foods as preservatives. Phenolic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxyl toluene (BHT) are commonly used to prevent changes in flavor quality and nutritive value that result from the oxidation of unsaturated fats and fat-containing products.

Moreover, they also have antimicrobial activity. Some studies have show that phenolic antioxidants react with the cellular membrane impairing both its function and integrity. The interaction of phenolic antioxidants with the membrane may cause intracellular leakage or may induce changes in cellular lipids. The concentration of phenolic antioxidants that had antimicrobial activity in food products was in the range of 30-10,000 ppm. Although, in general, these compounds are permitted in concentrations of up to 200 ppm (Raccach, 1984).

According to consumer demand, less use of chemicals being applied in the food process due to their toxicity and tumor promoting tendencies, so the consideration of synthetic antioxidants is the trend to decrease their use.

a)



b)

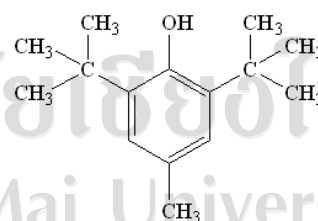


Figure 1.4 Structures of a) BHA and b) BHT

1.6 PLANT USED IN THIS STUDY (Guenther, 1950: Uhl, 2000)

1.6.1 *Anethum graveolens* L.

Botany of *Anethum graveolens* L.

Kingdom Plantae

Subkingdom Tracheobionta

Superdivision Spermatophyta

Division Magnoliophyta

Class Magnoliopsida

Subclass Rosidae

Order Apiales

Family Apiaceae

Genus *Anethum* L.

Species *Anethum graveolens* L.

Synonym: *Anethum sowa*

Common name: European dill, American dill, Indian dill, insilal (Amharic), shabath (Arabic), samit (Armenian), dille (Dutch), shivit and sheveed (Farsi), aneth odorant (French), dill/till (German), shamir (Hebrew), suwa/suwa patta (Hindi), adas manis/adas cina (Indonesian/Malaysian), aneto (Italy), diru (Japanese), phak si (Laotian), shih-lo (Mandarin), dill (Norwegian, Swedish), endro (Portuguese), ukrop (Russian), endure (sinhalese), eneldo (Spanish), satakuppi sompa (tamil) and pak chee lao (Thai).

Local name: ผักชีลาว (Pak chee lao), เทียนตาดักเตน (Tian ta tukatan)

Description: Dill is grown to a height of 3 to 4 ft. and its flowers are yellow. The entire plant is aromatic, but most of the volatile oil is contained in the seed (fruit). The seed is tiny, oval and flat (Fig. 1.5 a).

Distribution: Southern Mediterranean, Southern Russia, United States, England, Poland, Scandinavia, Turkey, Northern India, Japan.

Uses: Dill leaves are used as a seasoning for soups, sauces and particularly pickles. The seed is employed as a condiment; relieves stomach pains and hiccups because of its soothing effect on the digestive system. Chewing on the seeds can clear halitosis and stimulates appetite and induce sleep.

Review of biological activity:

Anethum graveolens (dill) were the most common and most-used spice antioxidants and antimicrobial agents. The dill oils exerted protection against *A. aegypti* (Choochote *et al.*, 2007). Macheboeuf *et al.* (2008) have been used dill oil and carvone (major component of the oil) as modulators of rumen fermentation. They suggested that carvone-based essential oil produced a linear non-threshold profile (characterized by a virtual stop of fermentation when doses negatively affected end-products of fermentation were higher than the threshold level). Moreover, Hosseinzadeh *et al.* (2002) suggested that *A. graveolens* seed extracts (aqueous and ethanol) have significant mucosal protective and antisecretory effects on the gastric mucosal in mice.

1.6.2 *Cuminum cyminum* L.

Botany of *Cuminum cyminum* L.

Kingdom Plantae

Subkingdom Tracheobionta

Superdivision Spermatophyta

Division Magnoliophyta

Class Magnoliopsida

Subclass Rosidae

Order Apiales

Family Apiaceae

Genus *Cuminum* L.

Species *Cuminum cyminum* L.

Synonym: *Cuminum nigrum*

Common name: Cumin—green cumin, white cumin and cumin (English), kemun (Amharic), kammun (Arabic), ziya (Burmese), komijn (Dutch), sire (Farsi), cumin/cumin blanc (french), romicher kummel (German), kamun (Hebrew), jeera (Hindi), comino/comino bianco (Italian), kumin (Japanese), jintan putih (Malay, Indonesia), jeeragam (Malayalam), Hsiao hui hsiang (Mandarin), spisskummen (Norwegian), cominho (Portuguese), kmin (Russian), comino/comino blanco (Spanish), spiskummin (Swedish), jiragum (Tamil), met yeeraa (Thai), kimyon (Turkish) and jirah (Urdu).

Local name: ยี่หระ (Yeeraa)

Description: *Cuminum cyminum* L. is a slender, rather pretty annual growing to a height of 1 ft. or less and browning and bearing very finely divided leaves. The aromatic, dried ripe fruits (seeds) are of elongated, oval shape about 5 to

6 mm. long. Flavor warm, slightly bitter, and somewhat disagreeable (Fig. 1.5 b).

Distribution: Eastern Mediterranean, Turkey, Egypt, Syria, China, India, Iran, Mexico, Argentina

Uses: The dried fruit is widely used as a condiment; it forms one of the most important flavoring constituents in East Indian curries, employed in native dishes of Central and South America, and for the flavoring of certain types of sausage and cheese. Moreover, cumin is used for its medicinal properties to aid digestion and to treat dysentery, stimulates circulation, dispels gas in the abdomen and relieves cramping.

Review of biological activity:

Derakhshan *et al.* (2008) investigated the activity of cumin seed oil and alcoholic extract against *Klebsiella pneumoniae* ATCC 13883 and clinical *K. pneumoniae*. In the presence of sub-minimum inhibitory concentrations of *C. cyminum* essential oil and alcoholic extract, *K. pneumoniae* showed a markedly altered capacity for capsule expression. In the study of various enzymatic and antioxidant activities, the presence of cumin extract including saline and hot aqueous showed a maximum increase in amylase, protease, lipase and phytase activities, along with antioxidant activity (Milan *et al.*, 2008).

1.6.3 *Foeniculum vulgare* Mill.

Botany of *Foeniculum vulgare* Mill.

Kingdom Plantae

Subkingdom Tracheobionta

Superdivision Spermatophyta

Division Magnoliophyta

Class Magnoliopsida

Subclass Rosidae

Order Apiales

Family Apiaceae

Genus *Foeniculum* Mill.

Species *Foeniculum vulgare* Mill.

Synonym: *Foeniculum vulgare* Var. dulce

Common name: Bitter fennel (United state), insilal (Amharic), shamar (Arabic), samong saba (Burmese), venkel (Dutch), fenouil (French), fenchel (German), shumar (Hebrew), motisaunf (Hindi), finocchio (Italian), urikyō (Japanese), jintan manis (Malaysian, Indonesian), perum jeerakam (Malayalam, Tamil), hui-hsiang (Mandarin), funcho (Portuguese), fenkhel (Russian), maduru (Sinhalese), hinojo (Spanish), fankal (Swedish), yira (Thai) and sonf (Urdu).

Local name: เทียนข้าวเปลือก (Tian-kae-plerk)

Description: Fennel is grown to a height of 4 to 5 ft. and bears 3-4 leaves pinnate into almost thread-like segments, small flowers containing 5 petals. The oval shaped, greenish fruit or seed varies in length from 4 to 10 mm (Fig. 1.5 c). The seeds are oval and ridged. They are bright or pale green to yellowish brown in color. They can be slightly curved or straight.

Distribution: France, Italy, Morocco, India, Europe, Russia, Germany, Hungary, Argentina

Uses: The seeds are widely employed in culinary preparation for flavoring bread and pastry, in candies and alcoholic liquors. It is used to complement rich fish sauces, roast pork, mutton and lamb curries, sweet and sour dishes, roast duck and cabbages.

Review of biological activity:

Fennel oil has been investigated for its antimicrobial and antiplasmid activities. These oils exhibited antimicrobial activities against gram positive (*Staphylococcus epidermidis*) and gram negative bacteria (*Escherichia coli* F' lac K12 LE140) and on two yeast strains, *Saccharomyces cerevisiae* 0425δ/1 and 0425 52C and has antiplasmid action on *E. coli* F' lac bacteria strain (Schelz *et al.*, 2006). Tognolini *et al.* (2007) evaluated fennel oil and anethole (the major component) in guinea pig plasma to be as potent as fennel oil in inhibiting arachidonic acid-, collagen-, ADP- and U46619- induced aggregation. The results demonstrate fennel oil and anethole showed significant antithrombotic activity in preventing the paralysis induced by collagen-epinephrine intravenous injection.

1.6.4 *Cinnamomum* sp.

Botany of *Cinnamomum* sp.

Kingdom Plantae

Subkingdom Tracheobionta

Superdivision Spermatophyta

Division Magnoliophyta

Class Magnoliopsida

Subclass Magnoliidae

Order Laurales

Family Lauraceae

Genus *Cinnamomum* sp.

Synonym: *Cinnamomum zeylanicum* Nees., *Cinnamomum loureirii* Nees.,
Cinnamomum cassia (Nees) Nees ex blume

Common name: Ceylon cinnamon/true cinnamon, Cassia/Chinese cinnamon

Local name: อบเชย (Op-choey)

Description: The species of *Cinnamomum* have aromatic oils in their leaves and bark. The genus contains over 300 species; there are three principal types of cinnamon.

1. Ceylon cinnamon: Ceylon cinnamon is the dried inner bark of the shoots of coppiced trees of *Cinnamomum zeylanicum* Nees.. The British Pharmacopoeia of 1948 recognizes the same drug under the collective term cinnamon, and specifies that the essential oil distilled from it contain cinnamic aldehyde not less than 50 percent and not more than 65 percent by weight.

2. Saigon cinnamon: The U. S. Pharmacopeia, Thirteenth Revision, recognized as the Saigon variety, i.e., the dried bark of *Cinnamomum loureirii* Nees. (Figure 1.5 b). Saigon cinnamon tree is of medium height, and originally native to China. Most of the bark comes from branches or from the trunks of young trees, but chips of thicker bark are occasionally included. The Saigon cinnamon bark is used only as a spice, and not for the commercial distillation of oil.

3. Cassia cinnamon: The U. S. Pharmacopeia, Thirteenth Revision, recognizes cinnamon oil, or cassia oil, as the volatile oil distilled with steam from the leaves and twigs of *Cinnamomum cassia* (Nees) Nees ex blume, rectified by distillation. The oil contains total aldehydes not less than 80 per cent by volume.

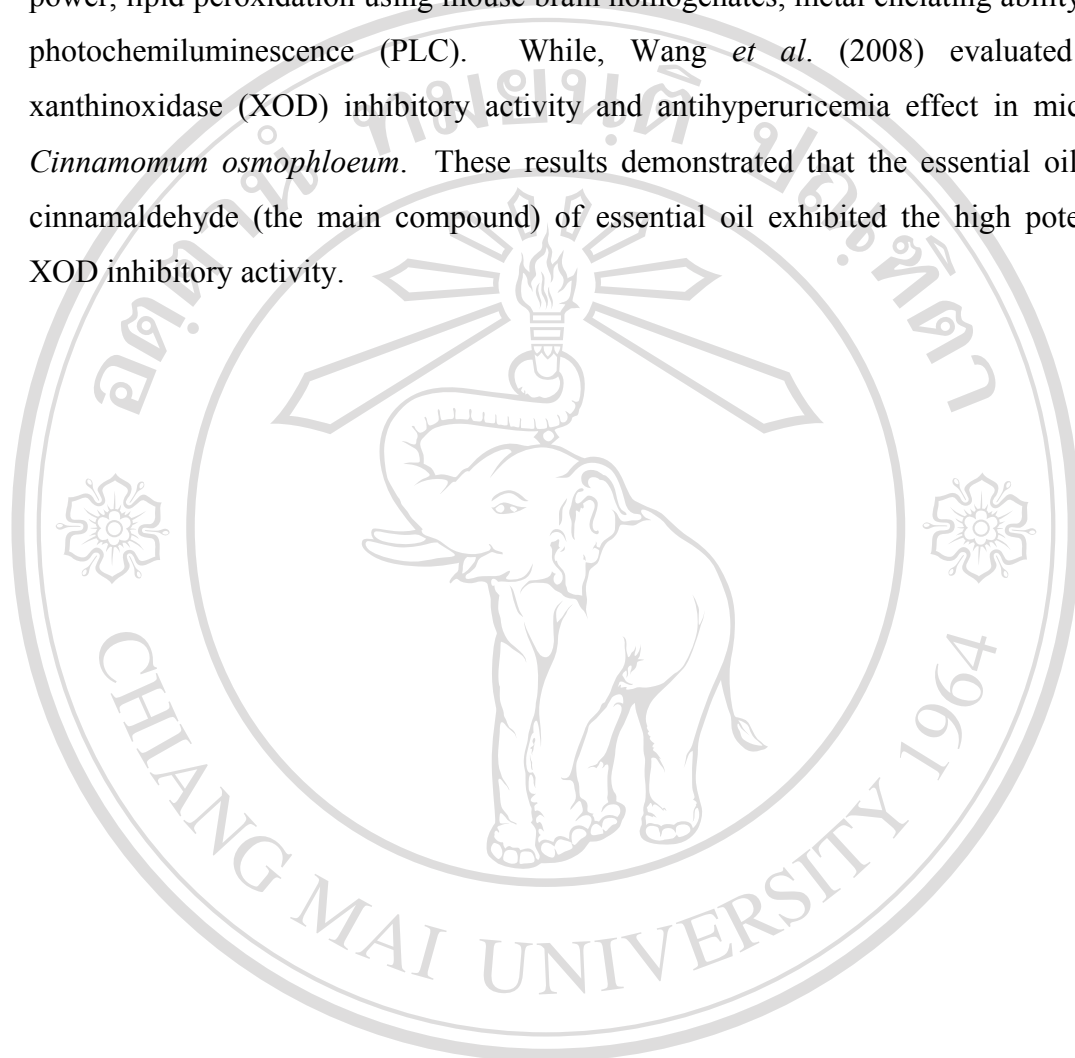
Distribution: North America, Central America, South America, Asia, Oceania and Australasia

Uses: Cinnamon bark is widely used as a spice. It is principally employed in cookery as a condiment and flavoring material. It is also used in the preparation of some kinds of desserts, such as apple pie and cinnamon buns as well as spicy candies, tea, hot cocoa, and liqueurs. Cinnamon can also be used in pickling. Cinnamon bark is one of the few spices that can be consumed directly and cinnamon powder has long been used in a variety of thick soups, drinks, and sweets.

Review of biological activity:

Singh *et al.* (2007) evaluated the antioxidant, antifungal and antibacterial activities of volatile oils and oleoresin of *Cinnamomum zeylanicum* Blume involving leaf and bark. They found that oleoresins has excellent activities for the inhibition of primary and secondary oxidation products in mustard oil. In antimicrobial investigations, the leaf and bark volatile oils have been found to be highly effective against all bacteria and fungi tested and the major components were (E)-cinnamaldehyde (49.9%), along with several other minor components. Chua *et al.*

(2008) have reported that ethanolic extracts of *C. osmophloeum* twigs have excellent antioxidant activities in various antioxidant assays such as DPPH, NBT, reducing power, lipid peroxidation using mouse brain homogenates, metal chelating ability and photochemiluminescence (PLC). While, Wang *et al.* (2008) evaluated the xanthinoxidase (XOD) inhibitory activity and antihyperuricemia effect in mice of *Cinnamomum osmophloeum*. These results demonstrated that the essential oil and cinnamaldehyde (the main compound) of essential oil exhibited the high potential XOD inhibitory activity.



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a)

b)



c)

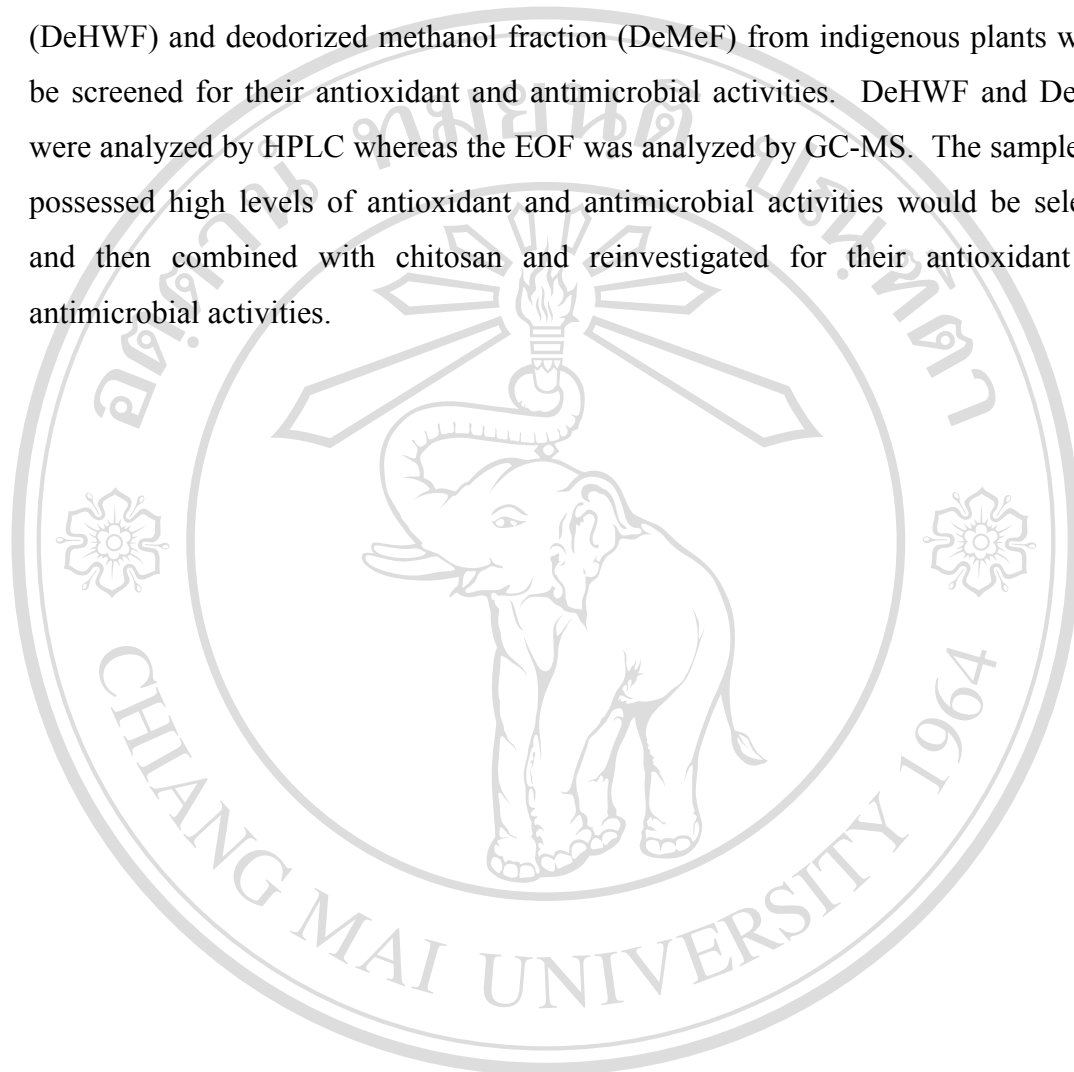
d)

Figure 1.5 The plant used in this study

(a) : *Anethum graveolens* L.(b) : *Cuminum cyminum* L.(c) : *Foeniculum vulgare* Mill.(d) : *Cinnamomum* sp.

1.7 PURPOSE OF THE STUDY

In this study, the essential oil fraction (EOF), deodorized hot water fraction (DeHWF) and deodorized methanol fraction (DeMeF) from indigenous plants would be screened for their antioxidant and antimicrobial activities. DeHWF and DeMeF were analyzed by HPLC whereas the EOF was analyzed by GC-MS. The sample that possessed high levels of antioxidant and antimicrobial activities would be selected and then combined with chitosan and reinvestigated for their antioxidant and antimicrobial activities.



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