CHAPTER 1

INTRODUCTION

1.1 STATEMENT AND SIGNIFICANCE OF THE PROBLEM

Incidences of infectious diseases are still a major problem, especially in tropical countries. The situation is alarming due to the indiscriminate use of antibiotics which has led to a drug resistance of several bacterial and fungal strains. The drug-resistant bacterial and fungal pathogens have further complicated the treatment of infectious diseases. The appearance of microbial resistance and occurrence of fatal opportunistic infections associated with AIDS, antineoplasic chemotherapy, and transplants have necessitated a search for new effective antimicrobial substances from other sources, including plants. The word antimicrobial is defined as an agent which destroys microorganisms or suppresses or prevents their multiplication or their growth (Gennaro *et al*., 1979). The substances that can either inhibit the growth of pathogens or kill them and have no or little toxicity to host cells are considered candidates for developing new antimicrobial drugs. There is considerable interest in the possible use of natural antimicrobials from plants. Many naturally occurring compounds, particularly polyphenols or phenolic compounds, have been studied for their antimicrobial activities. Plant polyphenols are mainly secondary metabolites derived from aromatic amino acid phenylalanine and from intermediate products of the shikimate pathway (Goodwin and Mercer, 1983). They are involved in the development of color, taste, and palatability (Strack, 1997). In various cases, these substances serve as plant defence mechanisms against predation

by microorganisms, insects, and herbivores (Cowan, 1999). In addition, the phenolics appear to have desirable medicinal properties. Some have been reported to possess antioxidant properties (Croft, 1999; Larson, 1988; Liégeois *et al*., 2000; Pietta, 2000; Rice-Evan and Miller, 1996), antitumor activities (Miki *et al*., 2001), anti-hyperglycemic activities (Lin Hsu *et al*., 2000), anti-inflammatory activities (Fernández *et al*., 1998; Kroes *et al*., 1992; Moreira *et al*., 2000), antimicrobial activities (Ahmad and Beg, 2001; Akiyama *et al*., 2001; Binutu *et al*., 1996; Fernández *et al*., 1996; Puupponen-Pimiä *et al*., 2001; Senji *et al*., 2000; Vijaya *et al*., 2001), stimulation of the phagocytic cells and anti-infective action (Cowan, 1999).

Plants, especially herbs and spices, are known to be able to prevent or delay the growth of microorganisms (Basílico and Basílico, 1999; Hayashi *et al*., 1995; Ibrahim and Osman, 1995; Masuda *et al*., 1991), and many researchers consider plant phenolic compounds as those responsible for antimicrobial activity (Azis *et al*., 1998; Cowan, 1999; Scalbert, 1991). In recent years, the antimicrobial properties of medicinal plants have been increasingly reported from different parts of the world. In Thailand, where there is a great diversity of plant species, several plants have been used in traditional medicines, particularly being used for skin infections. However, little is known about the nature of antimicrobial compounds. This attracted us to be interested in local Thai plants for new sources of antimicrobials. Therefore, the study of polyphenols and their nature in some local Thai plants, related to their antimicrobial properties is possible so that many of these compounds, either as individuals or in conjunction with other compounds, have pharmaceutical applications.

1.2 NATURAL ANTIMICROBIALS FROM PLANTS

It is estimated that there are 250,000 to 500,000 species of plants on the earth (Borris, 1996). A relatively small percentage (1 to 10%) of these is used as foods by both human and other animal species. It is possible that even more are used for medicinal purposes (Moreman, 1996). Plants have an almost limitless ability to synthesize aromatic substances; most of them are phenols or their oxygen-substitued derivative. Most are secondary metabolites, of which at least 12,000 substances have been isolated, a number estimated to be less than 10% of the total (Cowan, 1999). In many cases, these substances are called phytochemicals. They are not vitamins or minerals, but chemicals that are founds in plants. “Phyto” is the Greek word for plant. The phytochemicals appear to work alone and in combination, and perhaps in conjunction with vitamins and other nutrients in food, to prevent, halt or lessen disease. Phytochemicals are often found in the coloring agent in fruits and vegetables, so eating the brighter colored types may have benefits. However, there are also several beneficial phytochemicals in colorless or less colorful fruits and vegetables as well, for example, onions and corn are both full of phytochemicals.

Many naturally occurring compounds, such as essential oil, phenolic compounds, terpenoids, and alkaloids possess antimicrobial activity. Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditionally antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug. Moreover, the exploration of naturally occurring antimicrobials for food preservation receives increasing attention due to consumer awareness of natural food products, and a growing concern of microbial

resistance towards conventional preservatives. The numerous phytoantimicrobial agents, such as phenolics, flavonoids, saponins, terpenes, phytoalexins, etc. have been isolated from edible plants, herbs, spices, fruits, vegetables, or essential oil from medicinal plants. These agents have not only been used in food preservation, but may also provide health benefits. The plants containing antimicrobial activity are listed in Table 1.1 (Cowan, 1999). Several phytoantimicrobial agents provide dual functions, such as natural phytoantimicrobial-colorants (*e.g.* tumeric), phytoantimicrobial-flavorants (*e.g.* cinnamic aldehyde), phytoantimicrobial-antioxidants (*e.g.* allyl isothiocyanates), and phytoantimicrobial-nutraceuticals (*e.g.* flavonoids from cranberry). The antimicrobial spectrum of phytoantimicrobial agents is summarized in Table 1.2 (Naidu, 2000).

Table 1.1 Plants containing antimicrobial activity (Cowan, 1999)

Plant	Compound	Class	Activity[a]
Apple	Phloretin	Flavonoid derivative	General
Basil	Essential oil	Terpenoid	*Salmonella* sp., bacteria
Bay	Essential oil	Terpenoid	Bacteria, fungi
Black pepper	Piperine	Alkaloid	Fungi, *Lactobacillus* sp., *Micrococcus* sp.
Cashew	Salicylic acid	Polyphenol	Bacteria, fungi
Chamomile	Anthemic acid	Phenolic acid	Bacteria
Chili peppers	Capsaicin	Terpenoid	Bacteria
Clove	Eugenol	Terpenoid	General
Dill	Essential oil	Terpenoid	Bacteria
Eucalyptus	Tannin	Polyphenol	Bacteria, virus
Garlic	Allicin, ajoene	Sulfoxide, sulfated terpenoids	General
Gotu kola	Asiatocoside	Terpenoid	*Mycobacterium leprae*
Henna	Gallic acid	Phenolic	*Staphylococcus aureus*
Hops	Lupulone, humulone	Phenolic acid	General
Licorice	Glabrol	Phenolic alcohol	*S. aureus*, *M. tuberculosis*
Olive oil	Hexanal	Aldehyde	General
Onion	Allicin	Sulfoxide	Bacteria, *Candida* sp.

Table 1.1 (Continued)

Plant	Compound	Class	Activity[a]
Papaya	Latex	Mix of terpenoids, organic acids, alkaloids	General
Peppermint	Menthol	Terpenoid	General
Poppy	Opium	Alkaloid and others	General
Quinine	Quinine	Alkaloid	*Plasmodium* sp.
Rosemary	Essential oil	Terpenoid	General
Thyme	Caffeic acid, thymol, tannins	Phenolic, phenolic alcohol, polyphenols	Bacteria, fungi, virus
Tumeric	Cucurmin, tumeric oil	Terpenoids	Bacteria, protozoa
Willow	Salicin, tannins, essential oil	Phenolic glycoside, polyphenols, terpenoid	General
Wintergreen	Tannins	Polyphenols	General

[a] "General" denotes activity against multiple types of microorganisms (e.g. bacteria, fungi, and protozoa), and "bacteria" denotes activity against Gram-positive and Gram-negative bacteria.

Table 1.2 Antimicrobial spectrum of phytoantimicrobial agents (Naidu, 2000)

Phytoantimicrobial source	Susceptible microorganism
Essential oils	
Anise oil	*Lactobacillus curvatus* / *Saccharomyces cerevisiae*
Sweet linalool	*Pseudomonas* sp.
Basil methyl chavicol	*Aeromonas hydrophila* / *Pseudomonas fluorescens*
Bay and thyme oils	*Campylobacter jejoni*
Nutmeg oil	*Listeria monocytogenes*
Dill oil	*Lactobacillus buchneri* / *S. vini*
Achillea fragrantissima	*Candida albicans*
Cedronella canariensis	*Bordetella brochoseptica* / *Cryptococcus albicus*
Hoslundia opposita	*Aspergillus niger* / *Acinetobacter calcoacetical* / *Bronchothrix thermospactal* / *Flavobacterium* sp.
Camphor / camphene	*Escherichia coli* / *Aspergillus* sp. / *C. albicans* / *Trichophyton mentagrophytes* / *Pseudomonas* sp.
Ducrosia ismaelis	*Staphylococcus aureus* / *Bacillus subtilis*
Spices	
Cinnamon	*B. subtilis* / *Candida* sp. / *Rhodotorula* sp.
Cloves	*S. aureus* / *C. albicans*
Rosemary	*Salmonella typhimurum* / *Clostridium botulinum* / *S. aureus*
Mustard	*Vibrio parahaemolyticus*
Turmeric	*B. cereus* / *S. aureus* / *E. coli*

Table 1.2 (Continued)

Phytoantimicrobial source	Susceptible microorganism
Aframomum danielli	*S. enteritidis* / *Pseudomonas* sp. / *S. aureus* / *Aspergillus* sp. / *Proteus vulgaris* / *Streptococcus* sp.
Fruit / vegetables	
Carrots	*Listeria monocytogenes* / *E. coli* / *S. aureus* / *Pseudomonas* sp. / *C. lambica*
White potatoes	*A. parasiticus*
Cabbage	Enterobacteriaceae
Soybeans	*E. coli* / *S. aureus* / *Streptococcus pyogenes*
Garlic	*B. subtilis* / *Serratia marcescens* / *Mycobacterium* sp. / *P. aeruginosa* / *S. aureus* / *S. typhimurium* / *Shigella* sp. / *E. coli* / *Helicobacter pylori* / *Klebsiella* sp. / *Xanthomonas maltophila*
Herbs	
Green tea	*S. mutans* / *S. sobrinus* / *Porphyromonas* sp./ *Actinomyces viscosus* / *S. faecalis* / *E. coli* / *S. aureus* / Influenza virus / Vaccinia virus / Polio virus / Herpes Simplex virus / Coxasackie virus /
Yucca shidigera	*S. bovis* / *Butyrivibrio fibrisolvens*
Bridelia ferruginea	*Staphylococcus* sp. / *Streptococcus* sp. / *Klebsiella* sp. / *E. coli* / *Proteus* sp. / *C. albicans*
Camillia sinensis	*Microsporum audouinii*

Table 1.2 (Continued)

Phytoantimicrobial source	Susceptible microorganism
Arctotis auriculata	*Mycobacterium smegmatis* / *Pseudomonas* sp.
Uvaria chamae	*S. aureus* / *B. subtilis* / *M. smegmatis*
Amaranthaceae	*M. phlei*
Tecoma stans	*C. albicans*
Tagetes minuta	*Lactobacillus* sp. / *Zymomonas* sp.
Ceanothus americanus	*A. viscosus* / *Porphyrlomonas gingivalis* / *Prevotella intermedia* / *S. mutans*
Prosopis juliflora	*Candida* sp. / *Streptococcus* sp. / *B. subtilis* / *Corynebacterium diphtheriae* / *Shigella* sp. / *Vibrio* sp. / *Salmonella* sp. / *Aeromonas* sp.
Ziziphus abyssinica	*S. aureus* / *E. coli* / *C. albicans*
Arctotis hilotica	*Clostridium perfringens* / *E. coli* / *Salmonella* sp.
Vicia fava	*S. cerevisiae* / *C. albicans*

Useful phytoantimicrobial agents can be divided into several categories, described below (Cowan, 1999). Some of their structures are shown in Fig. 1.1.

1. Phenolic compounds

1.1 Simple phenols and phenolic acids

Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds which are effective against viruses, bacteria, and fungi. Catechol and pyrogallol both are hydroxylated phenols, also

shown to be toxic to microorganisms. Catechol has two hydroxyl groups, and pyrogallol has three. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity.

Phenolic compounds possessing a C_3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils, and often cited as antimicrobial as well. Eugenol is a well-characterized representative of phenolic essential oils. It is considered bacteriostatic against bacteria.

1.2 Quinones

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature, and are characteristically highly reactive. These compounds, being colored, are responsible for the browning reactions in cut or injured fruits and vegetables, and are an intermediate in the melanin synthesis pathway in human skin. Their presence in henna gives that material its dyeing properties. The switch between diphenol (or hydroquinone) and diketone (or quinone) occurs easily through oxidation and reduction reactions. The individual redox potential of the particular quinone-hydroquinone pair is very important in many biological systems; witness the role of ubiquinone (coenzyme Q) in mamalian electron transport system. Vitamin K is a complex napthoquinone. Its antihemorrhagic activity may be related to its ease of oxidation in body tissues.

Quinones are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Probable targets

in the microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes.

1.3 Flavones, flavonols, and flavonoid

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl group yields a flavonol. Flavonoids are also hydroxylated phenolic substances but occur as a C_6-C_3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins, and to complex with bacterial cell wall, as described above for quinones.

1.4 Tannins

Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. They are found in almost every part of plants; bark, wood, leaves, fruits, and roots. They are divided into two groups, hydrolysable and condensed tannins. Hydrolysable tannins are based on gallic acid, usually as multiple esters with D-glucose, while the more numerous condensed tannins (often called proanthocyanidins) are derived from flavonoid monomers. Tannins may be formed by condensation of flavan derivatives which have been transported to woody tissues of plants. Alternatively, tannins may be formed by polymerization of quinone units. This group of compounds has received a great deal of attention in recent years, since it was

suggested that the consumption of tannin-containing beverage, especially green tea and red wines, can cure or prevent a variety of ills.

Many human physiological activities, such as the stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions have been assigned to tannins. One of their molecular actions is to complex with proteins through so-called non-specific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial action, as described in the section on quinones (see above), may be related to their ability to inactivate microbial adhesins, enzymes, cell envelop transport proteins, etc.

1.5 Coumarins

Coumarins are phenolic substances made of fused benzene and α-pyrone rings. They are responsable for the characteristic odor of hay. As of 1996, at least 1,300 species had been identified. Their fame has come mainly from their antithrombotic, anti-inflammatory, and vasodilatory activities. Warfarin is a particularly well-known coumarin which is used both as an oral anticoagulant and, interestingly, as a rodenticide. It may also have antiviral effects. Coumarins are known to be highly toxic in rodents and therefore, are treated with caution by the medical community.

2. Terpenoids and essential oils

The fragrance of plants is carried in the so called *quinta essentia*, or essential oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure. They are called terpenes, their general chemical structure is $C_{10}H_{16}$, and they occur as diterpenes (C_{20}), triterpenes (C_{30}), and

tetraterpenes (C_{40}), as well as hemiterpenes (C_5) and sesquiterpenes (C_{15}). When the compounds contain additional elements, usually oxygen, they are termed terpenoids.

Terpenoids are synthesized from acetate units, and as such they share their origins with fatty acids. They differ from fatty acids in that they contain extensive branching, and are cyclized. Examples of common terpenoids are menthol and camphor (monoterpenes), and farnesol and artemisin (sesquiterpenoids). Artemisin and its derivative α-arteether, also known by the name qinghaosu, find current use as antimalarials.

Terpenes or terpenoids are active against bacteria, fungi, viruses, and protozoa. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds.

3. Alkaloids

Heterocyclic nitrogen compounds are called alkaloids. The first medically useful example of alkaloids was morphine, isolated in 1805 from the opium poppy *Papaver somniferum*; the name morphine comes from the Greek Morpheus, god of dream. Codein and heroin are both derivatives of morphine. Diterpenoids alkaloids, commonly isolated from the plants of the Ranunculaceae, or buttercup family, are commonly found to have antimicrobial properties. Berberine is an important representative of the alkaloid group. The mechanism of action of highly aromatic planar quaternary alkaloids such as berberin and harmane is attributed to their ability to intercalate with DNA.

4. Lectins and polypeptides

Peptides which are inhibitory to microorganisms were first reported in 1942. They are often positively charged and contain disulfide bonds. Their mechanism of action may be the formation of ion channels in the microbial membrane, or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors.

The larger lectin molecules, which include mannose-specific lectins from several plants are inhibitory to viral proliferation (HIV, cytomegalovirus), probably by inhibiting viral interaction with critical host cell components.

5. Other compounds

Many phytochemicals not mentioned above have been found to exert antimicrobial properties *e.g.* polyamines (in particular spermidine), isothiocyanates, thiosulfinates, and glucosides.

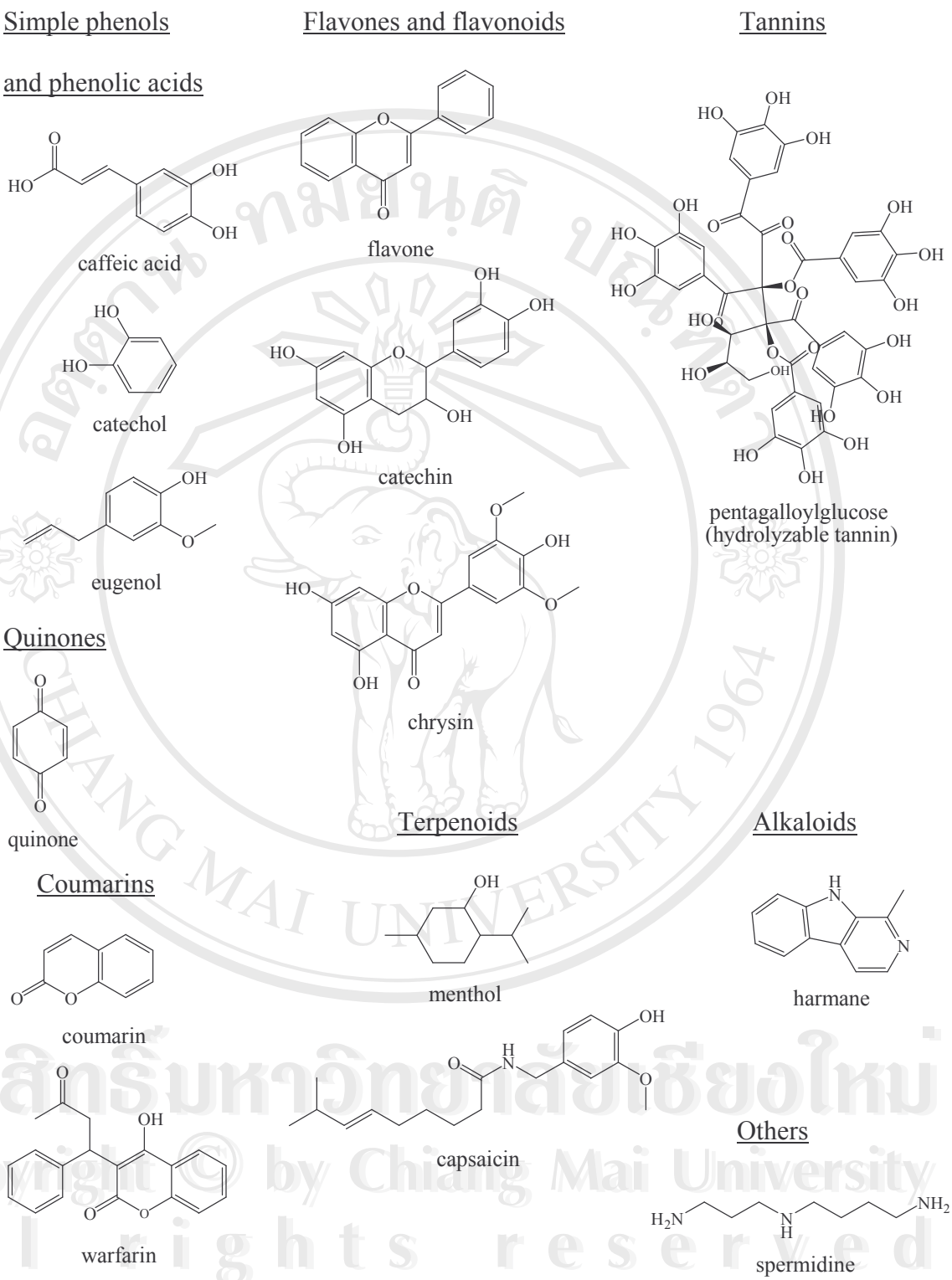

Figure 1.1 Some structures of common antimicrobial plant chemicals

1.3 PHENOLICS AND THEIR BIOLOGICAL PROPERTIES

The term “phenolic” is used to define substances that possess one or more hydroxyl substituents bonded onto an aromatic ring. Some also contain other substituents including carboxyl group, methoxyl groups, and other non-aromatic ring structures. The name derives from the simple parent substance, phenol (Fig. 1.2; a). Compounds that have many phenolic hydroxyl substituents are often referred to as polyphenols. However, it must be recognized that not all hydroxyl groups are phenolic; they are equally likely to occur bonded to non-aromatic cyclic or to non-cyclic structures e.g. ethanol and cholesterol (Fig. 1.2; b and c, respectively), in which case they do not have the properties of a phenol (Waterman and Mole, 1994). Phenolic compounds are amphipathic molecules. The phenolic hydroxyl group is hydrophilic, whilst the aromatic ring is hydrophobic in character (Haslan, 1998). Phenolic compounds rarely occur in the free state in living plant tissues; they are practically always present in conjugated form. In the simplet instance, they are bound to sugar as β-D-glucopyranosides but a wide array of other bound forms are known such as methyl esters, esters (*e.g.* hydroxycinnamic acids occur more frequently as esters than glycosides) (Harborne, 1979). The phenolics can be divided into major classes according to the number of carbon atoms in their skeleton (Table 1.3) (Goodwin and Mercer, 1983). The phenolics which occur in plants include low molecular mass phenolics (simple phenols, hydroxybenzoic and hydroxycinnamic acids, flavonoids, stilbenes, and lignans) and oligo- and polymeric forms (hydrolysable and condensed tannins, and lignins). Naturally occurring phenolic compounds from different plants are listed in Table 1.4 (Nychas, 1995). Goodwin and Mercer (1983) explained the details of biosynthesis of various plant phenolics. They

reported that all, except flavonoids, arise from a common biosynthetic intermediate phenylalanine or its close precursor shikimic acid. In the case of the flavonoids, its biosynthesis is unique in that the two component aromatic rings arise via different pathways. One aromatic ring and its C_3 side chain arise from phenylalanine, whilst the other arises from acetyl-Co A *via* the polyketide pathway.

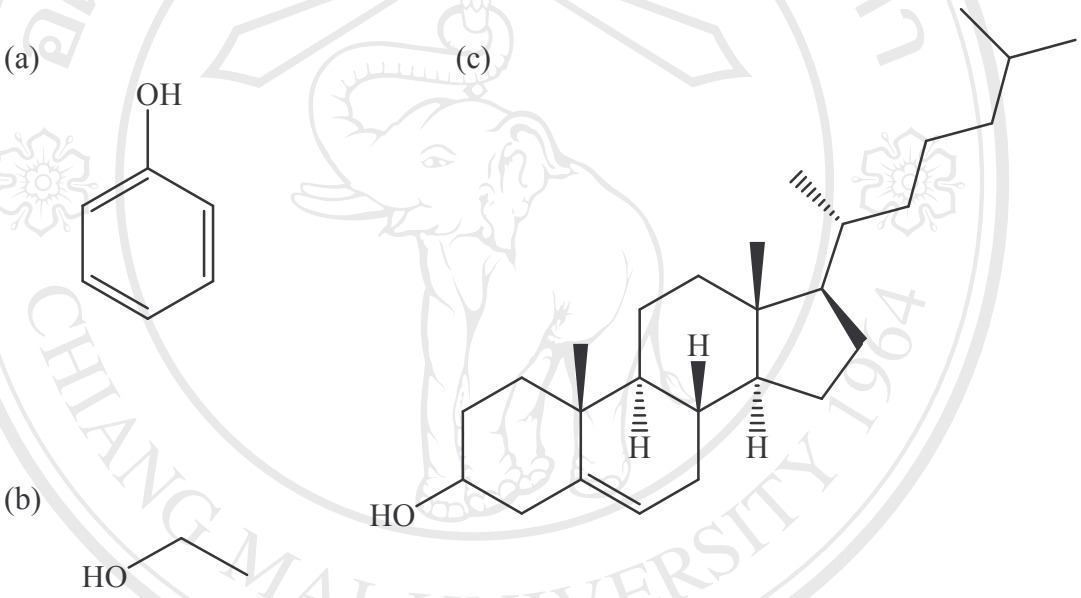

Figure 1.2 Structures of phenol (a), ethanol (b), and cholesterol (c)

Table 1.3 The major classes of plant phenolics (Goodwin and Mercer, 1983)

No. of C atoms	Basic skeleton	Class	Example
6	C_6	Phenols	Catechol
7	C_6-C_1	Phenolic acids	*p*-Hydroxybenzoic acid
8	C_6-C_2	Phenylacetic acids	2-Hydroxyphenylacetic acid
9	C_6-C_3	Hydroxycinnamic acids	Caffeic acid
		Phenylpropenes	Myristicin
		Chromones	Eugenin
10	C_6-C_4	Naphthoquinones	Juglone
13	C_6-C_1-C_6	Xanthones	Mangiferin
14	C_6-C_2-C_6	Stilbenes	Lunularic acid
		Anthraquinones	Emodin
15	C_6-C_3-C_6	Flavonoids	Catechin
18	$[C_6$-$C_3]_2$	Lignans	Pinoresinol
		Neolignans	Eusiderin
30	$[C_6$-C_3-$C_6]_2$	Biflavonoids	Amentoflavone
n	$[C_6$-$C_3]_n$	Lignins	
	$[C_6$-C_3-$C_6]_n$	Condensed tannins (flavolans)	

Table 1.4 Naturally occurring phenolic compounds from different plants (Nychas, 1995).

Phenolic compounds	Plant
Apigenin-7-glucoside	Oleaceae
Benzoic acid	Brassica oilseeds
Berbamine	*Berberis* sp.
Berberine	*Berberis* sp.
Caffeine	Tea
Caffeic acid	Oleaceae, avocado, artichoke, apple
3-*o*-Caffeylquinic acid	Artichoke, plum, carrot
4-*o*-Caffeylquinic acid	Artichoke, plum, carrot
5-*o*-Caffeylquinic acid	Plum
Caryophelene	Hop
Catechin	Skin and seeds of wine grape, some spices
Cinnamic acid	Brassica oilseeds
Chlorogenic acid	Brassica oilseeds, artichoke, apple
Chelldonic acid	*Berberis* sp.
Chicorin	Chicory
Columbamine	*Berberis* sp.
Coumarin	Spices
p-Coumaric acid	Oleaceae, avocado, Brassica oilseeds, apple
o-Coumaric acid	Avocado
m-Coumaric acid	Avocado

Table 1.4 (Continued)

Phenolic compounds	Plant
Cynarine	Artichoke
Dihydrocaffeic acid	Oleaceae
Dimethyloleuropein	Oleaceae
Esculin	Chicory
Ferulic acid	Oleaceae, avocado, Brassica oilseeds
Gallic acid	Oleaceae, skin and seeds of wine grape, avocado, Brassica oilseeds
Gingerols	Spices
Humulon	Hop
Hydroxytyrosol	Oleaceae
4-Hydroxybenzoic acid	Oleaceae, avocado, Brassica oilseeds, vanilla, carrot
4-Hydroxycinnamic acid	Oleaceae, carrot
Isovanillic	Avocado
Linalool	Boldo
Lupulon	Hop
Luteoline-5-glucoside	Oleaceae
Ligustroside	Oleaceae
Myricetin	Tea
3-Methoxybenzoic acid	Oleaceae
Oleoside	Oleaceae
Oleuropein	Oleaceae

Table 1.4 (Continued)

Phenolic compounds	Plant
Paradols	Spices
Protocatechuic acid	Oleaceae, avocado
o-Protocatechuic acid	Avocado
Quercetin-3-rutinoside (rutin)	Oleaceae, tea
Quercetin	Tea
α-Resocrylic	Avocado
β-Resocrylic	Avocado
Sesamol	Sesame oil
Shogoals	Spices
Syringic acid	Brassica oilseeds
Sinapic acid	Avocado, Brassica oilseeds
Tannins	Skin and seeds of wine grape, spices, Brassica oilseeds, boldo, herbs (ginger, gentian)
Tannic acid	Skin and seeds of wine grape
Thymol	Thymol
3,4,5-Trimethoxybenzoic acid	Oleaceae
3,4,5-Trihydroxyphenylacetic acid	Oleaceae
Tyrosol	Oleaceae
Verbascoside	Oleaceae
Vanillin	Spices, vanilla
Vanillic acid	Oleaceae, avocado, Brassica oilseeds, vanilla

Plant polyphenols have attracted much interest recently because many studies suggest that they have a variety of beneficial biological properties. They are potent antioxidants, which may play an important role in the maintenance of human health. There are several studies and reviews on the antioxidant activity of plant polyphenols, especially flavonoids and phenolic acids (Larson, 1988; Liégeois *et al*. 2000; Pietta, 2000; Rice-Evan and Miller, 1996). Liégeois *et al*. (2000) determined the antioxidant activity of 12 compounds (including some flavonoids and phenolic acids, thiols, sulfites, vitamins, Trolox, furaneol, and BHT). The efficiency of antioxidants of 12 recognized compounds have been compared. It was found that phenolic compounds (quercetin dihydrate, caffeic acid, ferulic acid, and catechin hydrate) proved to be the most efficient. Furthermore, the phenolics also exhibited various physiological and pharmacological activities that can be used for pharmaceutical purposes. For examples, antitumor effect of phenolic compounds was studied by Miki *et al*. (2001). They investigated the *in vivo* antitumor activity of orally administered gallic acid on LL-2 lung cancer cells transplanted in mice. The result showed that gallic acid reduced cell viability of the cancer cells, with a IC50 value of around 200 mM. They suggested that the combination of gallic acid with an anti-cancer drug, including cisplatin, may be an effective protocol for lung cancer therapy.

Caffeic acid, the cinnamic acid derivative, was found to possess antihyperglycemic activity. Lin Hsu *et al*. (2000) extracted and isolated caffeic acid from the fruits of *Xanthium strumarium*. Then, the antihyperglycemic effect of caffeic acid was investigated. The assay was determined in diabetic rats that received an intravenous injection of an aqueous solution containing caffeic acid at desired doses. Plasma glucose was measured every 30 minutes and then compared with the control

group that was injected with the same volume of a vehicle solution. It was found that after the injection of caffeic acid into diabetic rats of both streptozotocin-induced and insulin-resistant models, a dose-dependent decrease of plasma glucose was observed. They concluded that an increase of glucose utilization by caffeic acid seems to be responsible for the lowering of plasma glucose.

Moreira *et al*. (2000) studied the anti-inflammatory activity of extracts from *Gochnatia polymorpha*. The aqueous and ethanolic extracts from the leaves of this species, and further fractions which were obtained from the latter extract using solvents with increasing polarity, were investigated by carrageenan-induced pedal edema formation. The result demonstrated that the aqueous and ethanolic extracts and the ethyl acetate fraction demonstrated significant anti-inflammatory activity. The chemical investigation of the latter fraction was determined, and it revealed the presence of caffeic acid, chlorogenic acid, 3-*o*-methylquercetin, hyperosid, and rutin.

One of the major biological properties of phenolic compounds is their antimicrobial activity, and it is often assumed that their main role is to act as protective compounds against disease agents such as bacteria, fungi, and viruses. Scalbert (1991) reviewed the antimicrobial property of tannins in 1991. According to his report, tannins can be toxic to filamentous fungi, yeast, and bacteria. Akiyama *et al*. (2001) examined the antibacterial action of several tannins on plasma coagulation by *Staphylococcus aureus* and the effect of conventional chemotherapy combined with tannic acid below the MIC. Coagulation was inhibited in plasma containing tannic acid (100 mg/l), gallic acid (5000 mg/l), ellagic acid (5000 mg/l), (-)-epicatechin (1500 mg/l), (-)-epicatechin gallate (5000 mg/l), or (-)-epigallocatechin gallate (200 mg/l) after incubation for 24 h. The antibacterial effects of tannins were

also investigated by Senji *et al*. (2000). They examined the inhibitory action of green tea tannins towards the development and growth of bacterial spores. It was found that, among the tested Bacillus bacteria, tea tannins showed an antibacterial effect towards *Bacillus stearothermophilus*, which is a thermophilic spore-forming bacterium. The heat resistance of *B. stearothermophilus* spores was reduced by the addition of tea tannins. Moreover, *Clostridium thermoaceticum*, an anaerobic spore-forming bacterium, also exhibited a reduced heat resistance of its spores in the presence of tea tannins. The strong antibacterial effect was observed on (-)-epigallocatechin gallate, which is the main component of tea polyphenols. It showed a strong activity against both *B. stearothermophilus* and *C. thermoaceticum*.

Phenolic acids have also shown an antimicrobial activity. Binutu *et al*. (1996) isolated compounds from methanolic extracts of the root and fruits of *Kigelia pinnata*. The individual isolated compounds were identified and investigated for their antimicrobial activity. They found that naphthoquinones and some phenolic acids such as *p*-coumaric acid, ferulic acid, and caffeic acid showed antibacterial and antifungal activities. Vijaya *et al*. (2001) studied antifungal properties of origanum oil which possesses a high content of phenolic derivatives, especially carvacrol and thymol. In this study, the antifungal activity of origanum oil was examined against *Candida albicans*, both *in vitro* and *in vivo*. The results showed that the origanum oil at 0.25 mg/ml was found to completely inhibit the growth of *C. albicans* in culture. Growth inhibitions of 75% and more than 50% were observed at 0.125 mg/ml and 0.0625 mg/ml, respectively. In addition, they found that both the germination and the mycelial growth of *C. albicans* were found to be inhibited by origanum oil and carvacrol in a dose-dependent manner. Fernández *et al*. (1996) isolated the phenolic

acids fractions of two species of genus *Scrophularia*, (*S. frutescens* and *S. sambucifolia*). Ferulic, isovanillic, *p*-hydroxycinnamic, *p*-hydroxybenzomic, syringic, caffeic, gentisic, and protocatechuic acids were isolated from *S. frutescens*, and ferulic, *p*-coumaric, vanillic, *p*-hydroxybenzomic, and syringic acids were isolated from *S. sambucifolia*. The antimicrobial effect of these fractions was investigated by disc diffusion method. The result exhibited that the phenolic acid fractions of both species showed more activity against Gram-positive bacteria, specifically against *Bacillus* sp. Puupponen-Pimiä *et al*. (2001) investigated the antimicrobial properties of phenolic compounds presented in Finnish berries against probiotic bacteria and other intestinal bacteria, including pathogenic species. They found that phenolic extracts of eight berries inhibited the growth of Gram-negative, but not Gram-positive bacteria. Recently, ethanolic extracts of 45 Indian medicinal plants traditionally used in medicine were studied for their antimicrobial activity against certain drug-resistant bacteria and a yeast *C. albicans* of clinical origin. This study was reported by Ahmad and Beg (2001). The results of screening are encouraging as out of the 45 plants, 40 extracts showed antibacterial activity against one or more test bacteria while 24 extracts showed anticandidal activity. They also reported qualitative phytochemical test results, thin layer chromatography and TLC-bioautography of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts, including phenols, tannins, and flavonoid as major active constituents.

The antimicrobial activities of polyphenols are thought to be related to their oxidation. Some scientists have found that more highly oxidized phenols are more inhibitory (Rama Raje Urs and Dunleavy, 1975; Scalbert, 1991). The oxidized phenols occur easily through the oxidation reaction of polyphenols in the presence of

enzyme polyphenol oxidases. Like polyphenols, enzyme polyphenol oxidases commonly occur in plants. This enzyme can oxidize polyphenols to quinones. Thus, it seems logical that the antimicrobial mechanism may be caused by the oxidation of phenols to toxic quinones. Mihopoulos *et al*. (1999) reported that the oxidation of hydroquinone to quinone increased the antibacterial effect. Quinones, the oxidized compounds, are known to combine irreversibly with nucleophilic amino acids in proteins to become complex compounds, leading to the inactivation or loss of function of proteins. For this reason, the potential range of quinone antimicrobial effect is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes (Cowan, 1999). Some phenolics (flavonoids and tannins) have the ability to combine with proteins and bacterial cell walls (Cowan, 1999; Scalbert, 1991), as described above for quinones. Thus, the mode of antimicrobial action of polyphenols may be related to their ability to inactivate microbial adhesins, membrane-bound enzymes, or cell envelop transport proteins.

1.4 ANTIOXIDANTS AS ANTIMICROBIALS

Antioxidants are classified into two groups, namely primary or chain-breaking antioxidants, which can react with lipid radicals to convert them to more stable products, and secondary or preventive antioxidants, which reduce the rate of chain initiation in lipid oxidation by a variety of mechanisms (Gordon, 1990). The phenolic substances, that terminate the free radical chains in the lipid oxidation belong to the group of primary antioxidants, and function as electron donors (Kochhar and Rossell, 1990).

The antimicrobial effects of phenolic antioxidants were firstly investigated using butylated hydroxytoluene (BHT) in 1967. However, it was not until 1975 that the major reserch thrust in this area began with a work on butylated hydroxyanisole (BHA) in 1975. Previously, these compounds were used strictly as antioxidants, that is, to prevent oxidative rancidity in lipids and lipid-containing foods. Since these compounds were phenolic in nature, and since phenolics were known to possess antimicrobial activity, it was reasoned that they may also possess inhibitory powers against microorganisms (Davidson, 1993).

In case of medicinal role, the combination of immuno-deficiency, inflammatory process, and nutritional status that is characteristic of infective and food-borne illness is more evident in chronic diet- and environment-influenced chronic diseases such as diabetes, cardiovascular disease, cancer, arthritis, and neuro-degenerative diseases. In addressing the challenges of the above diseases, a significant role for dietary phytochemicals is emerging. Among the diverse groups of phytochemicals, phenolic antioxidants and antimicrobials from plants are being targeted for designed dietary intervention to manage major oxidation-linked disease such as diabetes, cardiovascular disease, arthritis, and cancer. Phenolic phytochemicals are also being targeted to manage bacterial infections associated with chronic disease such as peptic ulcer, urinary tract infections, dental caries, and food-borne bacterial infections (Shetty, 2004).

1.5 PLANTS USED IN THIS STUDY

1.5.1 *Caesalpinia mimosoides* Lamk.

Botany of *Caesalpinia mimosoides* Lamk. (McMakin, 1988; Smitinand, 1984)

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Order Rosales

Family Leguminosae

Genus *Caesalpinia*

Species *Caesalpinia mimosoides*

Synonym : -

Common name : -

Local names : Cha-lueat (ช้าเลือด), Phak-pu-ya (ผักปู่ย่า), Nam-pu-ya (หนามปู่ย่า), Phak-kha-ya (ผักขะยา), Phak-kad-ya (ผักกาดย่า)

Description : Erect or climbing shrub, up to 1 m high, common in old clearings, scrub areas and mixed deciduous forest. Plant densely thorny. Leaves with a 25-40 cm-long rachis; pinnae 10-30 pairs, 3-5 cm long; leaflets 10-20 pairs, opposite, oblong, rounded and mucronate at the tip, unequal at the base, 1 cm long, 4 mm wide. Inflorescence of racemes, terminal, pubescent, spiny, 20-40 cm long; flowers with unequal petals, the upper one obovate, 1-2 cm long, 7 mm wide, the others suborbicular, 1.2-2 cm long, 1-1.8 cm wide, yellow; from October to

February. Pod rounded-truncate, with a beak at the top; seeds ellipsoid, about 1 cm long, 7 mm wide, (Fig. 1.3; a).

Distribution : India, Burma, Laos, Vietnam, China (Yunnan), Thailand

Uses : The young shoots and leaves are locally consumed as fresh vegetable with nam-phrik or sliced and added to larp. The flower buds have a bitter taste and are used in the same ways.

Review of biological activity :

The antioxidant activity of *C. mimosoides* was determined by using a *β*-carotene bleaching method (Chanwitheesuk *et al*., 2002). The activity was measured by a rate of carotene bleaching in the coupled oxidation of linoleic acid and *β*-carotene. This plant exhibited a moderate level of antioxidant activity.

1.5.2 *Coccinia grandis* Voigt.

Botany of *Coccinia grandis* Voigt. (Jacquat, 1990; Nasir and Ali, 1981)

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Order Violales

Family Cucurbitaceae

Genus *Coccinia*

Species *Coccinia grandis*

Synonym : *Coccinia indica* Wight & Arn.

Common name : Ivy gourd

Local names : Tam-lueng (ตำลึง), Phak-khaep (ผักแคบ)

Description : Herbaceous climber, common in hedges or around houses, wild and cultivated. Leaves broadly ovate-cordate, more or less deeply palmatilobate, serrate, 5-10 cm across; petiole 3-6 cm long; tendrils slender. Flowers dioeciously, solitary, bell shaped, 1.5-2 cm long, 1-1.2 cm wide, with 5 ovate, acuminate lobes, white. Berry oblong-ovoid, 2.5-6 cm long, 1.5-3.5 cm wide, red when ripe; seeds pyriform, 6-7 mm long, 3 mm wide, (Fig. 1.3; b).

Distribution : India, Pakistan, Tropical Africa, Asia

Uses : - The tender shoots are an excellent vegetable served with nam phrik, with noodles or fried. They can be used in kaeng-liang or soup made with minced pork. The young green fruit is also an ingredient in kaeng-liang, while the fermented young fruit is mixed in soup, eaten with boiled rice or fried.

- Various preparations of roots, stem, and leaves of ivy gourd have been mentioned in indigenous systems of medicine as being efficacious in the treatment of skin diseases, bronchial catarrh, bronchitis, and diabetes.

Review of biological activity :

Ivy gourd has been used in Ayurveda and Unani system of medicine for treatment of diabetes. Grover *et al*. (2002) reviewed 45 such plants, including ivy gourd, and their products (active, natural principles and crude extracts) that have been mentioned or used in the Indian traditional system of medicine, and have shown experimental or clinical anti-diabetic activity. The plant has shown varying degree of hypoglycemic and anti-hyperglycemic activity.

Kar *et al*. (2003) evaluated comparative hypoglycemic activities of the organic parts (in the form of 95% ethanolic extract) of 30 hypoglycemic medicinal plants on alloxan-diabetic albino rats. The result showed that ivy gourd possessed a significant blood glucose lowering effect within 1 week using only a single dose of its ethanolic extract (250 mg/kg body weight).

The ethanolic extract of ivy gourd was also studied for antioxidant activity in streptozotocin-diabetic rats (Venkateswaran and Pari, 2003). Oral administration of ivy gourd leaf extract (200 mg/kg body weight) for 45 days resulted in a significant reduction in thiobarbituric acid reactive substances and hydroperoxides. The extract also caused a significant increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-s-transferase in liver and kidney of streptozotocin-diabetic rats, which clearly shows the antioxidant property of ivy gourd.

1.5.3 *Gymnema inodorum* Decne.

Botany of *Gymnema inodorum* Decne. (Prodr, 1984)

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Subclass Asteridae

Order Gentianales

Family Asclepiadaceae

Genus *Gymnema*

Species *Gymnema inodorum*

Synonyms : *Cynanchum inodorum* Lour., *Gymnema tingens* Roxb. ex Spreng.

Common name : -

Local names : Phak-sieng-da (ผักเซียงดา)

Description : Lianas to 10 m. Stems glabrous; young branchlets pale gray, lenticellate, puberulent. Petiole 2-6 cm; leaf blade membranous, ovate-oblong to ovate or broadly ovate, glabrous or thin puberulent along veins, base rounded to shallowly cordate, apex acuminate to caudate; lateral veins 4-6 pairs. Inflorescences eventually racemelike with sessile umbel-like cymes arranged in spiral, up to 4 cm; peduncle 1-2 cm. Pedicel 1-1.5 cm. Sepals oblong, 2-3x1.4 mm, shorter than corolla tube, puberulent, ciliate, (Fig. 1.3; c).

Distribution : China, India, Nepal, Philippines, Vietnam, Thailand

Uses : - The young shoots and leaves are served raw with nam-phrik or larp. They are also used in the mixed vegetable soup, kaeng-liang.

- All parts are used medicinally for hyperglycemia, infantile paralysis, and pulmonary tuberculosis.

Review of biological activity :

G. inodorum, which belongs to the same group as *G. sylvestre*, has been known to have the same effects on some diseases as *G. sylvestre* does, including diabetes, rheumatoid arthritis, and gout. The effects of glucose availability of some saponin fractions extracted from the leaves of of *G. inodorum* were studied on the high K^+-induced contraction of guinea-pig intestinal smooth muscle, O_2 consumption on guinea-pig ileum, glucose-evoked transmural potential difference of guinea-pig

everted intestine, and blood glucose level in glucose tolerance tests on rats (Shimizu *et al*., 1997). The results showed that the extracts of the plants suppressed the intestinal smooth muscle contraction, decreased the O_2 consumption, inhibited the glucose evoked-transmural potential, and prevented the blood glucose level. Their studies suggest that the component of *G. inodorum* inhibits the increase in the blood glucose level by interfering with the intestinal glucose absorption process.

Shimizu *et al*. (2001) evaluated the pharmacological properties of the four components from *G. inodorum* leaf extracts, which were purified by their single peaks on HPLC, in experiments on the high K^+-induced contraction of ileal longitudinal muscle of guinea-pig, measurement of glucose evoked-transmural potential difference in an inverted intestine isolated from guinea-pig, and the glucose telerance test by oral administration of glucose in rats. The extracts from *G. inodorum* leaves inhibited glucose absorption in the isolated intestinal tract, and suppressed the increased blood glucose in rats. They also examined the relationship between chemical structure and pharmacological activity of the purified components, which were derivatives of (3β, 4α, 16β)-16, 23, 28-trihydroxyolean-12-en-3-yl-β-D-glucopyranosiduroic acid. The study suggests that the inhibitory effect of triterpenoids from *G. inodorum* leaves on glucose absorption from the intestinal tract is associated with $-CH_2OH$ at 4β of aglycone.

1.5.4 *Pimpinella anisum* Linn.

Botany of *Pimpinella anisum* Linn. (Flück, 1988; Grieve, 1974, Stobart, 1977; Uhl, 2000)

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Order Apiales

Family Apiaceae

Genus *Pimpinella*

Species *Pimpinella anisum*

Synonym : -

Common name : Anise

Local name : Tian-pom (เทียนป้อม)

Description : Anise is a dainty, white-flowered umbelliferous annual, about 18 inches high, with secondary feather-like leaflets of bright green. Leaves and seeds are produced in large, loose clusters. Lower leaves entire, rounded or reniform, the upper ones dissected with 2 or 3 lobes, each with a pointed apex. Small white flowers in slender umbels. Ripe fruits brownish-gray with distinct ribs. The entire plant has a characteristic aromatic odor, (Fig. 1.3; d).

Distribution : Mediterranean countries, Western Asia, Southern Europe

Uses : - Anise is used in flavoring sweets and creams, also in cakes and bread in Europe. It is also used in certain types of curry in India.

- Traditionally, Europeans used anise to treat epilepsy and to ward off evil. The Aztecs drank tea made from its flowers and leaves to relieve coughing. Anise can dispel gas and aid digestion, improve appetite, and alleviate cramps, nausea, and colic in infants. Anise is commonly used in lozenges and cough syrups because it is a mild expectorant. It also soothes insect bites, and is chewed to induce sleep. In India, anise seeds are served after meals to aid digestion and sweeten breath.

Review of biological activity :

Anise has been used as a traditional aromatic herb in many drinks and baked foods because of the presence of volatile oil in its fruits commonly known as seeds (anise seed or aniseed). Several biological properties of anise seed oil have been reported. Pourgholami *et al*. (1999) investigated anticonvulsant effects of an essential oil of anise against seizures induced by pentylenetetrazole (PTZ) or maximal electroshock (MES) in male mice. The essential oil suppressed tonic convulsions induced by PTZ or MES. It also elevated the threshold of PTZ-induced clonic convulsions in mice.

Boskabady and Ramazani-Assari (2001) studied the relaxant effect of anise on isolated guinea-pig tracheal chains. In this study, relaxant (bronchodilatory) effects of essential oil, aqueous and ethanolic extracts of the plant in comparison with both saline and theophylline were examined by a more standard method. This report showed a relatively potent relaxant effect of anise on the tracheal chains of guinea-pig. The result also showed that the relaxant effect of this plant is not due to an inhibitory

effect of histamine or stimulatory effect of β_2-adrenergic receptors, but due to inhibitory effects on muscarinic receptors.

Kreydiyyeh *et al*. (2003) studied the effect of anise oil on transport processes through intestinal and renal epithelia, and determined its mechanism of action. This work has demonstrated a stimulation of the Na^+-K^+ ATPase activity in the jejunum and kidney which resulted in an increase in glucose absorption and a decrease in the volume of urine produce. The oil did not, however exert any effect on water absorption from the colon nor on its ATPase activity. The incorporation of aniseed oil in the diet will thus make a greater portion of ingested glucose available in blood for use by the cells, and in a hot and dry weather, the oil would help conserve water in the body and prevent dehydration.

Ponce *et al*. (2003) investigated the antimicrobial activity of several essential oils on the native microflora of Swiss chard. They reported that the essential oils of anise seed, including *Eucalyptus globules* (eucalyptus), *Melaleuca alternifolia*, and *Syzygium aromaticum* (clove) exhibited the highest level of antimicrobial activity.

The antioxidant and antimicrobial activities of aqueous and ethanolic extracts of anise seed were investigated by Gülçin *et al*. (2003). Both extracts of anise seed showed strong antioxidant activity, reducing power, free radical (1,1-diphenyl-2-picryl-hydrazyl (DPPH), superoxide anion, and hydrogen peroxide) scavenging, and metal chelating activities when compared with different standards such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol. In addition, 250 μg of both anise seed extracts possessed noticeable antimicrobial activity against Gram positive and Gram negative bacteria when compared with

standard and strong antimicrobial compounds such as micronazole nitrate, amoxicillin-clavulanic acid, ofloxacin, and netilmicin.

1.5.5 *Polygonum oderatum* Lour.

Botany of *Polygonum oderatum* Lour. (Jacquat, 1990)

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Subclass Caryophyllidae

Order Polygonales

Family Polygonaceae

Genus *Polygonum*

Species *Polygonum oderatum*

Synonym : *Persicaria oderata* Lour.

Common name : Vietnamese mint

Local names : Phak-phai (ผักไผ่), Chan-chom (จันทน์โฉม), Phrik-ma (พริกม้า)

Description : Annual herbaceous weed, creeping and erect, 30-35 cm high, usually found in ditches or on the banks of ponds. Stem furrowed; adventitious roots at the nodes. Leaves alternate, entire, lanceolate or ovate-lanceolate, attenuate at the base in a short petiole, with hairy margin and nervures; ocreae membranous. Inflorescence of long slender spikes

or clusters; perianth of the flowers white or purplish-pink. Fruit trigonal, acuminate at both ends, 1.5 mm long, shiny, (Fig. 1.3; e).

Distribution : Southeast Asia

Uses : The shoots and leaves have a strong odor and are eaten raw with nam-phrik or sliced and put into larp or curries.

1.6 PURPOSE OF THE STUDY

In this study, the phenolic extracts from some indigenous plants will be screened for antimicrobial activity against both bacteria and fungi. The plants which possess high level of the activity will be selected and then their antimicrobial components will be investigated. The antimicrobial compounds of the selected plants will be separated, purified (or partially purified), and identified. The structure of the active substance will be chemically modified in order to enhance the activity. The antimicrobial activity of the modified substance will then be examined compared with that of its parent structure.

(a)

(b)

(c)

(d)

(e)

Figure 1.3 The plants used in this study

(a) : *Caesalpinia mimosoides* Lamk.; (b) : *Coccinia grandis* Voigt.;

(c) : *Gymnema inodorum* Decne.; (d) : *Pimpinella anisum* Linn.;

(e) : *Polygonum oderatum* Lour.