CHAPTER II

LITERATURE REVIEW

I Botanical, Chemical and Pharmacological Aspects of *Garcinia mangostana* Linn.

1. Botanical aspects of *Garcinia mangostana* Linn.

Garcinia mangostana Linn. is in the family of Guttiferae, its common name is mangosteen (Figure 1). It is a slow-growing tree usually progate by seeds and mostly proliferates in hot and humid climate, preferably with a short dry season such as in India, Thailand, Indonesia and Philippines.

Garcinia mangostana Linn. is a tree, 7-12 m high, having straight trunk, brown to blackish bark, young branch quadrangular, exuding yellow gum-resin. Inner bark exudes an opaque yellow gum resin. Leaves are simple without stipules and decussate, opposite, ovate or elliptic-oblong, 6-11 cm wide, 15-25 cm long, with dark green and grossy above, yellowish green below. Flowers are solitary or in pair near the twig ends, yellowish green with red edges or almost entirely red. Fruit is globose 4-7 cm in diameter, having short and thick stalk, dark purple with four persistent sepals at the base. Seed embedded into a thick, whitish, edible and juicy pulp (Fransworth, and Bunyapraphatsara, 1992; Wong, 2002).

2. Chemical components of *Garcinia mangostana* Linn.

The chemical studies on the constituents of the fruit rind of *Garcinia mangostana* Linn. have revealed that the major substances are xanthones, the others component are cathecol triterpenoid, benzophenone (maclurin) and anthocyanin glycosides (Fransworth, and Bunyapraphatsara, 1992).

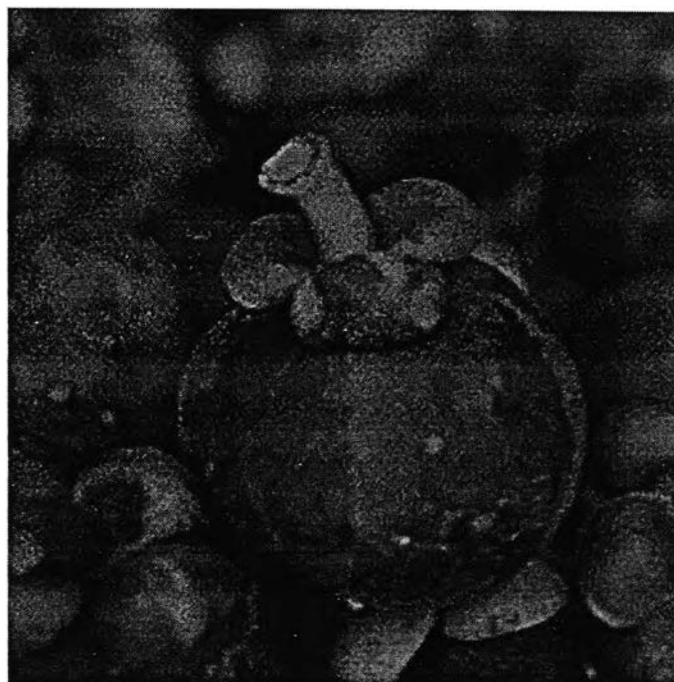

Figure 1 *Garcinia mangostana* fruit

Xanthones are highly active plant phenols found in a limited number of plants. They are formed by the condensation of a phenylpropanoid precursor. Hydroxyxanthones are mainly found in Guttiferae and Gentianaceae. Xanthones show considerable biological activities such as strong antimicrobial, anti-inflammatory and antitumor activities (Wong, 2002).

The major xanthones from fruit rind of *Garcinia mangostana* Linn. are α, β, and γ- mangostin, gartanin, 8-deoxygartanin and garcinones A, B, C and E, all of them have mono or diprenylated skeleton (Mahabusarakam, Wiriyachitra, and Tayler, 1987; Sen et al., 1980, 1981).

Mangostin or α-mangostin (1,3,6-trihydroxy-7-methoxy-2,8-bis-(3-methyl-2-butenyl)-9*H*-xanthen-9-one; $C_{24}H_{26}O_6$), a prenylated xanthone is the most active component. It is isolated from various parts of *Garcinia mangostana*. Isolated from fruit rind by extracted with hexane or benzene, it appears as yellow crystals with melting point of 181.6 – 182.6 °C and molecular weight of 410.46. It is soluble in alcohol, ether, acetone, chloroform and ethyl acetate but practically insoluble in

water. UV max in ethanol is 243, 259, 318 and 351 nm. (Mahabusarakam, Wiriyachitra, and Tayler, 1987; Budavari, 2001).

Moreover, various chemical compounds from fruit rind were reported as follows: (Sen et al., 1980; Mahabusarakam, Wiriyachitra, and Taylor, 1987; Balasubramanian and Rajagopalan, 1988; Parveen et al., 1991; Fransworth, and Bunyapraphatsara, 1992; Asai et al., 1995; Chairungsrilerd, Takeuchi et al., 1996; Gopalakrishnan, Banumathi, and Suresh, 1997; Gopalakrishnan and Balaganesan, 2000; Huang et al., 2001; Nilar and Harrison, 2002; Suksamrarn et al., 2002).

- 1-isomangostin
- 1-isomangostin hydrate
- 3-isomangostin
- 3-isomangostin hydrate
- BR-xanthone-A and B
- normangostin
- mangostin-3,6-di-O-glucoside
- cyanidin-3-O-β-D-sophoroside
- kolanone
- 1,5-dihydroxy-2-isopentenyl-3-methoxy xanthone
- 1,7-dihydroxy-2-isopentenyl-3-methoxy xanthone
- 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylut-2-enyl)-2(H),6(H)-pyrano-(3,2,6)-xanthen-6-one
- 3-O-methyl mangostin
- n-triacontane
- mangostin triacetate
- D-fructose and D-glucose
- garcimangosone A B C and D
- garcinone
- gartanin
- mangostanol
- mangostenol
- mangostenone A and B

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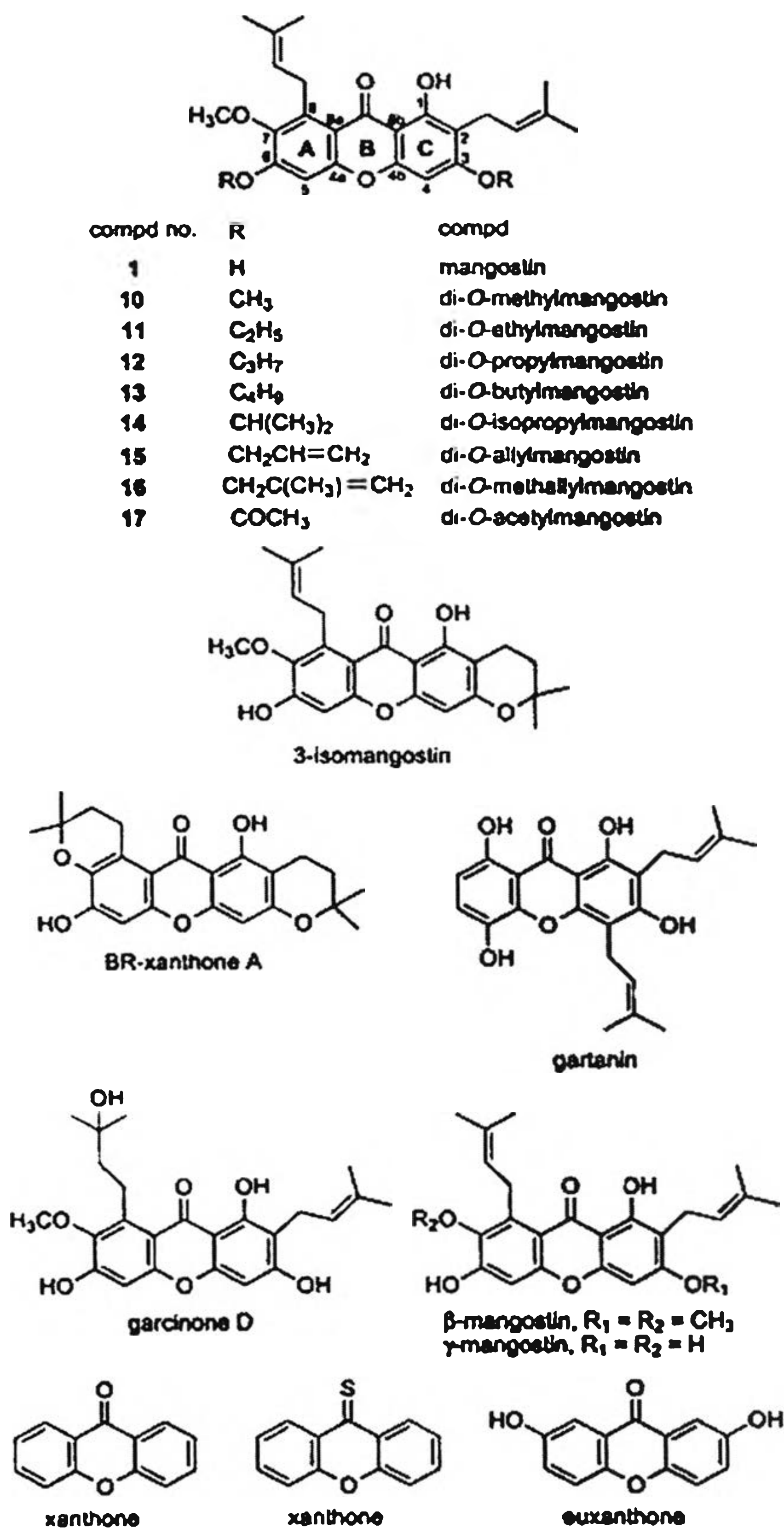

Figure 2 Chemical structures of some components in *Garcinia mangostana* Linn.

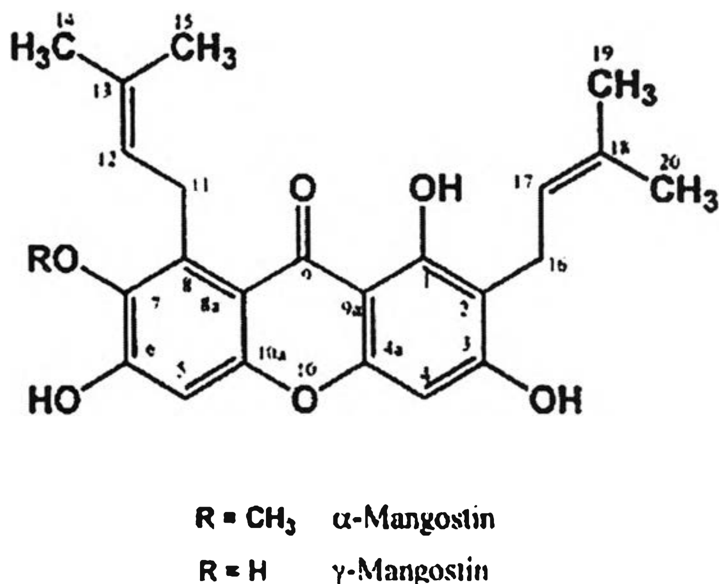

Figure 3 Chemical structure of α-mangostin and γ-mangostin

3. Pharmacological activities and toxicities of *Garcinia mangostana* Linn.

3.1 Pharmacological activities

There are many investigations on pharmacological activities of xanthones from *Garcinia mangostana* Linn.

3.1.1 Antimicrobial activity

There are many reports on the antimicrobial activity of extracts of fruit rind and other parts from *Garcinia mangostana*. Mangostin and its derivatives exhibited inhibitory effect against *Staphylococcus aureus*, bacteria causing wound infection (Mahabusarakam et al., 1983; Sundaram et al., 1983). It was found that the extracts have an intense antimicrobial activity against both methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) nearly equal to vancomycin (Fransworth, and Bunyapraphatsara, 1992; Iinuma et al., 1996; Voravuthikunchai and Kitpipat, 2005). Other studies showed that α-mangostin was found to be active against vancomycin resistant *Enterococci* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA), two leading causes of nosocomial infections (Phongpaichit et al., 1994; Sakagami et al., 2005).

The extract from *Garcinia mangostana* was tested for the activity against *Streptococcus mutans*, bacteria causing dental caries, and *Streptococcus sanguis* (a clinical isolate). The results showed that the extract exhibited significant antibacterial activity against both bacteria. (Hiranras, 2001; Tan, 2004).

The ethyl acetate extract from fruit hulls was determined antimicrobial effect against *Porphyromonas gingivalis* W50 with the MIC value of 20 μg/ml and the MBC value of 40 μg/ml. *Porphyromonas gingivalis*, which are gram-negative anaerobic bacteria generated volatile sulfur compounds caused oral malodor. Moreover, this bacteria has important role in periodontal diseases (Tan, 2004).

Moreover, the activity against dysenteric bacteria and diarrheal infecting bacteria such as *Shigella dysenteriae, Shigella sonnei, Escherichia coli, Salmonella typhimurium, Vibrio cholera, Pseudomonas aeruginosa* and *Bacillus subtilis* have been reported. (Sundaram et al., 1983; Sindermsuk and Deekijsermphong, 1989; Fransworth, and Bunyapraphatsara, 1992).

3.1.2 Antimycobacterial activity

Prenylated xanthones isolated from the fruit hulls, edible arils and seeds of *Garcinia mangostana* were tested for their antituberculosis potential, α-mangostin, β-mangostin and garcinone B exhibited strong inhibitory effect against *Mycobacterium tuberculosis* with the MIC value of 6.25 μg/ml (Suksamrarn et al., 2003).

3.1.3 Antifungal activity

Mangostin and its derivatives showed the activity against *Trichophyton mentagrophytes, Microsporum gypseum, Alternaria solani, Cunninghamella echinulata* and *Epidermophyton floccosum* but all test compounds had no effect on *Candida albicans* (Fransworth, and Bunyapraphatsara, 1992; Mahabusarakam, Phongpaichit and Wiriyachitra, 1983; Sundaram et al., 1983). Moreover, the activity against three phytopathogenic fungi, *Fusarium oxysporum vasinfectum*, *Alternaria tenuis* and *Dreschlera oryzae*, have been reported (Gopalakrishnan, Banumathi and Suresh, 1997).

3.1.4 Anti-inflammatory activity

Mangostin and its derivatives exhibited anti-inflammatory activity both by intraperitoneal and oral administration in normal and bilaterally adrenalectomized rats when tested by the carageenan-induced pedal edema, cotton pellet implantation and granuloma pouch technique (Fransworth, and Bunyapraphatsara, 1992).

Mangostin, 1-isomangostin and mangostin triacetate produced anti-inflammatory activity both by intraperitoneal and oral routes in rats when tested by carrageenin-induced hind paw edema, cotton pellet implantation and granuloma pouch techniques. These compounds did not produce any mast cell membrane stabilizing effect and the degranulation effect of polymixin B, diazoxide and Triton X-100 on rat peritoneal mast cells in vitro was not prevented. These compounds did not alter the prothrombin time of albino rats. Only mangostin produced significant anti-ulcer activity in rats (Shankaranarayan, Gopalakrishnan and Kasemswaran, 1979).

The crude extracts from fruit hulls were examined the effect on prostaglandin E_2 (PGE_2) synthesis. The study found that the 40% ethanol extracts potently inhibited A23187-induced prostaglandin E_2 synthesis in C6 rat glioma cells in a concentration-dependent manner (Nakatani, Atsumi et al., 2002). In further study, γ-mangostin was isolated and examined the effect on arachidonic acid cascade in C6 rat glioma cells. The results indicated that γ-mangostin exhibited potent inhibitory activity of prostaglandin E2 release induces by A23187, a Ca^{2+} ionophore and competitively inhibited the activities of both constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2) in concentration-dependent manner (Nakatani, Nakahata et al., 2002). These results suggest that γ-mangostin is new useful lead compound for anti-inflammatory drug development.

3.1.5 Antihistamine and antiserotonin activities

A crude methanolic extract inhibited the contractions of isolated thoracic rabbit aortas induced by histamine and serotonin. The extract has been fractionated by siliga gel chromatography. The active compounds were α-mangostin

and γ-mangostin. The results suggested that α-mangostin and γ-mangostin are a histaminergic and a serotonergic receptor blocking agent, respectively. A further study revealed that α-mangostin completely inhibited not only the histamine H_1 receptor-mediated smooth muscle contraction but also the [^{3}H]mepyramine blocking directly to histamine H_1 receptor sites on intact smooth muscle cells. It is suggested that α-mangostin is novel type of histamine H_1 receptor antagonist and may become a valuable leading compound for the development of antihistamines (Chirungsrilerd, Furukawa et al., 1996a, 1996b).

The 40% ethanol extract of mangosteen fruit hull was examined the inhibitory effect on histamine release. The study found that the extract inhibited IgE-mediated histamine release from rat basophilic leukemia (RBL-2H3) cells displayed properties of mucosal-type mast cells. This result suggests that the mangosteen extract may be useful crude drug for treatment of allergy (Nakatani, Atsumi et al., 2002).

3.1.6 Central nervous system depressant activity

Mangostin and its derivatives produced CNS depressant characterized by ptosis, sedation, decreased motor activity potentiation of pentobarbital sleeping time and ether anesthesia in mice and rats. None of the compounds exhibited analgesic, antipyretic and anticonvulsant effects (Shankaranarayan, Gopalakrishnan and Kameswaran, 1979; Fransworth, and Bunyapraphatsara, 1992).

3.1.7 Cardiovascular effect

Mangostin-3,6-di-O-glucoside exhibited significant effects on the cardiovascular systems of frogs and dogs by produced myocardial stimulation and increased the blood pressure which was partially blocked by propanolol (Shankaranarayan, Gopalakrishnan and Kameswaran, 1979; Fransworth, and Bunyapraphatsara, 1992).

3.1.8 Inhibitory effects on HIV-1 protease and viral reverse transcriptase

The ethanol extract of *Garcinia mangostana* showed potent inhibitory activity against HIV-1 protease and viral reverse transcriptase. The activity-guided

purification of the extract resulted in the isolaton of two active, mangostin and γ–mangostin with the IC_{50} values of 5.12±0.41 μM and 4.81±0.32 μM, respectively (Chen, Wan, and Loh, 1996).

3.1.9 Anti-tumor activity

The xanthone compounds from *Garcinia mangostana* were tested the cytotoxic effects on a panel of 14 different human cancer cell lines; 6 types of hepatoma, 4 types of lung carcinoma and 4 types of gastric carcinoma cell lines. The results have shown that garcinone E has a very broad spectrum of cytotoxic effect and cell lines tested could be totally killed. This study suggests that garcinone E may be potential useful for the treatment of hepatocellular carcinoma, lung cancers and gastric cancers (Ho, Huang and Chen, 2002).

The six xanthones from pericarps of mangosteen were investigated the in vitro antileukemic activity and were examined the participation of apoptosis in the cell growth inhibition of human leukemia cell line HL60 using Hoechst 33342 nuclear staining and nucleosomal DNA gel electrophoresis. The results showed that all xanthones exhibited significantly growth inhibition of human leukemia cell line HL60 at 72 hours after the start of treatment of concentrations from 5 to 40 μM. Among them, α-mangostin showed the strongest activity to completely growth inhibition of all leukemia cell lines, especially HL60, NB4 and U937 at 10 μM through the induction of apoptosis (Matsumoto et al., 2003). In another research, the cytotoxic effect against K562, P3HR1, Raji and U937 leukemia cells was evaluated by the colorimetric XTT assay. Results indicated that the extract showed a significant cytotoxic effect against K562 and Raji cells ($p<0.05$). It also possessed moderate anti-U937 activity, but was less effective against P3HR1 cells (Chiang et al., 2004).

The crude methanolic extract from the pericarps of *Garcinia mangostana* was determined of the antiproliferative, apoptic and antioxidative properties using human breast cancer (SKBR3) cell line as a model system. These investigations suggested that the methanolic extract had strong antiproliferation, potent antioxidation and induction of apoptosis. Thus, it indicated that this substance has potential for cancer chemoprevention, which was dose dependent as well as

exposure time dependent (Moongkarndi, Kosem, Kaslungka et al., 2004). In another study, the ethanolic pericarp extracts were tested for antiproliferative activity against SKBR3 human breast adenocarcinoma cell lines using MTT assay. It found that the extracts showed more potent antiproliferative activity at 48 hours than of paclitaxel, which is antitumor drug with the IC_{50} values of 15.45 ± 0.50 and 22.20 ± 2.30 μg/ml, respectively (Moongkarndi, Kosem, Luanratana et al., 2004).

α-mangostin, a prenylated xanthone was investigated apoptotic activity, programmed cell death in PC12 rat pheochromocytoma cells. The study found that α-mangostin had potent apoptotic effect with the EC_{50} value of 4 μM. α - mangostin showed the feature of Ca^{2+}-ATPase inhibition to induce cytochrome C release from mitochondria, resulting in apoptosis with time- and concentration-dependent manners. These results suggest that α -mangostin may become potential pharmacological tool for clarifying the Ca^{2+}-ATPase-dependent apoptotic mechanism (Sato et al., 2004).

The crude α -mangostin was examined the short-term chemopreventive effects on putative preneoplastic lesion involved in rat colon carcinogenesis by dietary administration. This finding showed that crude α - mangostin has potent chemopreventive effects in short-term colon carcinogenesis bioassay system and suggests that longer exposure might result in suppression of tumor development. Therefore, the results suggest that α-mangostin may be a valuable chemopreventive or chemotherapeutic agent (Nabandith et al., 2004).

α -mangostin, isolated from *Garcinia mangostana* revealed highly selective inhibitory activity against acidic sphingomyelinase closely related to apoptosis induction. This active substance and its derivatives were useful in cancer chemotherapy. Deprenyl and benzofenone type congeners of α -mangostin have been synthesized and investigated its activity. The results suggested that removal of the prenyl group of the right side of α -mangostin structure caused loss of the selectivity between acidic sphingomyelinase and neutral sphingomyelinase while the prenyl group of the left side appeared to increase the inhibitory activities (Iikubo et al., 2002; Hamada et al., 2003).

3.1.10 Antioxidant activity

The methanol extract of the fruit hulls was found to exhibit a potent radical scavenging effect. By monitoring the radical scavenging effect, two xanthones, α -mangostin and γ-mangostin were isolated. The antioxidative activity of these two xanthones was measured by the ferric thiocyanate method. It was found that γ-mangostin showed more potent antioxidative activity than BHA and α -tocopherol (Yoshikawa et al., 1994).

Mangostin was investigated the antioxidant effects on metal ion dependent (Cu^{2+}) and independent (aqueous peroxyl radicals) oxidation of human low density lipoprotein (LDL). Mangostin prolonged the lag time to both metal ion dependent and independent oxidation of LDL in a dose dependent manner. Formation of thiobarbituric reactive substances (TBARS), generated in LDL after oxidation, was inhibited with 100 microM of mangostin. Mangostin (100 μM) significantly inhibited the consumption of α-tocopherol in the LDL during Cu^{2+} initiated oxidation. From these results, conclude that mangostin is acting as a free radical scavenger to protect the LDL from oxidative damage which is a critical role in cardiovascular and other chronic diseases (William et al., 1995). In further study, mangostin and structural modification of mangostin were tested the antioxidant activity on an isolates LDL and plasma assay. The results of this study show that structural modification of mangostin can have a profound effect on antioxidant activity. Derivatisation of C-3 and C-6 with aminoethyl derivatives enhances antioxidant activity, which may be related to change in solubility (Mahabusarakam, Proudfoot, and Croft, 2000).

3.1.11 Other activities

Polysaccharides from the pericarps of *Garcinia mangostana* were composed of mainly D-galacturonic acid and a small amount of neutral sugar (L-arabinose as the major one and L-rhamnose and D-galactose as the minor ones). The extract of polysaccharides was studied for immunopharmacological activities by phagocytic test to intracellular bacteria (*Salmonella enteritidis*) and superoxide generation tests. The results showed that the number of *S. enteritidis* in cultured monocyte with extract of pericarps was killed. Superoxide generation test was also done by color reduction of cytochrome C. The extract was stimulating superoxide

production. This study suggests that polysaccharides in the extract can stimulate phagocytic cells and kill intracellular bacteria (*S. enteritidis*) (Chanarat et al., 1997).

3.2 Toxicities of *Garcinia mangostana* Linn.

Toxicological study of mangostin was performed by Sornprasit et al. (1987). The effect of mangostin on the activities of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) enzymes were tested by treated the rats with a high dose of mangostin (200 mg/kg body weight) by intraperitoneal injection. The activities of these two enzymes were increased and and reached the maximal level after 12 hr injection. The activities of enzymes were dose dependent. Another experiment in this study, the effect of mangostin which were forced fed to rats was compared with paracetamol in the same dose (1.5 g/kg body weight). It was found that paracetamol increased the activities of SGOT and SGPT much more than mangostin and the amounts of total liver protein of paracetamol treated rats decreased significantly, whereas mangostin treated rats did not change total liver protein. The results indicate that mangostin has lower hepatotoxicity than paracetamol.

Hepatotoxic effects of xanthones extracted from fruit rind of *Garcinia mangostana* were studied in isolated rat hepatocytes using the release of cellular transaminase (SGOT and SGPT) as the criteria for loss of cell membrane integrity. Malondialdehyde (MDA) formation, glutathione (GSH) content and aminopyrine N-demethylase activity were investigated for the preliminary information of xanthones hepatotoxic mechanism. Carbon tetrachloride (CCl_4) was selected as the reference hepatotoxin. Xanthones demonstrated the dose-related hepatotoxic effect, at 200 μg/ml, by increasing the release of cellular transaminases, decreasing MDA formation and GSH content with no change in aminopyrine N-demethylase activity. CCl_4 (0.08 mM) showed different result, especially the increase in MDA formation, illustrating its different hepatotoxic mechanism. Coadministration of xanthones and CCl_4 exhibited no additive cytotoxic effects. Pretreatment of xanthones (100 mg/kg/day, po., 5 days) prior to isolation of hepatocytes, caused no changes in transaminase activities and MDA formation with the increase in GSH content. In the presence of CCl_4, hepatocytes from xanthone and Tween (as the solvent used to dissolve

xanthones) treated rats demonstrated higher production of MDA than the control hepatocytes. Lowering of GSH content by CCl_4 was also observed, with similar reduction in hepatocytes from the control and Tween treated groups. In xanthone treated hepatocytes, the GSH content was similar to control level after incubation with CCl_4. in conclusion, the hepatotoxic mechanism of xanthones may be different from CCl_4 and may involve cellular GSH level (Sapwarobol, 1997; Pramyothin, Sapwarobol and Ruangrungsi, 2003).

Additionally, It has been reported that the application of 1.5% mangostin cream in the patients with chronic ulcer, produced no harmful side effects, allergic condition and irritation (Panchinda, 1992).

4. The medicinal uses of *Garcinia mangostana* Linn.

Garcinia mangostana Linn. has traditionally been used for a long time for the treatment of wounds and diarrhoea. The medicinal uses are as follows:

1. Root may be treated for irregular menstruation.
2. Bark has been used for washing and healing wounds, treatment of aphthous ulcer and diarrhea.
3. Leaves are useful for treatment of dysentery.
4. Fruit rind is the astringent and has been used for treatment of both normal and infectious wounds including aphthous ulcer, it was also used for treatment of diarrhea and dysentery.

As the traditional recipes of *Garcinia mangostana* Linn. in treatment of diarrhea, the dried fruit rind was boiled with water or saturated calcium hydroxide solution and the extract is taken. For treatment of wounds, the dried fruit rind was rubbed with saturated calcium hydroxide solution as a solvent the suspension was applied over the wound area (Fransworth, and Bunyapraphatsara, 1992).

Considering pharmacological activities of *Garcinia mangostana*, this medicinal plant exhibits many interesting activities. The extracts from this plant can be developed into the pharmaceutical and cosmetic products. However, only few

studies have been developed from this plant such as Hiranras (2001) designed the buccal mucoadhesive films for aphthous ulcer treatment. Panchinda (1992) evaluated the efficacy of 1.5% mangostin cream for chronic wound treatment and reported that it was effective for wound healing and no serious side effect. Kumjorn (2003) compared the healing of foot ulcers in diabetic patients by dressing with mangostin cream and normal saline wet dressing. The results showed that patients who received mangostin cream dressing treatment earned higher healing tares when compared with normal saline dressing treatment and recommended that the mangostin cream is new alternative dressing for promoting wound healing.

II Oral Conditions

The oral cavity is an extremely complicated system contained within a complicated organism. Bacteria are the most numerous, but yeasts (*Candida albicans*) and protozoa (*Entamoeba gingivalis*, *Trichomonas tenax*) occur in many individuals. Oral bacteria include streptococci, micrococci and diphtheroids, together with *Actinomyces israeli* and other anaerobic bacteria. Some of these are able to make very firm attachments to mucosal surfaces and others to teeth which provide a long term, non- desquamating surface (Mims et al., 1995; Mandell, Bennett, and Dolin, 2000).

Table 1 Predominant cultivatable bacteria from various sites of the oral cavity.

Type	Predominant Genus or Family	Total viable count (mean %)			
		Gingival crevice	Dental plaque	Tongue	Saliva
Facultative					
Gram-positive cocci	Streptococcus	28.8	28.2	44.8	46.2
	S. mutans	(0-30)	(0-50)	(0-1)	(0-1)
	S. sanguis	(10-20)	(40-60)	(10-20)	(10-30)
	S. mitior	(10-30)	(20-40)	(10-30)	(30-50)
	S. salivarius	(0-1)	(0-1)	(40-60)	(40-60)
Gram-positive rods	*Lactobacillus*, *Corynebacterium*	15.3	23.8	13.0	11.8
Gram-negative cocci	*Moraxella*	0.4	0.4	3.4	1.2
Gram-negative rods	*Enterobacteriaceae*	1.2	ND	3.2	2.3
Anaerobic					
Gram-positive cocci	*Peptostreptococcus*	7.4	12.6	4.2	13.0
Gram-positive rods	*Actinomyces*, *Eubacterium*, *Lactobacillus*, *Leptotrichia*	20.2	18.4	8.2	4.8
Gram-negative cocci	*Veillonella*	10.7	6.4	16.0	15.9
Gram-negative rods		16.1	10.4	8.2	4.8
	Fusobacterium	1.9	4.1	0.7	0.3
	Porphyromonas (or *Prevotella*)	4.7	ND	0.2	ND
	Bacteroides	5.6	4.8	5.1	2.4
	Campylobacter	3.8	1.3	2.2	2.1
Spirochetes	*Treponema*	1.0	ND	ND	ND

Abbreviation: ND, Not detected.

Essential influences upon oral condition such as oral cavity temperature, salivation, buffer capacity of the saliva, food intake, air flow and microbiological environment conditions. The oral conditions were as follows:

1. Dental pellicle

Within minutes to hours after a tooth has been thoroughly cleaned, dental pellicle, a film that deposits selectively from saliva, covers the tooth. It is this film to which the bacterial mass (known as dental plaque) adheres, and which stains when exposed to chromogenic materials. Dental pellicle consists of glycoproteins selectively absorbed from saliva. Toothbrushing alone is inadequate to remove pellicle and must be used in conjunction with an abrasive. Chemical removal is possible, but only at the risk of damaging the underlying enamel.

2. Dental plaque

Dental plaque is primarily a bacterial accumulation or complex microbial mass containing about 10^9 bacteria per gram. It occurs supragingivally (above the gum line) and subgingivally (below the gum line). Dental plaque generates acid from carbohydrate. And this acid attacks the tooth enamel and initiates formation of the carious lesion. The actions of self-administered oral hygiene procedures are usually limited to the supragingival plaque. Subgingival plaque is best cared by the dental professional (Mims et al., 1995).

Table 2 Selected bacterial species found in dental plaque

Types	Bacterias	
	Facultative	anaerobic
Gram positive	*Sreptococcus mutans*	
	Streptococcus sanguis	
	Actinomyces viscosus	
Gram negative	*Actinobacillus actinomycetemcomitans*	*Porphyromonas gingivalis*
	Capnocytophaga species	*Fusobacterium nucleatum*
	Eikenella corrodens	*Prevotella intermedia*
		Bacteroids forsythus
		Campylobacter species
Spirochetes		*Treponema denticola*

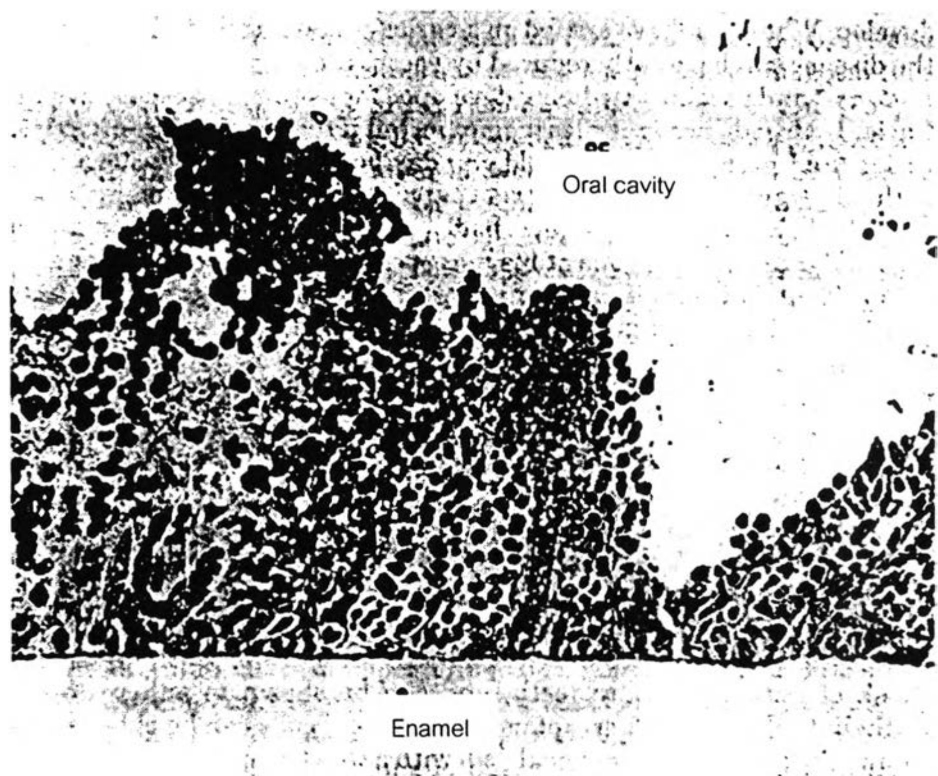

Figure 4 Electron microscope section through dental plaque at gingival margin of a child's tooth, showing microcolonies of cocci. Magnification x 4000.

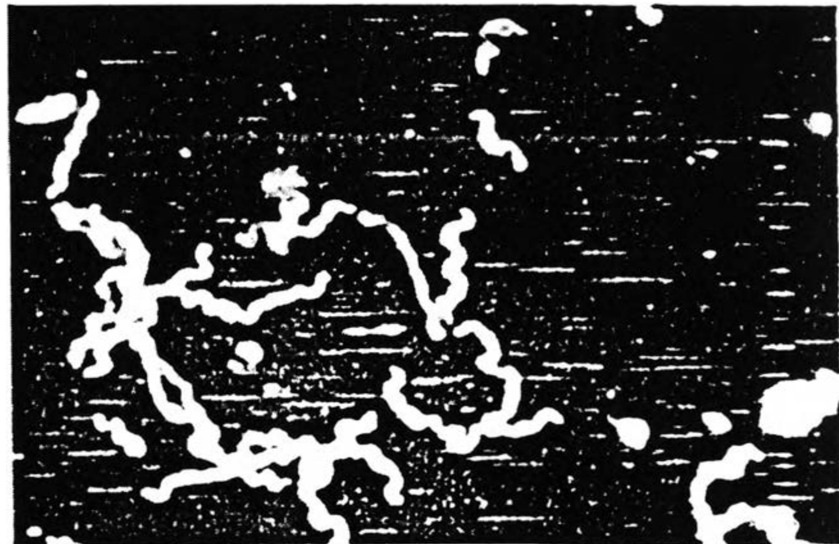

Figure 5 Dark-field microscopic examination of plaque showing small spirochetes.

3. Periodontal disease

Periodontal disease is the general description applied to the inflammatory response of the gingiva and surrounding connective tissue to the bacterial of plaque accumulations on the teeth. These inflammatory responses are divided into two general groupings: gingivitis and periodontitis. Gingivitis is extremely common, and is manifested clinically as bleeding of the gingival or gum tissues without evidence of bone loss or deep periodontal pockets. Pocketing is the term given to the pathologic loss of tissue between the tooth and the gingival, creating spaces that are filled by dental plaque. Periodontitis occurs when the plaque-induced inflammatory response in the tissue results in actual loss of collagen attachment of the tooth to the bone, to loss of bone and to deep periodontal pockets.

The microorganisms involved in periodontal disease are largely gram-negative anaerobic bacilli with some anaerobic cocci and a largely quantity of anaerobic spirochetes. The main organisms linked with deep destructive periodontal lesions are *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Actinomyces viscosis* and *Actinobacillus actinomycetemcomitans*. *Porphyromonas gingivalis* is more frequency detected in severe adult periodontitis.

Porphyromonas gingivalis is one of black-pigmented species that has been associated with dental infections. It could contribute to periodontal disease by releasing endotoxin, various organic acids such as butyric acid, and low molecular weight compounds such as hydrogen sulfide and ammonia into the pocket microenvironment (Gorbach, Bartlett, and Blacklow, 2004).

The level of oral hygiene correlates with the severity of periodontal disease. Periodontal pathology and attachment loss are most severe interproximally and least severe in the buccal sites. Supragingival plague removal by self-administered oral hygiene procedures slows the progression of periodontal disease.

Table 3 Relationship between clinical forms of periodontal disease and various bacterial species

Clinical entity	Bacterial factor
Gingivitis	
Experimantal	Plaque accumulation, streptococci, actinomycetes
Pregnancy	*Prevotella intermedia*
Puberty	*Prevotella intermedia*
Stress (acute necrotizing ulcerative gingivitis)	*Prevotella intermedia*, Spirochetes
Simple	Plaque accumulation, Spirochetes
Generalized severe	Spirochetes
Periodontitis	
Prepuberty	Spirochetes, black-pigmented species
Localized juvenile	*Actinobacillus actinomycetemcomitans*, Spirochetes
Early onset, adult and progressive	Spirochetes, *Porphyromonas gingivalis, Bacteroides forsythus* and *Treponema denticola*

4. Dental caries

Dental caries, which is also called tooth decay, is the gradual destruction of enamel of tooth and thus open a path for bacteria to reach the pulp. *Streptococcus mutans* is important gram positive bacteria that cause dental caries. It is able to form acids by fermenting dietary sugars and maintain sugar metabolism under extreme acidic environment such as carious lesion. The pH in an active caries lesion may be as low as 4.0. Unless the bacteria. the sugar are present, dental caries does not develop (Mims et al., 1995).

Both dental caries and periodontal disease are infectious diseases, caused by dental plaque. Therefore, elimination of bacteria in dental plaque is an essential factor in prevention and treatment of the diseases. Clinical study indicated that scaling and root planning, in combination with optimal oral hygiene, results in an alteration of the

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subgingival plaque which is sufficient to stop periodontal destruction. Thus oral hygiene is important for the clinical outcome of the treatment.

5. Oral malodor

Oral malodor is also commonly known as "bad breath," "foul breath," or "halitosis." It has been estimated that 9 of 10 persons exhibit oral malodor on arising in the morning. In healthy persons, over 90% of the malodors are produced by local oral conditions. "Morning mouth" has been attributed to reduced activity of the tongue and cheeks during sleep, along with reduced salivary flow, all of which enhance activity of the oral bacterial flora, some of which generate volatile, odoriferous sulfur compounds. These compounds consist primarily of hydrogen sulfide, methyl mercaptan, and dimethyl sulfide (Pader, 1988; Williams and Schmitt, 1996).

Oral microorganisms that play an important role in the production of malodor were *Peptostreptococcus, Eubacterium, Selenomonas Bacteroides*, and *Fusobacterium*. From these species, specific microorganisms such as *Porphyromonas gingivalis, Treponema Denticola,* and *Porphyromonas endodontalis* tend to be associated with periodontitis infections and are rarely found in a healthy mouth.

Mouth malodor can be controlled by vigorous oral hygiene practice. Most of the hydrogen sulfide and methyl mercaptan in periodontitis-free individuals derive from the dorsoposterior surface of the tongue and can be considerably reduced by tongue brushing, tooth brushing, food ingestion and use of an oral rinse that reduces oral bacterial populations.

III Oral Hygiene

The bacteria present in plaque produce organic acids that demineralize tooth enamel and cause cavities as well as endotoxins, which are the cause of gingivitis. Even the cleanest teeth are from a physiological standpoint still coated by a film of the saliva's glycoproteins, the so-called tooth cuticle or pellicle. When oral hygiene is

intermittent or totally lacking, a gradual collection of microorganisms begin to appear on this layer. This flora is aided by the settling of dead mucous membrane and vascular cells. The bacterial coating in considered to serve as the starting point of all periodontal diseases. Removal of this bacteria deposit provides a good means of prevention. Oral hygiene must be continued daily, since tooth deposits form continuously.

Oral hygiene practice plays two major roles

1. Maintenance of personal confidence in appearance and social contacts such as malodor and staining.
2. Prevention of dental caries and periodontal diseases by reducing the effects of bacterial population

As mentioned previously, toothbrushing is key, but inadequate when carried out by most persons, therefore supplements such as special mechanical aids and chemical agents applied through dentifrices and oral rinses are desired.

1. Oral hygiene products (Williams and Schmitt, 1996)

The role of oral hygiene products is to keep the teeth free of stain, to freshen the breath and to control dental plaque. Oral hygiene products have been categorized as cosmetic or therapeutic, depending on whether or not they contain a health benefit agent.

1.1 Toothbrush

The toothbrush has major functions to remove dental plaque and to work with toothpaste to remove dental stain. The toothbrush also provides a mechanism with which to apply toothpaste for its secondary benefits, such as anticalculus activity. The toothbrush can remove soft dental plaque.

1.2 Mechanical devices

Mechanical devices to cleanse the teeth are becoming increasingly sophisticated. These are available which not only cleanse the teeth, but also are able to deliver agents to the dentition, e.g. anti-plaque agents from water irrigators. The

purpose of special mechanical devices (other than the toothbrush) is to compensate for the inability of most consumers to realize the full potential of the toothbrush.

1.3 Toothpaste

Toothpaste fulfills two primary functions; removal of stain from the teeth and freshening of the breath and mouth. Mild abrasives and appropriate flavors, respectively, accomplish these objectives. These basic and essential functions are being supplemented, but not reduced in importance, as it has become recognized that additional functions can be beneficially built into toothpaste.

1.4 Tooth powder

Tooth powder is a forerunner of toothpaste. Once popular, it now has a very low level of popularity. Tooth powder is a physical mix of dental abrasive, flavor and foaming aid. Tooth powders differ from toothpaste in their lack of moisturizing agents such as glycerin, sorbitol and water, while abrasive content can reach 90% of their total volume (Umbach, 1991). Tooth powder is packed in a rigid container equipped with an orifice from which the powder can be shaken. Some users prefer to pour the powder onto the hand and scoop it up onto the toothbrush, others to dispense it directly onto the toothbrush. The disadvantages of tooth powder compared with toothpaste and the difficulty of obtaining predictable doses of health benefit agents at each use has led to the virtual demise of tooth powder as a major oral care product.

1.5 Oral rinses

Oral rinses are characterized by two common features as antimicrobial action and ability to freshen the breath. The antimicrobial activity is credited with several benefits such as reduction the dental plaque, maintain of periodontal health, and reduction of oral malodors of microbiological origin. Breath freshening is attributable to both the flavor and the cleansing action of the rinse.

Oral rinse products can be categorized broadly as aqueous, aerosol sprays, concentrates and specially forms, such as powders, to be reconstituted with water before use and fast dissolving strips.

2. Determination of the *in vitro* antimicrobial effect of oral hygiene preparations

It is especially important in assessing any mouth and tooth care preparation to know the intensity and scope of its antimicrobial action. The serial tube dilution test is selected to a quantitative method which enables a reasonably exact measurement of a preparation's Minimal Inhibition Concentration (MIC). The test results can, however, be influenced by a number of factors, the most important being the type, amount, rate of growth of the test strain, the type and chemical properties of the test substances, as well as the stability of the active ingredient in the reactive environment.

The microbial test including the usual indicator germs, such as *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, and oral germs such as *Streptococcus mutans,* the most serious caries inducer and *Streptococcus silvarius*, as well as *Lactobacillus odontolyticus*. Generally, it is not possible to make a direct comparison between, for instance, a gel toothpaste and a ready-to-use mouthwash regarding their antimicrobial effectiveness (Umbach, 1991).

IV Fast Dissolving Drug Delivery

Many pharmaceutical dosage forms are administered in the form of tablets and capsules that designed for swallowing or chewing to deliver a precise dosage of medication to patients. However, some patients, particularly pediatric and geriatric patients, have difficulty in swallowing or chewing solid dosage forms. In order to assist these patients, several fast dissolving drug delivery systems have been developed. Fast dissolving drug delivery systems are dosage forms which dissolve or disintegrate rapidly upon contact with the moist mucosal surfaces of the oral cavity without the need for water or chewing.

1. Types of fast dissolving dosage forms

Fast dissolving dosage forms were divided in 2 major units as fast dissolving tablet and fast dissolving films/strips.

1.1 Fast dissolving tablet (Rathbone, Hadgraft, and Roberts, 2003)

The oral fast dissolving tablets are also known as fast dispersing and quick disintegrating tablets, however the function and concept of all these dosage forms are similar. The original "fast dissolving tablets" are the molded tablets for sublingual use. These tablets generally consist of active drug and lactose moistened with an alcohol-water mixture to form a paste. The tablets were then molded, dried and packaged.

There currently are no standards that define a "rapidly dissolving tablet" but one that could be considered would be a tablet that disintegrates/dissolves within approximately 15-30 seconds in the mouth; anything slower than about 15-30 seconds would not really be categorized as "rapidly" dissolving (Ansel, Allen, and Popovich, 1999).

Zydis is the first fast dissolving dosage form in the market. This technology is based on the concept of forming an open matrix network containing active ingredient. These are freeze-dried products containing water soluble matrix material and drug, which is performed in blister pockets and freeze dried to remove the water by sublimation. The resultant structures are very porous in nature and rapidly disintegrate or dissolve upon contact with saliva (Kuchekar, Bhise and Arumugam, 2001).

There are a number of disadvantages and difficulties associated with formulating rapidly dissolving tablets, including drug loading, taste masking, manufacturing costs and stability of the product. In addition, many fast dissolving tablets are soft, friable, and/or brittle (such as the lyophilized dosage forms) so they often required specialized and individually expensive packaging and processing due to lacking the mechanical strength common to traditional tablets and more susceptible to degradation via temperature and humidity. These products can be manufactured by a variety of technologies, including direct compression, wet granulation, and freeze drying.

Currently, four fast-dissolving/disintegrating technologies have reached the U.S. market such as Zydis, Wowtab, Orasolv, and Durasolv. Each technology has

a different mechanism. Specific properties of the various fast dissolving technologies are listed in Table 4.

Table 4 Comparison of fast dissolving/disintegrating technologies

Technologies	Manufacturing	Handling/stroage	Drug release /bioavailability
Zydis (R.P. Scherer, Inc.)	Freeze dried	- Packaged in foil - Sensitive to degradation at humidities > 65%	- Dissolves in 2 to 10 seconds - May allow for pregastric absorption leading to enhanced bioavailability
Wowtab (Yamanouchi pharma technologies, Inc.)	Compressed	- Packaged in bottles - Avoid exposure to moisture or humidity	- Disintegrates in 5 to 45 seconds depending upon the size of the tablet -No significant change in drug bioavailability
Orasolv (Cima labs, Inc.)	Lightly compressed	packaged in patented foil packs	-Disintegrates in 5 to 45 seconds depending upon the size of the tablet -No significant change in drug bioavailability
Durasolv (Cima labs, Inc.)	Similar to orasolv, but better mechanical strength	- Packaged in foil or bottles - If packaged in bottles, avoid exposure to moisture or humidity	- Disintegrates in 5 to 45 seconds depending upon the size of the tablet -No significant change in drug bioavailability

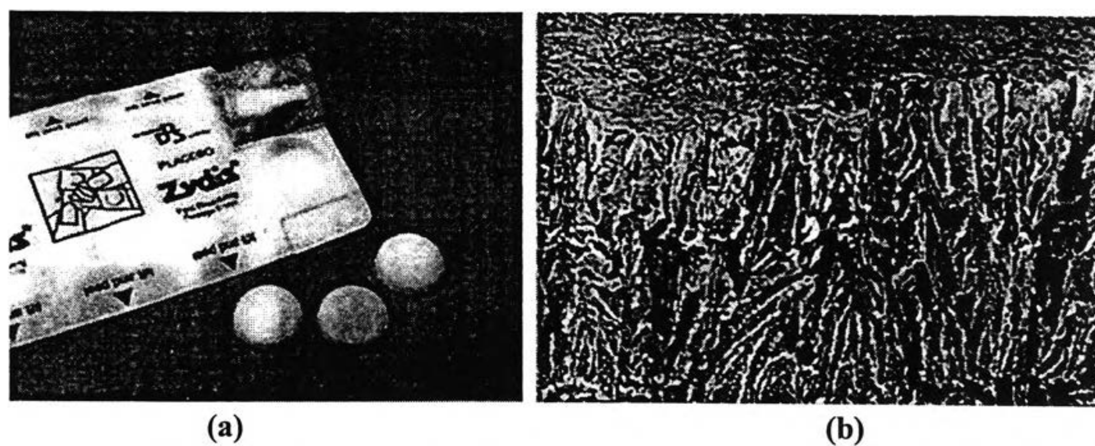

Figure 6 (a) Zydis dosage form and blister pack. (b) Scanning electron micrograph of cross section through a Zydis formulation.

Figure 7 Fast dissolving tablet containing acetaminophen and caffeine (http://www.excedrin.com)

1.2 Fast dissolving films/strips

Fast dissolving film is a thin, flexible, and quick dissolving film in oral cavity. The film is placed on the top or the floor of the tongue and is retained at the

site of application. It is rapidly release the active agent for local and/or systemic absorption. The fast dissolving film is the system that can deliver both pharmaceutical and cosmetic substances. Therapeutic effect of film is to deliver either water soluble or insoluble drug in the oral cavity without the need for water or chewing. Breath freshening films delivering volatile oils have antimicrobial effect by inhibiting microorganisms in oral cavity and enhanced breath freshening during day (Kulkarni, Kumar and Sorg, 2003).

The first step in the development of fast dissolving films is the selection and characterization of the appropriate film forming polymers that give the rapidly dissolving or disintegrating characteristic. The polymers that are commonly used for the development of fast dissolving films including low viscosity grade of cellulose derivatives (e.g. Sodium carboxymethylcellulose, hydroxypropylcellulose, and hydroxypropyl methylcellulose), polysaccharides from bacteria as pullulan, and synthetic polymers such as polyvinyl alcohol (Zerbe and Al-Khalil. 2003).

The fast dissolving films can be manufactured by one or a combination of following processes; hot melt extrusion, solid dispersion extrusion, rolling, semisolid casting, and solvent casting. These fast dissolving oral delivery systems can be packed using various options, such as single pouch, blister card with multiple units, multiple unit dispenser, and continuous roll dispenser, depending on the application and marketing objectives (Figures 8-12) (Borsadia, Halloran and Osborne, 2004).

Drug release from fast dissolving film (Rapifilm®) with the various drug substances such as ambroxol HCl, dextromethorphan HBr, metoclopramide HCl, ketotifen hydrogenfumarate and loperamide HCl were investigated by Mertin (1998). *In vitro* dissolution study showed that the drug was released completely within 2 to 3 minutes (Figure 13). In addition, comparison of loperamide release from Rapifilm® and Zydis® tablet showed that Rapifilm® released drug within 5 minutes completely and independently of the pH value of dissolution medium. Therefore, it can be absorbed immediately by the buccal mucosa faster than Zydis® form (Figure 14).

Figure 8 Fast dissolving films containing vitamin B_{12} (http://www.jamiesonvitamins.com)

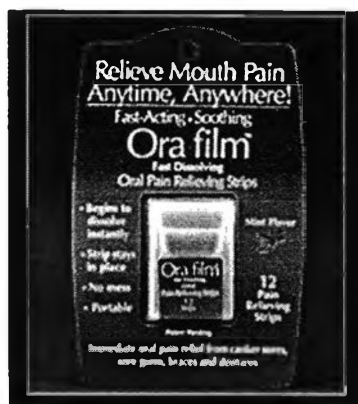

Figure 9 Fast dissolving films for relieve oral pain (http://www.apothecus.com)

(a) (b)

Figure 10 Fast dissolving films containing medicine; (a) dextromethophan HBr; (b) diphenhydramine HCl (http://www.triaminic.com)

Figure 11 Fast dissolving film containing vitamins (http://www.healthymoments.com)

Figure 12 Fast dissolving films containing breath freshening agent for pets (http://www.furlongspetsupply.com)

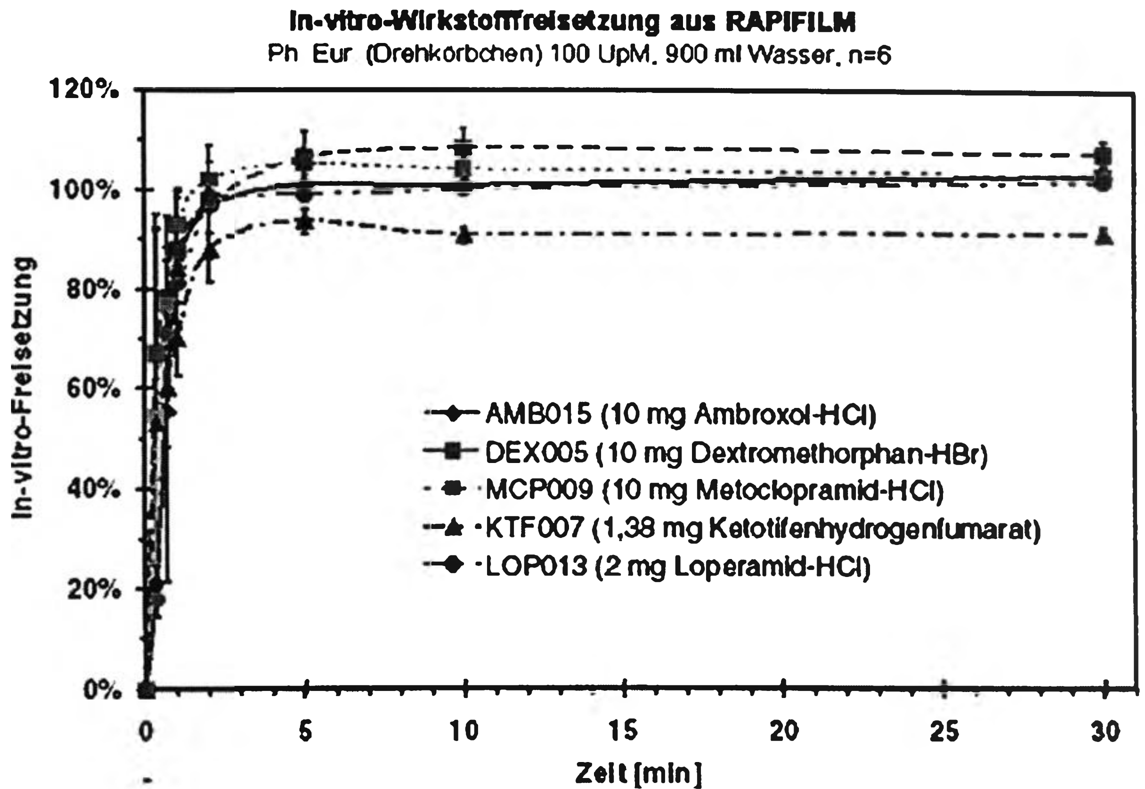

Figure 13 *In vitro* drug dissolution from Rapifilm® containing various drugs

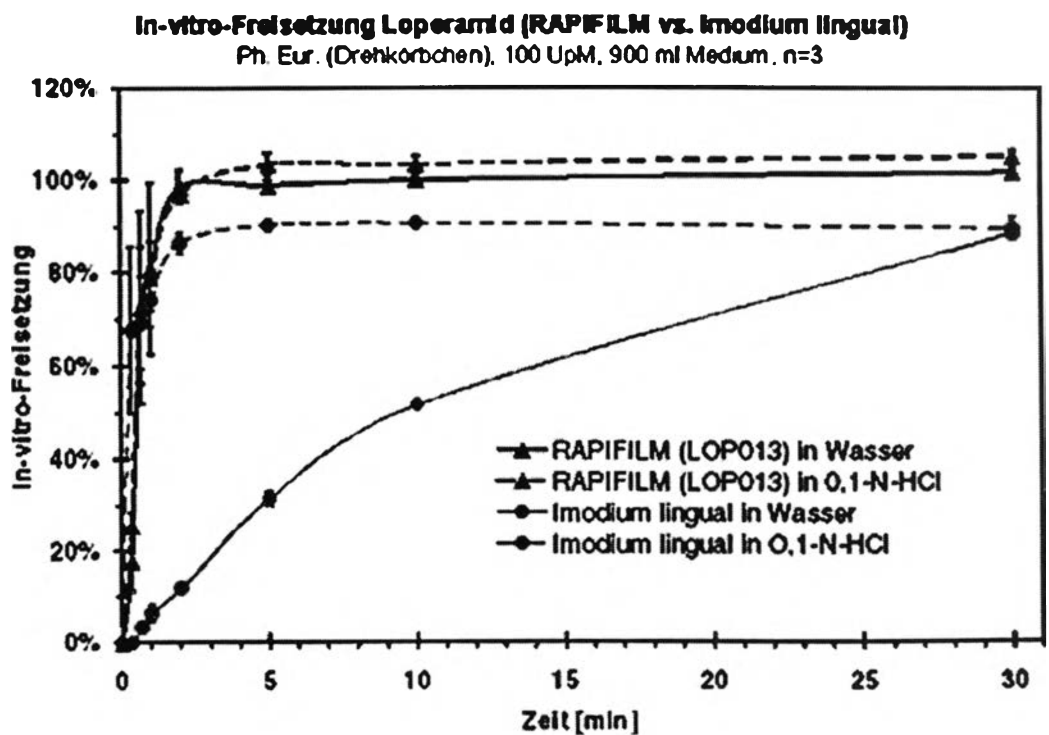

Figure 14 *In vitro* dissolution of loperamide from Rapifilm® and Imodium® lingual in water and 0.1 N HCl

V Evaluation of Films

The evaluation tests designed to study the characteristics and final properties of film bases are carried out by many utilizing laboratory techniques including thermal analysis, mechanical measurements, microscopic examination, and diffusion experiments (Peh and Wong, 1999; Mashru et al., 2005). The most commonly used methods are thermal analysis and mechanical property evaluations. Other test procedures are considered on the basis of needful information in that investigation.

1. Thermal analysis

Measurements of thermal analysis are conducted for the purpose of evaluating the physical and chemical changes that may take place in a heated sample. This requires that the operator interpret the observed events in a thermogram in terms of plausible reaction processes. The reactions normally monitored can be endothermic (melting, boiling, sublimation, vaporization, desolvation, solid-solid phase transitions, chemical degradation, etc.) or exothermic (crystallization, oxidative decomposition, etc.) in nature.

Thermal methods can be used to indicate the existence of possible drug-excipient interactions in a formulation and evaluate compound purity, polymorphism, solvation, degradation (Brittain, 1995).

Thermal properties of the films were examined by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and thermomechanical analysis (TMA). Thermogravimetric analysis is used to determine thermal stability of membranes and the upper limit of thermal stability is usually taken as the temperature at which weight loss of the sample begins. Differential scanning calorimetry is an extremely useful technique for measuring glass transition temperature (Tg), that means the temperature at which a glassy polymer becomes rubbery on heating and a rubbery polymer reverts to a glassy one on cooling. Whereas thermomechanical analysis measures deformation of a substance under a non-oscillatory load and can also conveniently measure transition from a glassy to a rubbery polymer.

Polymers in the rubbery state are very viscous liquids, with relatively high freedom of rotation around the carbon-carbon bonds in the backbone within the constraint of the tetrahedral bond angle. The temperature is high enough so that most bonds can overcome the potential energy barrier against rotation. Rotational freedom results in very flexible chains, segmental or micro-Brownian motion, and changing chain conformations as discussed for polymer solutions. Segmental mobility is considerably smaller in rubbery, liquid bulk polymers than in their solutions because of the much higher viscosity of the former (Martin, Swarbrick, and Cammarata, 1983).

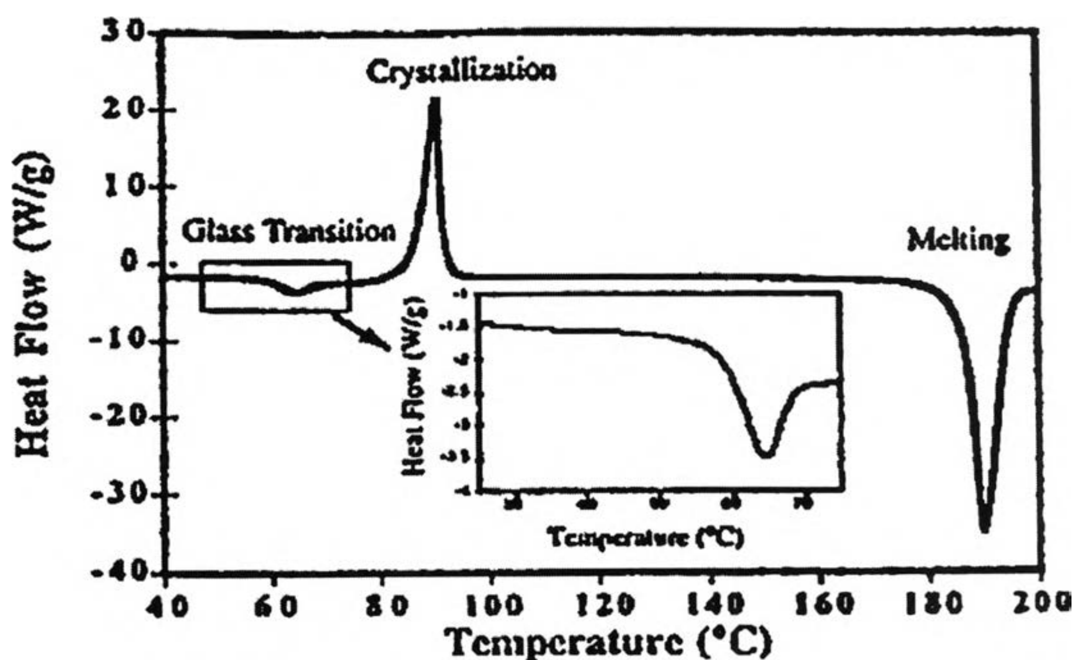

Figure 15 Typical DSC thermogram

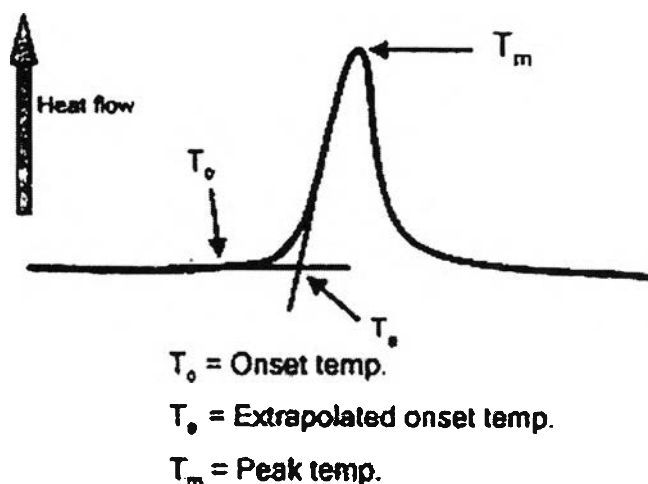

Figure 16 Peak of DSC thermogram

2. Mechanical properties of film (Aulton and Abdul-Razzak, 1981; Martin, Swarbrick, and Cammarata, 1983)

The mechanical properties, tensile strength, percentage of elongation at break and Young's modulus of various films can be evaluated by using a tensile-strength tester. The tensile testing process is to apply increasing tensile load at s constant rate to a film strip which know dimension perpendicular to the cross-section of the film strip until the failure takes place. The load at film failure will be measured in term of force per unit cross-section area of the film.

Polymers are divided into five categories according to a qualitative description of their mechanical behavior and corresponding stress-strain characteristics as showed in the Table 5 and typical mechanical properties of commercial elastomers, plastics and films/fibers as showed in the Table 6.

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Table 5 Qualitative description of polymer and its stress-strain characteristics

Polymer description	Characteristics of stress-strain curve			
	Young's modulus	Yield stress	Tensile strength	Elongation to break
Soft, weak	Low	Low	Low	Low to moderate
Soft, tough	Low	Low	Moderate	Very high (20-1000%)
Hard, brittle	High	None (break around yield point)	Moderate to high	Very low (<2%)
Hard, strong	High	High	High	Moderate (~5%)
Hard, tough	High	High	High	High (cold drawing or "necking")

Hard or stiff polymers are characterized by high moduli as opposed to soft ones. Strong (as opposed to weak) polymers have high tensile strengths. Tough (as opposed to brittle) polymers have large area under their stress-strain curves and require large amounts of energy to break under stress, combining high or at least modulate tensile strength with high elongation. The desirable hard, tough film must have a high yield stress large extension before breaking and high elastic modulus.

A typical stress-strain curve is shown in Figure 17. The ultimate tensile strength or breaking stress is the maximum applied at which the film breaks. Stress is calculated by dividing force by original cross-sectional area. Elongation or strain at break is a measure of the ductility of the films. Strain in tension called elongation. It is calculated by dividing the increase in length by original length. Elastic modulus or Young's modulus is the most basic and structurally important of all mechanical properties and is a measure of the stiffness and rigidity of the film. It is calculated as applied stress divided by the corresponding strain in the region of linear elasticity. Area under curve is a function of the energy or work necessary to break the film and is representative of the film's toughness or brittleness. The energy to break per unit

area is calculated by dividing the area under curve by the volume of the specimen between the clamps.

Table 6 Typical mechanical properties of commercial elastomers, plastics and films/fibers

End use	Tensile strength (psi x 10^{-3})	Young's modulus (psi)	Ultimate elongation (%)
Elastomers	1-4	10-400	300-900
Plastics	2-12	10^4-5 x 10^5	2-200
Films/Fibers	20-100	10^5-10^6	10-50

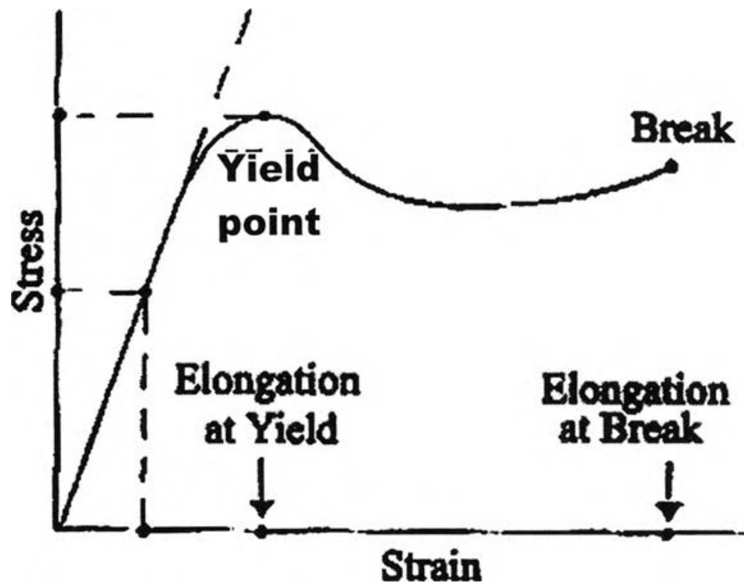

Figure 17 Typical stress-strain curve