

CHAPTER 2

LITERATURE REVIEW

2.1 *Phyllanthus emblica* L.

2.1.1 Taxa and Classification of *Phyllanthus emblica* L. (4, 27)

Kingdom	: Plantae (Plants)
Subkingdom	: Tracheobionta (Vascular plants)
Superdivision	: Spermatophyta (Seed plants)
Division	: Magnoliophyta (Flowering plants)
Class	: Magnoliopsida (Dicotyledons)
Subclass	: Rosidae
Order	: Euphorbiales
Family	: Euphorbiaceae (Spurge family)
Genus	: <i>Phyllanthus</i> L. (leaf flower)
Species	: <i>Phyllanthus emblica</i> L. (emblic)

Plant Synonyms : *Emblica officinalis* Gaertn.

Common names : Emblica, Amala, Indian gooseberry

Phyllanthus emblica Linn., formerly *Phyllanthus emblica* L., is known in Thai name as Ma-Kham-Pom (4, 5) and commonly called Emblica, Amala or Indian gooseberry. It has a habitat in tropical and subtropical forest in South East Asia and South Asia.

Description of the plant: (5, 6)

P. emblica is a small or medium-sized tree, up to 20 m high, deciduous, with crooked trunk and spreading branches; bark greenish gray, peeling off in conchoidal flakes; branchlets glabrous or finely pubescent 10 to 20 cm long.

Leaves imbricate when young, subsessile, 0.5 to 2.5 cm by 1.5 to 5.5 mm, closely set along the branchlets, distichous, light green, glabrous, narrowly-linear, obtuse, having appearance of pinnate leaves; stipules minute, ovate, finely acute.

P. emblica is flowering in May to July, male and female flowers borne in the same tree. The flowers are small, monoecious, apetalous, greenish yellow, in axillary fascicles on the leaf-bearing branchlets, often on the naked portion below the leaves, with fimbriate bracts at the base.

Male flowers are numerous, on short slender pedicel; calyx-lobes 6, oblong, obtuse, 1.2 mm long; anthers 3; filaments united in a short central column; disk-glands 6 alternating with the calyx-segments. Female flowers are few, subsessile or sessile; calyx as in the male; ovary 3-celled, half immersed in the lacerate, cup-shaped disk; style connate at the base; stigmata 3, beebled, lobes dilated, recurved.

Fruits are sessile, 1.3 to 2.7 cm in diameter, fleshy, globose or depress globose, with 6 longitudinal faint lines, glabrous, lucid, pale yellow; endocarp of triangular cocci, bony, dehiscent, with 3 short bundles of vascular tissue at the base. It has 6 seeds with trigonous. The scattered picture of female, male flower and fruiting twig of *P. emblica* is shown in Figure 1 and the figure of *P. emblica* fresh and dried fruits is shown in Figure 2.

Medicinal uses: The fruit of *P. emblica* is a rich source of vitamin C and phenolic compounds (18) It is used in anaemia, jaundice, and dyspepsia. It is a good liver tonic and also used as expectorant, antidiuretic, antidiarrheal, antipyretic, and antiscury (5).

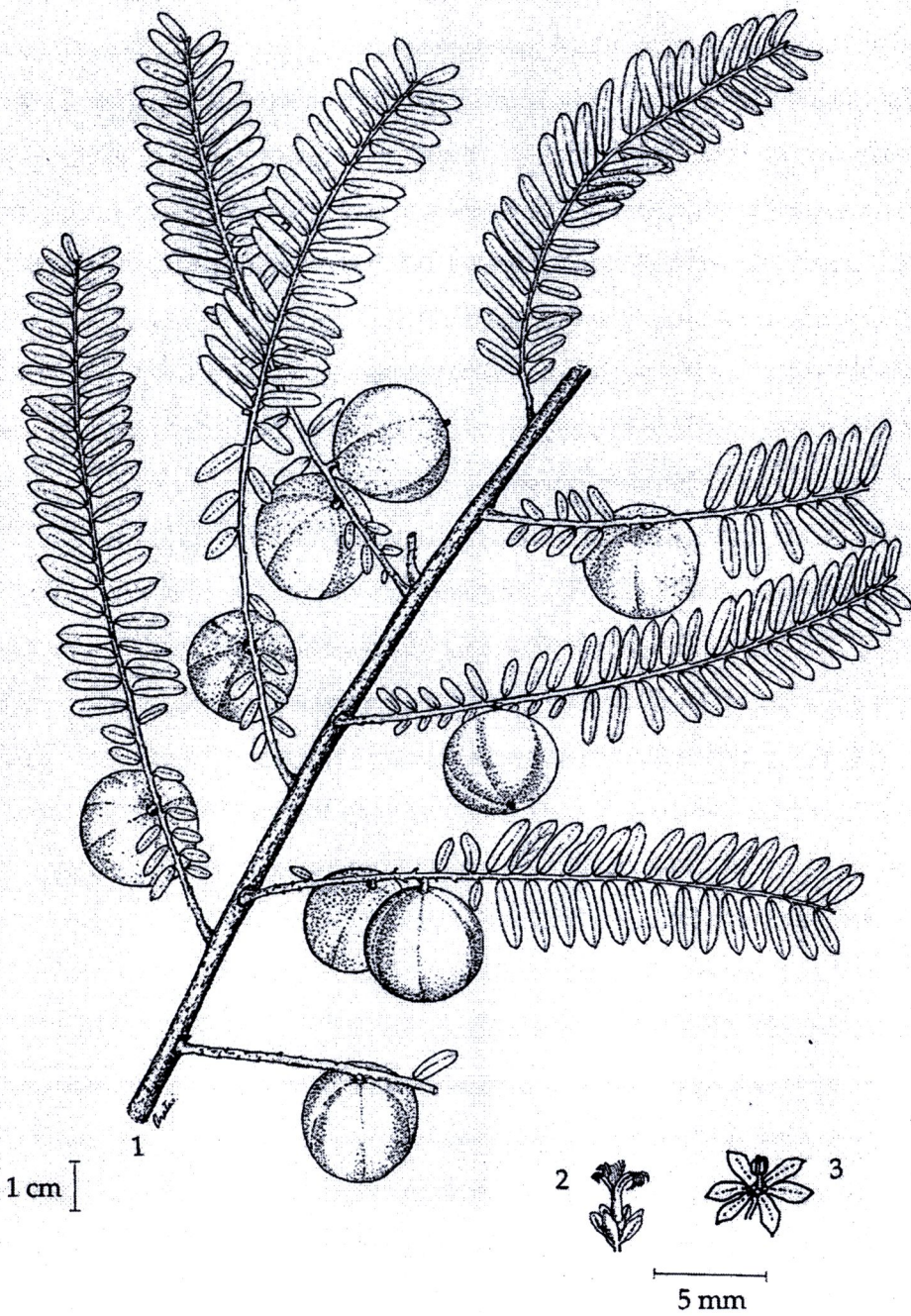


Figure 1 *P. emblica*. 1. fruiting twig, 2. female flower, and 3. male flower
modified from Thai herbal pharmacopoeia (5)



Figure 2 *P. emblica* fresh and dried fruits

2.1.2 Chemical composition from *P. emblica* extract:

The fruits of *P. emblica* consist of moisture 81.2%, protein 0.5%, fat 0.1%, mineral matter 0.7%, fiber 3.4%, carbohydrates 14.1%, Ca (0.05%), K (0.02%), Fe (1.2 mg:100g), nicotinic acid (0.2 mg:100g), phyllembelin, phyllemblic acid, gallic acid, emblicol, ellagic acid and pectin (28).

The pharmacological active composition of *P. emblica* was studied and elucidated in several studies but it had some conflicts about the composition of *P. emblica* extract. Until the 1990s, it was believed that *P. emblica* contained a large amount of vitamin C. However, Ghosal et al. published a paper where he asserted that the fruit of this plant does not contain any ascorbic acid, but contains emblicanins which are responsible for the biological activity of the fruit.

In 2002, Bhattacharya et al. (16) reported the aqueous-methanol (1:1) extract of *P. emblica* was composed of emblicanin A (37%), emblicanin B (33%), punigluconin (12%), pedunculagin (14%), rutin (3%) and gallic acid (1%). These bioactive tannoids have antioxidant activity against ischemia-reperfusion (IRI)-induced oxidative stress in rat heart. The structures of hydrolysable tannins present in *P. emblica* are shown in Figure 3.

In 2006, Kumar et al. (21) reported the high content of free and bound phenolic compounds from *P. emblica* extract and their high antioxidant activity. They concluded that gallic and tannic acid are the only phenolic acids present in both free and bound form in *P. emblica*. Hence, it is possible that tannoid derivatives reported earlier (16) may be constituted by enriched amount of gallic acid residues. In the same year Kumaran et al (17) reported the structures of purified compounds isolated from ethyl acetate fruit extract of *P. emblica* which identified by high performance liquid chromatography (HPLC) and fast-atom bombardment (FAB) mass spectroscopy compared with nuclear magnetic resonance (NMR) spectral data. These five compounds are a group of hydrolysable tannins with gallic acid substituent groups, that are gallic acid, methyl gallate, corilagin, furosin and geraniin as shown in Figure 4.

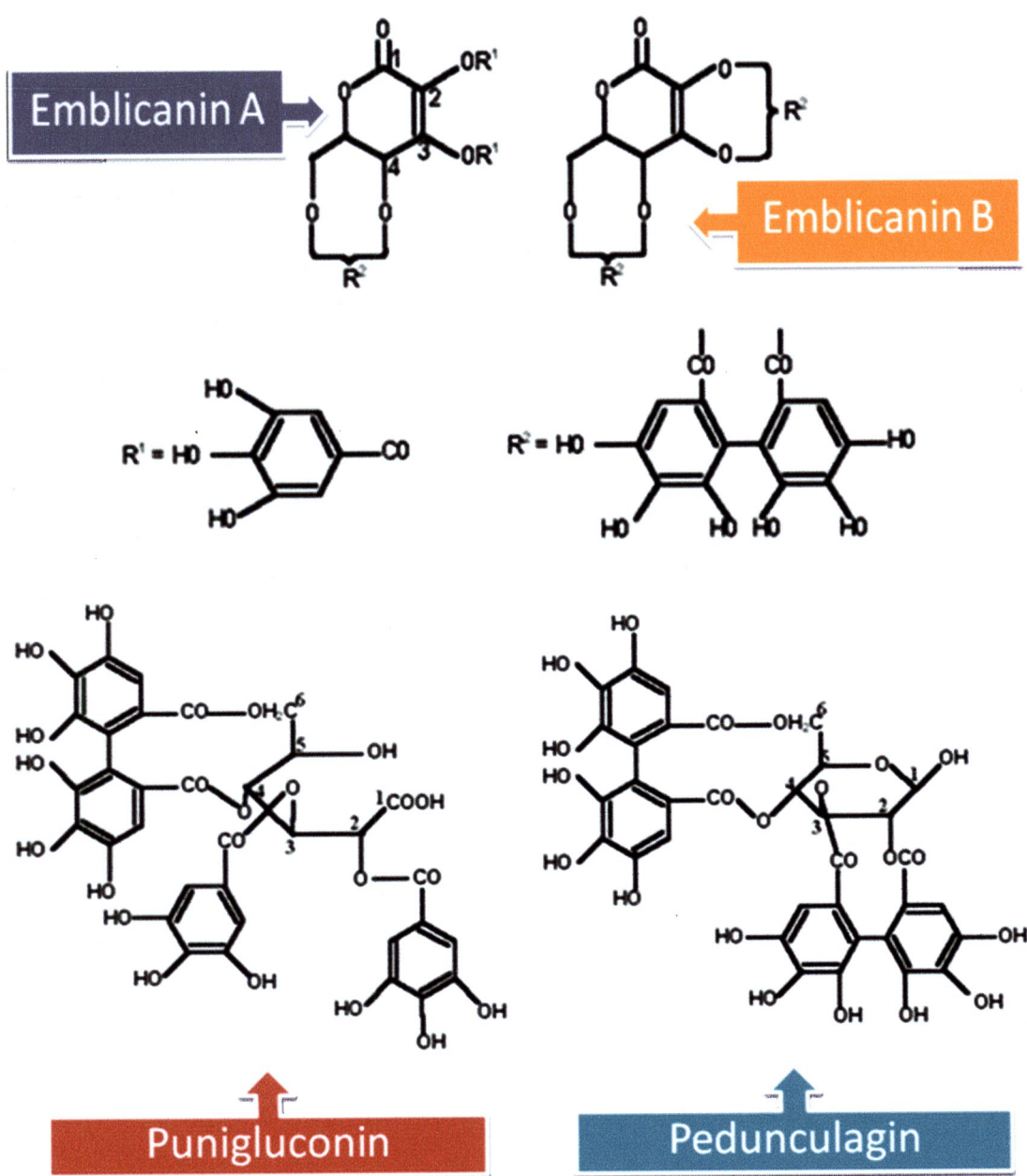


Figure 3 Hydrolysable tannins in *P. emblica* extract (16) modified from:

<http://www.molvis.org/molvis/v10/a20/suryanarayana-fig2.html>

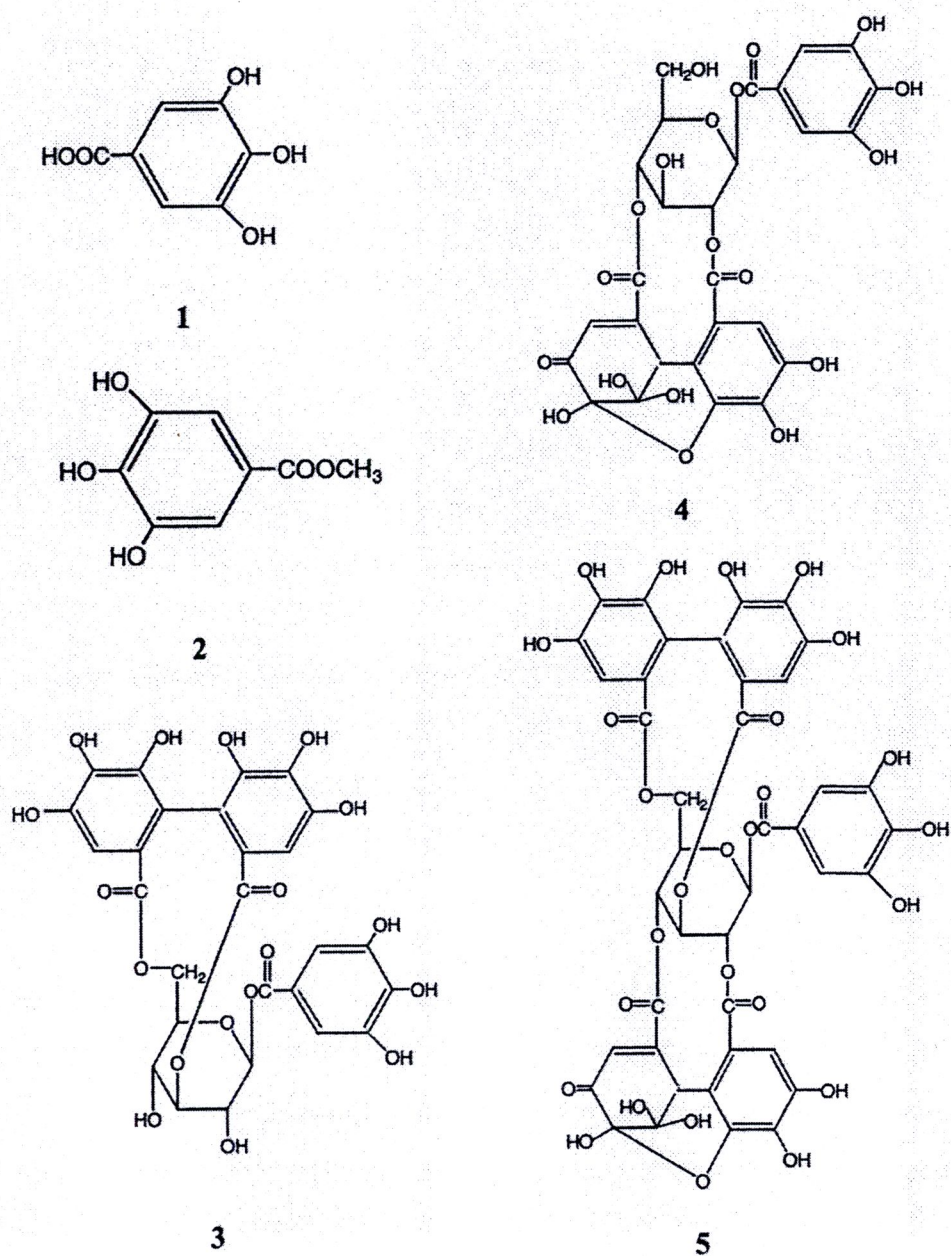


Figure 4 Chemical structure of compounds isolated from *P. emblica* (17)

1. Gallic acid, 2. Methyl gallate, 3. Corilagin, 4. Furosin, and 5. Gerniin

The study of Raghu et al. in 2007 (19) presented the comparison of ascorbic acid content of *P. emblica* fruits determined by different analytical methods. High ascorbic acid contents were found in this fruit extract. In 2008, Scartezzini et al. (18) reported vitamin C content and antioxidant activity of the fruits of *P. emblica* by HPLC with diode array detector (DAD). The results indicated that these fruits indeed contain ascorbic acid and also suggested that the high antioxidant activity is due to a large percentage of ascorbic acid. It is however possible that the tannins identified by Ghosal et al. favors the recycling of ascorbic acid, thereby increasing the antioxidant activity of the *P. emblica* fruits.

The recent study of Majeed et al. in 2009 (20) presented the detection of ascorbic acid in the lyophilized-extract of fresh fruits of *P. emblica* using multiple methods such as HPTLC, HPLC-DAD, preparative HPLC, liquid chromatography-mass spectrometry (LC-MS), and NMR. They suggested that the antioxidant hydrolysable tannins which were earlier reported as emblicanins A and B, corresponded to β -glucogallin and mucic acid 1,4-lactone 5-*O*-gallate, respectively (Figure 5).

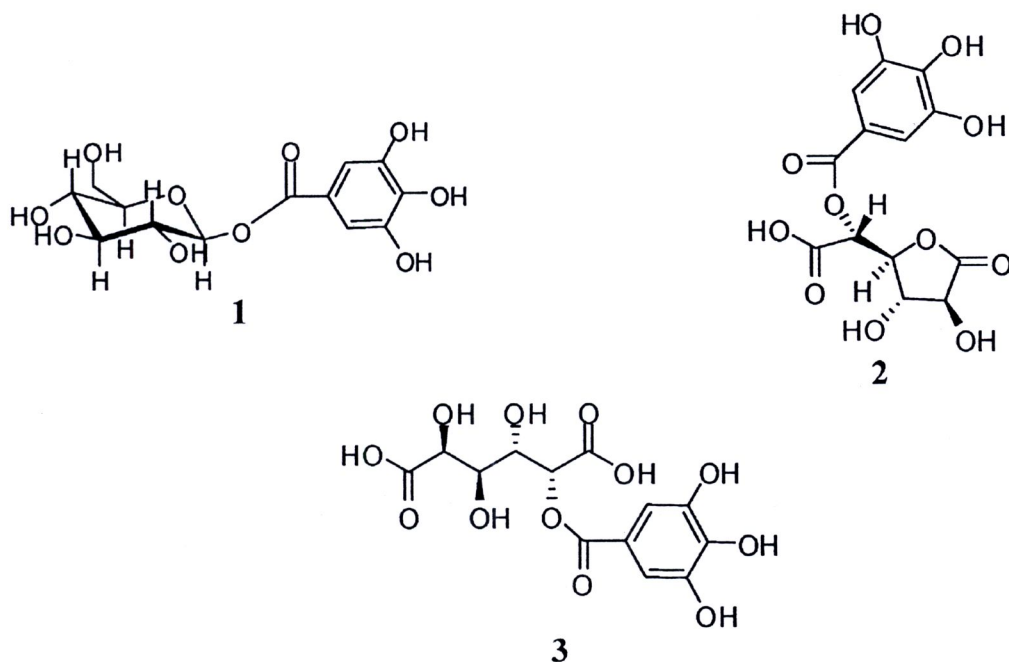


Figure 5 Compounds from *P. emblica* fruit extract (20): 1. β -glucogallin; 2. mucic acid 1,4-lactone 5-*O*-gallate; 3. mucic acid 2-*O*-gallate.

Although there is contradiction about composition of *P. emblica* extract from the previous studies, it can be concluded that *P. emblica* fruit extract consists of ascorbic acid and a group of polyphenolic compounds which are gallic acid derivatives. Moreover, the high antioxidant activity of this plant has been obviously established.

2.1.3 Pharmacological effects of *P. emblica* fruits

Phyllanthus emblica L. is a medicinal plant that has a long historic use in Chinese, Ayurvedic medicines, and also in Thai folk medicine as a tonic agent (6). The fruits of *P. emblica* are believed to help increase immune defense against diseases. It has beneficial roles in cancer, diabetes, liver treatment, heart trouble, ulcer, anemia and various diseases (28). It is often used in the form of 'Triphla', the well known Ayurvedic preparation which is a herbal formulation containing fruits of *P. emblica*, *Terminalia chebula*, and *Terminalia belerica* in equal proportions (29). The Pharmacological effects against many diseases *P. emblica* were reported in several studies as follows.

Lowering serum lipid level: Anila et al. (7) found that flavonoids from *P. emblica* effectively reduced lipid levels in serum and tissues of rats induced hyperlipidemia by inhibiting hepatic HMG CoA reductase enzyme activity in rats and enhanced degradation and elimination of cholesterol. The study of Mathur and colleagues (9) showed that *P. emblica* fresh fruit juice had lipid lowering and antiatherosclerotic effects on cholesterol-fed rabbits after administration 5 ml/kg body weight for 60 days and also reduced serum cholesterol, triglyceride, phospholipid and LDL levels, respectively. Faizal et al., (8) reported the significant hypoglycemic and hypolipidemic effects of an ayurvedic medicine "Rajanyamalakadi" composed of *P. emblica* in type 2 diabetic patients over a period of 3 months.

Antioxidant activity of *P. emblica*: Several researchers studied the antioxidant activity of many kinds of *P. emblica* extracts, and found that they had high antioxidant activity. Kumar et al. (21) reported the high antioxidant activity of phenolic extracts from *P. emblica*, which were ascorbic acid, gallic acid and tannic

acid. The extracts also exhibited significant protection to DNA against oxidative damage as evidenced by migration of DNA on the agarose gel. In 2008, Lui et al. (22) studied the phenolic contents and antioxidant activity of methanolic extracts of *P. emblica* fruits from six regions in China. The high correlation coefficient was existed between the phenolic content and superoxide anion radical scavenging activity. Methanolic extracts of *P. emblica* fruits from some selected regions exhibited stronger antioxidant activities compared to those of the commercial compounds (quercetin and BHA) and might be considered as a potential plant source of antioxidants.

Antidiabetic activity: The study of Sabu and Kuttan (23) showed the relationship between antidiabetic and antioxidant activity of methanolic extract (75%) of *Terminalia chebula*, *T. belerica*, *P. emblica* and their combination named 'Triphala'. The extracts had the significant antioxidant activity and their oral administration (100 mg/kg bodyweight) could reduce the blood sugar level in normal and in alloxan diabetic rats significantly within 4 h. Nevertheless, the mechanism of action of these drugs in reducing the diabetic was not known.

Antitumor activity: Several studies have been done on hepatoprotective and antitumor effects of *P. emblica*. Joes et al. (10) reported that aqueous extract of *P. emblica* was found to be cytotoxic to L 929 cells in culture and inhibited cell cycle regulating enzymes cdc 25 phosphatase in a dose dependent manner. The results suggested that antitumour activity of *P. emblica* extract may partially be due to its interaction with cell cycle regulation.

Antitussive activity: Nosálóvá et al. (11) reported the presence of antitussive activity of *P. emblica* in conscious cats, which was dose-dependent. Its activity was higher than the commonly used non-narcotic antitussive, dropropizine but was still lower than the classical narcotic antitussive drug, codeine.

Antipyretic activity: The study of Perianayagam et al. (12) showed that a single oral dose of ethanol and aqueous extracts of *P. emblica* fruits (500 mg/kg, i.p.) showed significant reduction in brewer's yeast induced hyperthermia in rats.



Additionally, the extracts also elicited pronounced inhibitory effect on acetic acid-induced writhing response in mice in the analgesic test.

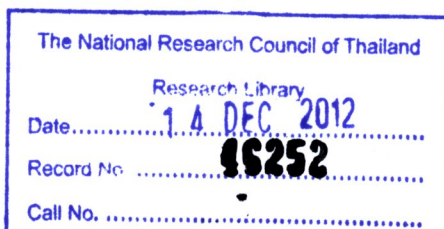
Anti-ulcerogenic activity: The ulcer protective potential of methanolic extract of *P. emblica* was assessed by Sairam et al. (13) using different acute gastric ulcer models. *P. emblica* extract, 10-50 mg/kg administered orally, twice daily for 5 days showed dose-dependent ulcer protective and significant ulcer healing effect in a dose of 20 mg/kg after 5 and 10 days treatment. This plant extract also showed significant antioxidant effect in stressed animals and did not have any effect on cell proliferation. The lyophilized aqueous extract of *P. emblica* L. fruits showed significant gastro-protective effect on post-treatment of the indomethacin induced ulcerated-rats at the 100 mg/kg bodyweight, orally for 7 continuous days (14).

Hepatoprotective effect: The *P. emblica* extract was found to inhibit the hepatotoxicity produced by acute and chronic carbon tetrachloride administration in rat model by decreased levels of serum and liver lipid peroxides, glutamate-pyruvate transaminase, and alkaline phosphatase. It also reduced liver fibrosis as seen from the decreased levels of collagen-hydroxyproline and pathological analysis significantly (15).

2.2 Free radicals and their role in diseases

Free radicals are chemical species having unpaired electrons. Electrons are present generally in pairs. However, under certain condition, molecules have unpaired electrons and such they are called free radicals. Unpaired electron usually seeks another electron to become paired. Thus, free radicals are in general reactive and attack other molecules, although some radicals are not reactive but stable enough to have long life (30).

Free radicals tend to rob electrons from the molecules in the immediate surrounding in order to replace their own losses. Reactive oxygen species (ROS) is a collective term, which includes not only the oxygen radicals ($O_2^{\bullet-}$, and $\bullet OH$) but also some non-radical derivatives of oxygen. These include hydrogen peroxide (H_2O_2),



hypochlorous acid (HOCl) and ozone (O₃) (31). The oxidative reaction is demonstrated in Figure 6.

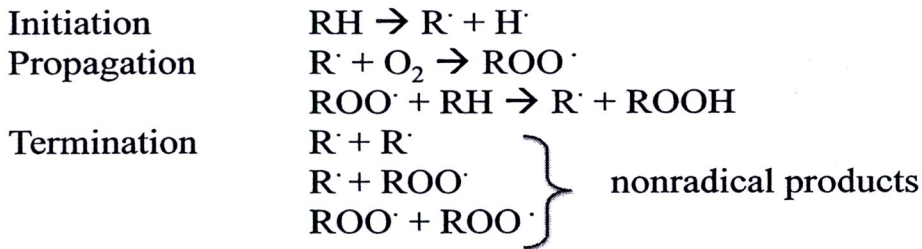


Figure 6 Oxidation reaction

Free radicals are natural by-products of metabolism in the body. They are electrically charged molecules that attack cells, tearing through cellular membranes to react and generate destruction with the nucleic acids, proteins, and enzymes present in the body. The attack by free radicals is collectively known as oxidative stress. The oxidative stress is capable of causing cells to lose their structure, function and can eventually destroy them. Moreover, this reaction is continuously produced by our body's use of oxygen such as in respiration and some cell-mediated immune functions. They are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air-pollution, pesticides, etc. (1).

Normally there is a balance between the amount of free radicals generated in the body and the antioxidant defense systems that scavenge/quench these free radicals preventing them from causing deleterious effects in the body. The antioxidant defense systems in the body can only protect the body when the amount of the free radicals is within the normal physiological level. But when this balance is shifted towards more of free radicals, increasing their burden in the body either due to environmental condition or produced within the body, it leads to oxidative stress, which may result in tissue injury and subsequent diseases (2).

Over about 100 disorders like rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases, gastrointestinal and ulcerogenesis have been reported as ROS mediated.

Some specific examples of ROS mediated diseases include Alzheimers disease, Parkinson's disease, atherosclerosis, cancer, and ischemic reperfusion injury in different tissues including heart, liver, brain, kidney and gastro intestinal tract (3).

2.2.1 Antioxidant defense

Aerobic organisms are protected from oxidative stress induced by free radicals and active oxygen species by an array of defense system. Various kinds of antioxidants with different functions play an important role in these defense systems. The preventive antioxidants acting in the first defense line suppress the formation of free radicals and reactive oxygen species. The radical scavenging antioxidants are responsible in the second defense line and inhibit chain initiation and/or break the chain propagation. The antioxidant enzymes such phospholipase, proteases, DNA repaired enzyme, and transferases act as the third line defense (32).

Antioxidant defenses comprise:(3)

- Agents that catalytically remove free radicals and other reactive species like super oxide dismutase (SOD), catalase (CAT), peroxidase and thio specific antioxidants
- Proteins that minimize the availability of peroxidase such as iron ions, copper ions and heam
- Proteins that protect biomolecules against oxidative damage, for example heat shock proteins
- Low molecular mass agents that scavenge ROS and RNS, for example GSH, ascorbic acid and tocopherol

2.2.2 Natural antioxidants

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (33). Plants and herbs have been used for a large range of purposes including medicine, nutrition, flavorings, beverages, dyeing, repellents, fragrances, cosmetics, and industrial uses. Since prehistoric times, some plants were the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth

century (34). In general, there are two basic categories of antioxidants, natural and synthetic. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants.

Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease, cancer and other health problems is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (35).

Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs. Flavonoids and other plant phenolics, such as gallic acid, phenolic acids, stilbenes, tannins, lignans, and lignin, are especially common in leaves, flowering tissues, and woody parts such as stems and barks (36). They are important in the plant for normal growth development and defense against infection and injury. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. It can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (37).

The fruit of *P. emblica* is one of the well known plant material that have a long historic use in traditional medicine. This fruit is a high source of ascorbic acid and phenolic compounds which have high antioxidant activity. It is now interested for use as a functional food, nutraceutical, medicinal, and cosmetic purposes.

2.2.3 Antioxidant activity testing

As an importance of antioxidant substance, there is an increasing interest in antioxidants, particularly in those intended to prevent the corrosive effects of free radicals in the human body. A preference for antioxidants from natural rather than from synthetic sources, therefore stimulate a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants.

The models available to test antioxidant activity of the substances are described by the antioxidation reaction of the substance into major 2 groups, these are

- Hydrogen atom transfer based method (HAT): This method test the antioxidant activity of substances to eliminate free radicals by hydrogen atom donating mechanism; for example, β -carotene bleaching method.
- Electron transfer based method (BAT): This method test the electron transferring ability of substances to reduce the free radicals, metal ions, and electron receipting groups; for example,
 - Free radical scavenging activity assay such as DPPH (2,2-diphenyl-1-picrylhydrazyl) method, and ABTS (2,2'-azin-bis-(3-ethylbenzo-thiazone-6-sulfonic acid) diammonium salt) method
 - Reducing ability assay such as ferric reducing antioxidant power (FRAP) method

The model used to test antioxidant activity of substances is depending on the mechanism of action and the properties of the substance. Normally, more than two methods will be used to confirm the activity and the mechanism of action of the antioxidants. In this study, the free radical scavenging activity on DPPH method and reducing ability via FRAP method were used to test the antioxidant activity of *P. emblica* crude and fractional extracts.

2.2.3.1 Free radical scavenging activity assay (38)

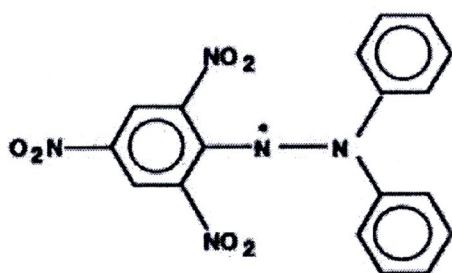
This model indentifies the antioxidant activity of substance by scavenging activity on free radical molecule from radical to non radical form and detect the change via specific method such as spectroscopy, high performance liquid chromatography or specific enzymatic assay. The key of importance of this method is

the free radical molecule. The commonly used molecules are 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azin-bis-(3-ethylbenzo-thiazine-6-sulfonic acid) diammonium salt (ABTS). In this study, we used DPPH as a free radical molecule to study the scavenging activity.

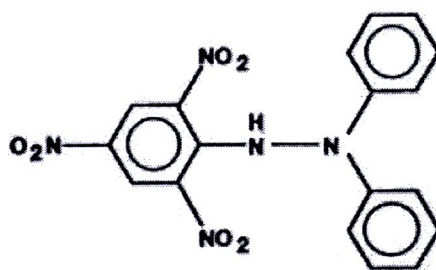
Scavenging on 2,2-diphenyl-1-picrylhydrazyl or DPPH method

The molecule of 2,2-diphenyl-1-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerize, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 520 nm.

When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present). The figure of DPPH radical form and the non radical form is showed in Figure 7.



1: Diphenylpicrylhydrazyl (free radical)



2: Diphenylpicrylhydrazine (nonradical)

Figure 7 DPPH molecule (38)

2.2.3.2 Reducing ability assay

Ferric reducing antioxidant power (FRAP)

This method is used to evaluate the reducing ability of antioxidant compound to reduce ferric ion to the ferrous form. The molecule of 2,4,6-Tris (2-pyridyl)-s-triazine; TPTZ (Figure 8) react with ferric chloride (FeCl_3) and form the colorless complex compound, Fe^{3+} -TPTZ. When Fe^{3+} -TPTZ is reduced by antioxidant compound, it will form the blue complex compound, Fe^{2+} -TPTZ. The change in color of the molecule is detected by spectrophotometric method like UV-visible spectrophotometry at 593 nm.

The result can be calculated by the change in absorbance of the colorless to the blue compound and expressed in term of EC value, the concentration of antioxidant having a ferric reducing ability equivalent to that of 1 mM FeSO_4 .

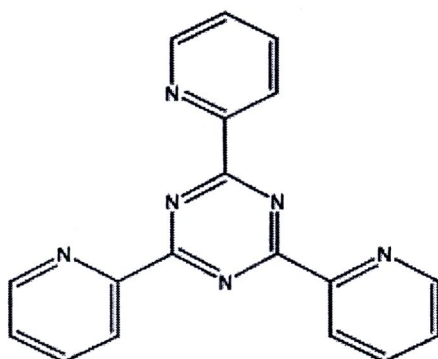


Figure 8 Structure of TPTZ

2.3 Glycation reaction

Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eyes, nerves, blood vessels, skin, and kidneys. Increased glycation of proteins and accumulation of advanced glycation endproducts (AGEs) have been implicated in the pathogenesis of diabetic complications. Glycation and AGEs formation are also accompanied by formation of free radicals via autoxidation of glucose and glycated proteins (39). The study of glycation reaction and AGEs formation is tended to the understanding and preventing of diabetic complication.

2.3.1 Diabetes Mellitus (24, 25, 40)

Diabetes mellitus (DM) is the most common endocrine disorder. It is a chronic condition, characterized by chronic hyperglycemia due to impaired insulin secretion with or without insulin resistance. Diabetes mellitus may be classified according to etiology to type 1 and type 2 diabetes.

Type 1 diabetes (insulin-dependent diabetes mellitus or IDDM) is a disease that causes destruction of the insulin-producing pancreatic β -cell. In over 90% of case, β -cell destruction is associated with autoimmune disease. Type 1 diabetes usually develop in young (below the age of 30), although it can develop in older adults and it usually associated with faster onset of symptoms leading to dependency on extrinsic insulin for survival.

Type 2 diabetes (non-insulin dependent diabetes mellitus or NIDDM) is more common above the age of 40, although it is being increasingly seen in young people and even children. It is caused by a relative insulin deficiency and/or insulin resistance. Symptoms are generally slower in onset and less marked than those of type 1. Type 2 diabetes may be incidental finding, particularly when patients present with complication associated with the disease.

Diabetes affects 1–2% of the population, and there are around 100 million diabetic patients worldwide. This figure is expected to double over the next 10–15 years (39). Individuals affected by diabetes are prone to long-term complications such as retinopathy, cataract, neuropathy, atherosclerosis, nephropathy, embryopathy, and delayed healing of wounds (25). The long term hyperglycemia is a key role in the pathogenesis of diabetic complications. Diabetic patients with poor blood glucose control are more likely to develop chronic complications (24).

2.3.2 Glycation reaction and Advanced glycation endproducts

The precise mechanism underlying pathogenesis of diabetic complications is unclear. It has been suggested that during hyperglycemia, a direct reaction, referred to as the Maillard, or browning, reaction occurs between body proteins and sugars. Food scientists have been interested in the Maillard reaction for many years because it is associated with food spoilage, altered taste, and loss in nutritional value (39) The importance of Maillard-like reactions *in vivo* began in the mid-1970s. This started

with studies of hemoglobin A1c (HbA1c), a naturally occurring minor human hemoglobin that is elevated in diabetics. HbA1c was known to be a post-translational adduct of glucose with the N-terminal valine amino group of the β chain of hemoglobin, in which glucose was thought to be attached via a nonenzymatically formed Schiff base structure. Measurement of the elevation of HbA1c in diabetics allowed assessment of the degree of glucose control integrated over several weeks (41).

Protein glycation is the first part of the Maillard reaction and occurs when a sugar carbonyl group reacts with a protein amino group to form a labile Schiff base that subsequently rearranges to a stable Amadori product. Formation of the Schiff base occurs over a period of hours, whereas Amadori products form over a number of days. Glycation occurs spontaneously whenever proteins are exposed to reducing sugars and is dependent on the degree and duration of hyperglycemia *in vivo*.

Glycated proteins can undergo further reactions involving dicarbonyl intermediates such as 3-deoxyglucosone to form complex heterogeneous, cross-linked and fluorescent molecules called advanced glycation endproducts (AGEs) (Figure 9).

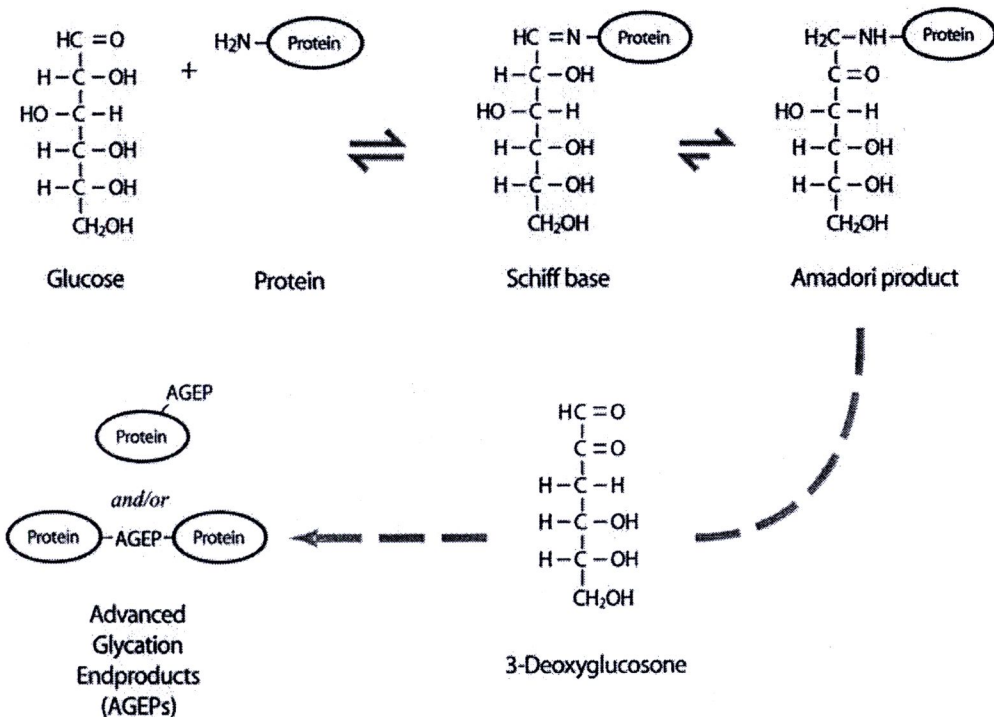


Figure 9 Glycation reaction and AGEs formation (39)

Little is known about the chemistry of AGEs, and only a few have been characterized. In general, AGEs can be fluorescent crosslinked structures such as pentosidine, pentodilysine, nonfluorescent crosslinked structures such as imidazolium dilysines, or non cross-linked structures such as pyrraline imine (Figure 10). When [Lys] represents a desamino-lysine residue, and [Arg] represents a desguanidino-arginine residue.

Glycation and free radicals. Glucose and Amadori products can undergo autoxidation in the presence of transition metals to generate free radicals. Autoxidation of glucose (autooxidative glycation) generates a ketoimine capable of reacting with proteins to form AGEs and also generates free radicals such as the superoxide radical in the process. Similarly, Amadori products undergo metal-catalyzed oxidation (glycoxidation), generating reactive dicarbonyl compounds capable of forming AGEs and free radicals (26). Free radicals can damage proteins, lipids, and nucleic acids and might contribute toward tissue damage in diabetes.

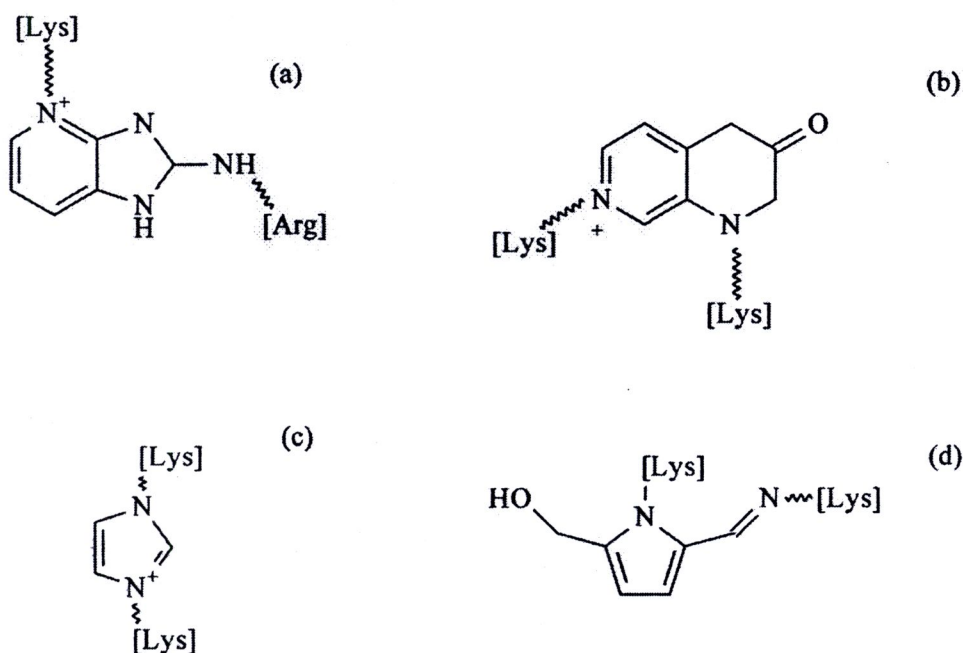


Figure 10 Structure of some known advanced glycation endproducts, pentosidine (a), pentodilysine (b), imidazolium dilysines (c), and pyrraline imine (d)

AGEs and diabetic pathology. Increased glycation and accumulation of tissue AGEs can alter protein conformation and impair function by altering enzyme activity, modifying protein half-life, altering immunogenicity, and cross-linking of structural proteins (42, 43). Many cells possess receptors for advanced glycation endproducts (RAGEs), and the interaction of AGEs with their receptors can generate intracellular oxidative stress and activation of nuclear factor κ B (NF- κ B). The latter can stimulate generation of proinflammatory and adhesion molecules that in turn increase vascular permeability, which underlies the pathology of diabetic vascular complications (44). It is generally accepted that accumulation of tissue AGEs together with increased oxidative stress has an important role in the pathogenesis of diabetic complications (43).

2.3.3 Antiglycation compounds

There is considerable interest in inhibitors of glycation because of their therapeutic potential. Antiglycation compounds may act by blocking carbonyl groups on reducing sugars, Amadori products, and 3-deoxyglucosones to inhibit formation of AGEs (39). Certain enzymes can deglycate Amadori products and are referred to as Amadoriases. Recently drugs have been developed that can cleave AGE cross-links and perhaps open up the possibility of reversing diabetic complications (45-47). Much interest has been devoted to RAGE blockers and their ability to protect against diabetic complications (44). Antioxidants may protect against glycation-derived free radicals, whereas chelators remove transition metals, preventing autoxidation of glucose and Amadori products (26, 43).

The nucleophilic hydrazine compound aminoguanidine (Figure 11) has received the most interest and is effective in reducing AGE formation *in vitro* as well as in animal and human studies. The mechanism of action of aminoguanidine is still unclear, but it may act by blocking carbonyl groups on free sugars, Amadori products, or dicarbonyl intermediates such as 3-deoxyglucosone. However, phase III clinical trials in which aminoguanidine therapy was used to treat diabetic nephropathy proved unsuccessful because of the high toxicity encountered in many patients (46).

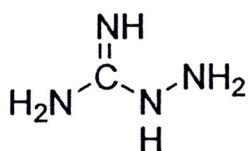


Figure 11 Structure of aminoguanidine

2.3.4 Antiglycation testing

The standard procedures of assay of glycation require specific equipments, methods and reagents like antibodies (48) and are time consuming requiring days and weeks (49, 50) because of the slow reaction of sugars with protein. Some attempts have been made to cut down assays duration by replacing glucose with reactive sugars like ribose and by increasing the incubation temperature (51). Until now, there is no standard method procedure to study the glycation reaction or detect the AGEs formation *in vitro*. Matsuura et al. (52) described an assay in which the rate of reaction of glucose with bovine serum albumin (BSA) was accelerated by performing the incubation at 60 °C. AGEs formation was detected by measuring quenching of intrinsic fluorescence and generation of the new fluorophore (excitation wavelength of 370, and emission wavelength of. 440 nm).

Studying the formation of AGEs *in vitro* by determining the fluorescence intensity of the reaction mixture between sugar (glucose, fructose, ribose) and protein (BSA) after incubation is the generally used method because it uses a simple technique and equipment. In this study, the method of Matsuura et al. (52) was used as a model to study the antiglycation activity of the *P. emblica* fruit crude and fractional extracts.

2.4 Solid dosage forms

Drug substances are most frequently administered as solid dosage formulations, mainly by the oral route. The drug substance's physicochemical characteristics, as well as the excipients added to the formulations, all contribute to ensuring the desired therapeutic activity. Tablets and capsules are the most frequently used solid dosage forms comprising a mixture of ingredients presented in a single rigid entity and usually containing an accurate dosage of a drug. Tablets can range

from relatively simple, single, immediate-release dosage form to complex modified-release system. Tablets offer advantages for both patients and manufacturers (Table 1).

2.4.1 Excipients in solid dosage formulations

In addition to the active ingredients, solid oral dosage forms will also contain a range of substance called excipients. The role of excipients is essential in ensuring that the manufacturing process is successful and that the quality of the resultant formulation can be guaranteed. The appropriate selection of excipients and their relative concentrations in the formulation is critical in development of a successful product. Excipients are categorized into groups according to their main function, although some may be multifunctional, and examples of common excipients used in the manufacture of tablets and capsules are detailed in Table 2.

Table 1 Advantages of tablet as a solid dosage form

Easy to handle
Variety of manufacturing methods
Can be mass produced at low cost
Consistent quality and dosing precision
Can be self-administrated
Enhanced mechanical, chemical, and microbiological stability compared to liquid dosage forms
Temperproof
Lend themselves to adaptation for other profiles, e.g., coating for sustained release

Table 2 Excipients used in solid dose formulations

Excipients category	Examples
Fillers/diluents	Lactose, sucrose, glucose, microcrystalline cellulose
Binders	Polyvinyl pyrrolidone, starch, gelatin, cellulose derivatives
Lubricants	Magnesium stearate, stearic acid, polyethylene glycol, sodium chloride
Glidants	Fine silica, talc, magnesium stearate
Antiadherents	Talc, corn starch, sodium dodecylsulfate
Disintegrants and superdisintegrants	Starch, sodium starch glycollate, cross-linked polyvinyl pyrrolidone
Colorants	Iron oxide, natural pigments
Flavor modifiers	Mannitol, aspartame

