# CHAPTER 2

## REVIEW of LITERATURE

### 1. Alpha-amylase (EC 3.2.1.1)

Alpha-amylase (EC 3.2.1.1) is an endo-acting enzyme which catalyzes the hydrolysis of the (1→4)-α-D-glycosidic linkages of starch, amylose, amylopectin, glycogen and various maltodextrins. Alpha-amylase is present in a wide variety of organisms including bacteria, fungi, plants, and animals. Two kinds of α-amylase are produced by various mammals *i.e.* salivary α-amylase from the parotid gland and pancreatic α-amylase from the pancreas. The digestion of food starch begins with salivary α-amylase in the mouth and the reaction of this enzyme can be stopped by low pH of gastric juice of stomach. When the food boluses from stomach pass into a small intestine, the pH of gastric juice is neutralized by pancreatic juice secreted from pancreas in which the digestion of the starch is completed by the reaction of a pancreatic α-amylase. Bacteria and fungi use starch for their growth by secreting alpha-amylases into their environment and the hydrolyzed products *i.e.* maltodextrins are transported into the cells and further converted to D-glucose or other metabolites . Plants produce α-amylase to degrade the starch, which is synthesized from photosynthesis. Digestion of starch by α-amylase is an important process in the utilization of the sun's energy by non-photosynthesizing organisms (Yoon and Robyt, 2003).

### 2. Alpha-amylase inhibitor

Alpha-amylase inhibitor (AI) is classified into two major groups: proteinaceous α-amylase inhibitor and nonproteinaceous α-amylase inhibitor.

### 2.1 Proteinaceous α-amylase inhibitor

The proteinaceous α-amylase inhibitors are founds in cereals and legumes, such as common beans (*Phaseolus valgalis*) (Gibbs and Alli, 1998; Lee *et al*., 2002), wheat (*Triticum aestivum*) (Franco *et al*., 2000), barley *(Hordeum vulgare)* (Abe *et al*., 1993), and corn seeds (Figueira *et al*., 2003). Wheat α-amylase inhibitor (>4 mg/ml) can reduce α-amylase activity more than 90% inhibition without affecting other enzymes that can digest starch in duodenum (Choudhury *et al*., 1996). Based on their tertiary structure proteinaceous α-amylase inhibitors were classified into six structural classes as shown in Table 1.

**Table 1.** Different structural classes of α-amylase inhibitor

| Structural class | Source | Amino acid residue numbers | Disulfide bonds | Names |
|---|---|---|---|---|
| Lectin-like | common beans | 240-250 | 5 | α-AI1 & α-AI2 |
| Knottin-like | amaranth | 32 | 3 | AA1 |
| Cereal-type | wheat, barley , millet | 124-160 | 5 | WRP25, WRP26, WRP27 & RBI |
| Kunitz-like | barley, wheat, rice | 176-181 | 1-2 | BASI, WASI, & RASI |
| Thaumatin-like | maize | 173-235 | 5-8 | Zeamatin |
| γ-Purothionin-like | sorghum | 47-48 | 5 | SIα1, SIα2, SIα3 |

From : Franco *et al*., 2002

## 2.2 Nonproteinaceous α-amylase inhibitor

The nonproteinaceous α-amylase inhibitor contains diverse types of organic compounds such as acarbose, acarbose analogues, hibiscus acid, tannins, flavonoid, glucopyranosylidene-spiro-thiohydantoin. The inhibitory activity of these compounds against α-amylase is due in part to their cyclic structures, which resemble substrates at catalytic sites of α-amylase (Franco *et al*., 2002).

Acarbose is a natural product produced by *Actinoplanes* sp. fermentation. It is a pseudotetrasaccharide with an unsaturated cyclitol [2, 3, 4-trihydroxy-5-(hydroxymethyl)-5, 6-cyclohexene in a D-*gluco*-configuration] attached to the nitrogen of 4-amino-4, 6-dideoxy-D-glucopyranose, which is linked α-(1→4) to maltose (Figure 1). Acarbose is a strong competitive inhibitor of several enzymes such as α-glucosidase, α-amylase, cyclomaltodextrin glucanyltransferase, glucoamylase and glucansucrases. The mechanism of inhibition for these enzymes has been postulated to be due to the cyclohexene ring and nitrogen linkage that mimics the transition state for the enzymatic cleavage of glycosidic linkages (Yoon and Robyt, 2003)

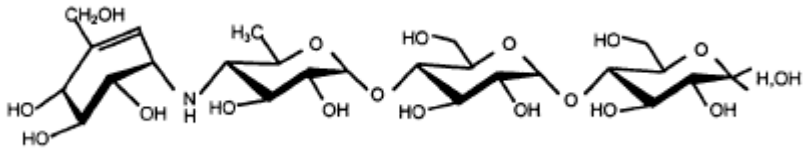


**Figure 1.** Molecular structure of acarbose

( From: http://www.rxlist.com/cgi./generic/acarbose.htm)

Recently, Yoon and Robyt (2003) reported that inhibition kinetics of the two acarbose analogues $G_6$-acarbose, $G_{12}$-acarbose and acarbose were mixed noncompetitive inhibitor of four different α-amylases from *Aspergillus oryzae*, *Bacillus*

*amyloliquefaciens*, human salivary and porcine pancreatic α-amylase. These analogues were synthesized by adding maltodextrin chains, maltohexaose ($G_6$), maltododecaose ($G_{12}$) and maltooctadecaose ($G_{18}$) to the C-4-hydroxyl group of the nonreducing end cyclohexene ring by the reaction of acarbose with cyclomaltohexaose (α-CD) catalyzed by cyclomaltodextrin glucanyltransferase (GTase) (Yoon and Robyt, 2003). $K_i$ values of each inhibitors against the four enzymes revealed that the two analogues were more potent than acarbose.

Hibiscus acid, a compound in roselle (*Hibiscus sabdariffa* Linn.) tea extract was found to have high inhibitory activity against porcine pancreatic α-amylase. Hibiscus acid and its 6-methyl ester (Figure 2) were respectively isolated as active principles from the 50% methanol and acetone extracts of roselle tea (Hansawasdi *et al*., 2000).

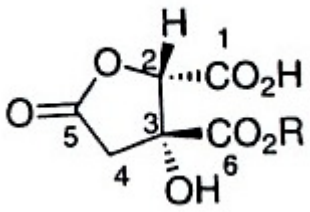


**Figure 2.** Molecular structure of Hibiscus acid : Hibiscus acid (R=H); Hibiscus acid 6-methyl ester ($R=CH_3$) ( From: Hansawasdi *et al*., 2000)

Tannin is a group of high molecular polyphenolic compounds produced by secondary plant metabolism. It is a colorless to pale yellow solid. It mainly consists of gallic acid residues that are linked to glucose *via* glycosidic bonds (Figure 3). Kandra *et al*. (2004) reported that commercial tannic acid could inhibited human salivary α-

amylase (HSA). This inhibition is a mixed noncompetitive type and only one molecule of tannin binds to the active site or the secondary site of the enzyme.

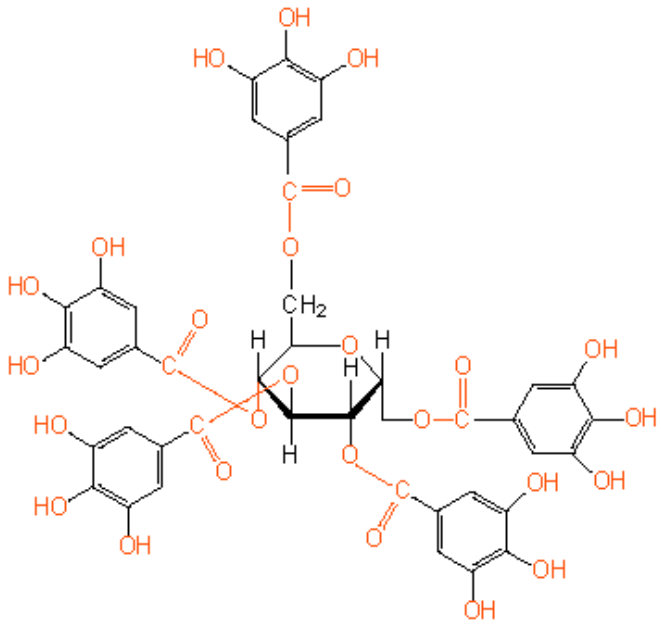

**Figure 3.** Basic structure of tannin (

From: http://www.biologie. uni-hamburg.de/b-online/e26/11.html)

Flavonoids are a ubiquitous group of polyphenolic substances widely distributed in most plants concentrating in seeds, fruit skin or peel, bark, and flowers. The structural components common to these molecules include two benzene rings on either side of a 3-carbon ring (Figure 4). Multiple combinations of hydroxyl groups, sugars, oxygens, and methyl groups attached to these structures create the various classes of flavonoids: flavanones, flavanols, flavones, flavan-3-ols (catechins), anthocyanins, and isoflavones. Flavonoids have been shown in a number of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals. Twenty-one naturally occurring flavonoids were tested for inhibitory activities against α-glucosidase and α-amylase. Luteolin, amentoflavone, luteolin 7-O-glucoside and daidzein were the strongest inhibitors among the compounds tested. Luteolin inhibited α- glucosidase by 36% at the concentration of 0.5 mg/ml and

was stronger than acarbose, the most widely prescribed drug. This inhibitory potency suggested that luteolin has the possibility to effectively suppress postprandial hyperglycemia in patients with non-insulin dependent diabetes mellitus. Luteolin also inhibited α-amylase effectively although it was less potent than acarbose (Kim *et al*., 2000)

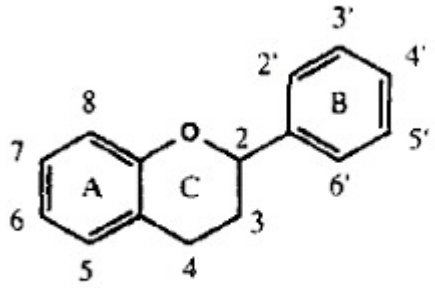


**Figure 4.** General structure and numbering pattern for common flavonoids (From: http://www.lpi.oregonstate. edu/ infocenter/ phytochemical/flavonids/basis flav.)

Glucopyranosylidene-spiro-thiohydantoin (G-TH) (Figure 5) was synthesised by Somsák *et al*. (2000) is an inhibitor on the 2-chloro-4-nitrophenyl-4-*O*-β-D-galactopyranosyl-maltoside ($GalG_2CNP$) hydrolysis catalysed by human salivary α-amylase. In spite of mixed-noncompetitive salivary amylase inhibitor of this compound Gyémánt *et al*. (2003) proposed G-TH as a supplementary drug for the treatment of sugar metabolic disorders.

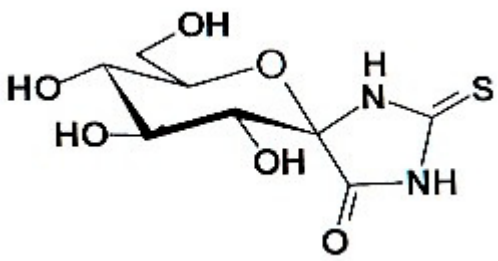

**Figure 5.** Molecular structure of glucopyranosylidene-spiro-thiohydantoin (G-TH)
(From: Somsák *et al.*, 2000)

Polyphenol structure is shown in Figure 6. Water-soluble extracts with optimized phenolic content of selected American and Asian foods had inhibitory activity against α-amylase and α-glucosidase which linked to hyperglycemia-associated hypertension. The experiment was perform by allowing porcine pancreatic α-amylase (PPA) to react with each phenolic-optimized food extract, and the derivatized enzyme-phytochemical mixtures obtained were characterized for residual amylase activity. The α-glucosidase were also determined in the presence of each phenolic-optimized food extract. The amylase activity was inhibited more than the glucosidase activity in the presence of these phytochemical extracts and more so by Asian foods than by American foods (McCue *et al.*, 2004).

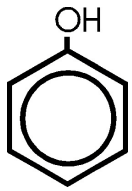


**Figure 6.** Common structure of phenol
(From: http://www.iaf.inrs.ca/gmre/Nos_realisations.html)

Figure 7 shows a molecular structure of stigmast-4-en-3-one, a plant steroid compound. A study on hyperglycemic effect of it in alloxan induced diabetic rats showed 84% blood glucose reduction at 100 mg of pericarp $kg^{-1}$ body weight with

the minimum effective dose at 50 mg of pericarp $kg^{-1}$ body weight, compare to 111% activity of gliben clamide at 5 mg $kg^{-1}$ body weight dosages. Result of this study (Jamaluddin *et al*., 1995) suggested this compound as a new hypoglycaemic agent from natural source.

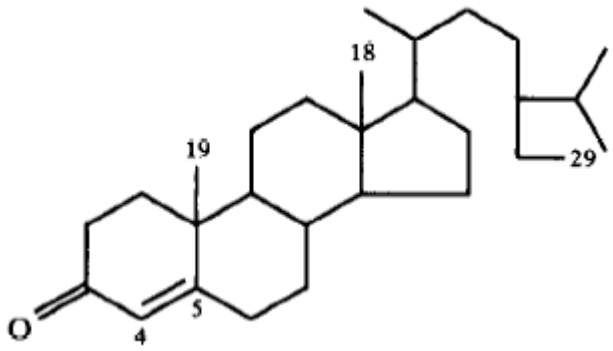


**Figure 7.** Molecular structure of Stigmast-4-en-3-one

(From: Jamaluddin *et al*., 1995)

### 3. Extraction of proteinaceous α-amylase inhibitor

Proteinaceous α-amylase inhibitors have been extracted from plant material with two methods including heat and non-heat extraction.

Heat extraction : Sasikiran *et al*. (2002) extracted proteinaceous inhibitors from sweet potato by homogenizing in 0.01 M sodium phosphate buffer pH 8.0 (ratio 1 : 5 w/v) in the presence of 1.0% polyvinyl pyrrolidone (PVP). The native proteases were inactivated by heating at 70°C for 10 min and the precipitated proteins were removed by centrifugation at 1,000 x g for 10 min. To optimize the recovery of proteinaceous inhibitor from the leaves and stem the crude extract was treated with 5% TCA. After the removal of the precipitated proteins by centrifugation at 5,000 x g, the pH was rapidly brought back to 8.0 and dialyzed overnight against in 0.01 M sodium phosphate buffer pH 8.0. Iulek *et al*. (2000) extracted proteinaceous inhibitor from rye

seeds in 1,000 ml of 70% ethanolic solution (v/v) with continuous stirring for 3 hours. The crude extract was filtered and centrifuged at 9,400 x g for 1 hour yielding a clear supernatant. The precipitate was discarded and supernatant was heated for 1 hour at 70°C, followed by another centrifugation step at 9,400 x g for 1 hour to eliminate the coagulated proteins, including the endogenous amylase. The remaining supernatant was extensively dialysed against 20 mM phosphate buffer pH 6.9 and further centrifuged at 9,400 x g for 1 hour. The supernatant was used for the inhibitor characterization. Grant *et al*. (1995) extracted proteinaceous inhibitor from various seeds available in Europe with 0.02 M sodium phosphate buffer pH 6.9 containing NaCl (9 g/litre$^{-1}$) (1 : 5 w/v, sample to buffer ratio) for 16 hours at 4 °C and finally centrifuged at 50,000 x g for 20 min. The supernatant was heated at 70°C for 10 min, centrifuged and supernatant was used for estimation of AI content.

Non-heat extraction : To obtain AI, wheat (*Triticum aestivum*) was extracted with 0.15 M NaCl (1 : 5 w/v, sample to buffer ratio) with continuous stirring for 5 hours at 4°C for 30 min (Franco *et al*., 2000). AI from common bean seeds (*P. vulgaris*) were extracted with 0.15 M NaCl and 0.1%HCl (1: 5 w/v, sample to buffer ratio) with continuous stirring for 5 hours at 4°C. The mixture was centrifuged at 10,000 x g at 4°C for 30 min. The precipitate was discarded and supernatant was submitted to fractionation with ammonium sulfate (Dayler *et al*., 2005). Corn seeds were 4-times defatted by shaking with acetone for 15 min followed by decanting. The defatted corn flour (100 g) was extracted for its AI with 500 ml of 0.1 M acetate buffer, pH 6.0 and continuously stirred for 12 hours at 4°C. The soluble proteins were obtained by centrifuging at 30,000 x g for 20 min at 4°C. This solution was used for the inhibitor characterization (Figueira *et al*., 2003).

**4. Extraction of nonproteinaceous α-amylase inhibitor**

Jamaluddin *et al*. (1995) extracted the nonproteinaceous α-amylase inhibitor, stigmast-4-en-3-one from empty pods of *P. speciosa* with petroleum ether, chloroform, dichloromethane, ethyl acetate, 25% ammoniacal chloroform and methanol. A general extraction procedure was followed for each solvent by soaking the powdered pods overnight, the solution filtered and the solvent then evaporated. The extraction was repeated three times, each using a fresh solvent.

McCue *et al*. (2004) studied potential inhibition of nonproteinaceous α-amylase inhibitor of selected American and Asian foods against α-amylase and α-glucosidase by homogenizing each of them in distilled water ($dH_2O$) for 1 min using a Waring laboratory blender set on "HIGH". The homogenate was centrifuged at 10,000 rpm at 4°C for 20 min. The supernatant was filtered through Whatman filter paper # 1 and subsequently optimized for phenolic content.

Hansawasdi *et al*. (2000) extracted hibiscus acid from a roselle (*H. sabdariffa* Linn.) tea with 50% aqueous methanol (10 ml / g fresh weigh) for 24 hours at room temperature. The methanol was evaporated from one part of the extract, the resulting residue was redissolved in dimethyl sulfoxide (10 ml/g fresh weigh) and subjected to a porcine pancreatic amylase (PPA) inhibitory activity assay.

Kim *et al*. (2002) isolated acarbose from *Actinoplanes* sp. by centrifugation at 8,000 x g for 15 min. The pH of the supernatant was readjusted to 6 with 0.1 M NaOH and concentrated by 10 folds using a rotary vacuum evaporator. The concentrated supernatant (20 ml) was precipitated by adding 80 ml of methanol. After removal of sediment by centrifugation at 4,000 x g for 5 min, 800 ml of ethanol was added to 100 ml of the supernatant. Finally, the high-molecular-weight acarbose was collected by centrifugation and dissolved in 200 ml of 50 mM sodium acetate buffer (pH 6.0).

**5. Purification and identification of proteinaceous α-amylase inhibitor**

Dayler *et al*. (2005) purified proteinaceous α-amylase inhibitor from crude extract of common bean seeds by ammonium sulfate precipitation followed by dialysis. The fraction obtained between 0% and 80% saturation was applied to an ionic exchange DEAE-cellulose column equilibrated with 20 mM $KPO_4$ buffer, pH 6.7 with a flow rate of 30 ml/hours. The retained proteins were removed with a 0-0.2 M NaCl linear gradient and futher applied onto an HPLC reversed phase analytical column (Vydac 218 TP 1022 C-18) at flow rate of 1.0 ml/min.

Figueira *et al*. (2003) purified corn amylase inhibitor by ammonium sulfate at 30 to 60%saturation. The precipitate was recovered by centrifuging at 30,000 x g for 20 min at 4°C, then the pellet was dissolved in 0.01 M Tris-HCl buffer pH 8.0 and concentrated using an ultrafiltration cell with a 30 kDa molecular mass exclusion membrane. The concentrated sample was fractionated on a Sephadex G-75 gel filtration column (166 cm x 1.6 cm), equilibrated with 0.01 M ammonium bicarbonate buffer pH 8.2 and the elution was carried out at a flow rate of 15 ml/hours. Protein content was monitored at 280 nm. The active fractions were pooled and dried. The pellet was solubilized in 0.01 M Tris-HCl buffer pH 7.5 and fractionated in an HPLC system fitted with a superose 12 HR 10/30 equilibrated with the same buffer. The elution was carried out at a flow rate of 0.4 ml/min and the active peaks was collected and concentrated by ultrafiltration cell using a 10 kDa molecular mass exclusion membrane. The active sample was applied into HPLC system fitted with an anionic exchange column Vydac 300 VHP 575 (0.75 cm x 5.0 cm) equilibrated with 0.01 M Tris-HCl buffer pH 7.5. Separation was performed using a linear NaCl gradient in the same buffer (0 to 0.5 M NaCl in 120 min). The separation was monitored at 220 nm then the active peak was collected and stored at -20°C.

Iulek *et al*. (2000) purified α-amylase inhibitor from rye by ammonium sulfate at 20-50% saturatation, followed by centrifugation at 9,400 x g for 1 hours, then redissolved in and extensively dialysed against 20 mM phosphate buffer pH 6.9. The resulting solution was first applied to a DEAE-Sepharose ion exchange (24 cm x 2.6 cm). Fractions were eluted stepwise using 20, 30 and 200 mM phosphate buffer pH

6.9, followed by a gradient of 0.0-1.0 M NaCl in the same buffer. A flow rate of 144 ml/hours was used throughout. Fractions with α-amylase inhibitory activity were then dialysed against 50 mM acetate buffer pH 5.0 and submitted to CM-Sepharose ion exchange column (57cm x 1.6 cm). Fractions were eluted using steps of 50, 100, 500 mM acetate buffer followed by a gradient of 0.0-1.0 M NaCl in the same buffer, at a flow rate of 138 ml/hours.

Yamada *et al*. (2001) purified and estimated molecular weight of αAI-Pa1 and αAI- Pa2 from *Phaseolus acutifolius* A. Gray by using column chromatography of DEAE–sephacel and CM-Sepharose and used sodium dodecyl sulfate polyacrylaminde gel electrophoresis (SDS-PAGE) of 13.5 % acrylamide. Molecular weight of αAI- Pa1 was determined as 39.6 kDa, while molecule weight of αAI- Pa2 was estimated as 28.1 kDa.

## 6. Purification and identification of nonproteinaceous α-amylase inhibitor

After the extraction, nonproteinaceous α-amylase inhibitors were purified by thin layer column chromatography (TLC).

Kim *et al*. (2002) purified acarbose from *Actinoplanes sp*. by thin layer column chromatography (TLC) using Whatman K6F silica gel plates and a solvent system of ethyl acetate/isopropyl alcohol/water (1:3:1, v/v/v). After the developing, the plate was dried and visualized by spraying with a solution containing 0.3% (w/v) *N*-(1-naphthyl)-ethylenediamine and 5% (v/v) $H_2SO_4$ in methanol and heated at 110 °C for 10 min.

Yoon and Robyt, (2003) prepared and purified acarbose analogues by adding B. macerans CGTase [EC 2.4.1.19] (15 IU) to 2.0 ml, containing 50 mM acarbose and 50 mM cyclomaltohexaose in 25 mM imidazolium / HCl buffer (pH 6.0). The enzyme reaction was carried out at 35 °C for 6 days with the periodic, stepwise addition of 1.0 ml of 100 mM cyclomaltohexaose solution every 1- 2 days. After stopping the enzyme reaction by heating in boiling water for 5 min, insoluble matter was removed

by centrifugation at 4,000 rpm for 10 min and then the supernatant was concentrated to 1.2 ml by rotary vacuum evaporation. The major reaction products, G6-Aca, G12-Aca and G18-Aca, were purified by Bio-Gel P2 column (1.5 cm x 100 cm) chromatography (flow rate 0.06 ml/min, fraction size 1.0 ml).

Jamaluddin *et al*. (1995) isolated and purified stigmast-4-en-3-one from the chloroform extract of *P. speciosa* by silica gel column chromatography (6.5 cm x 46 cm) eluted with petroleum ether/$CHCl_3$ (1: 1v/v) followed by a gradual increase with 5% MeOH at each successive addition of solvent. Fractions were analysed on TLC (silica gel). Fractions having similar TLC patterns were combined to give 12 fractions. When the fractions were screened, only fraction P-7 showed significant hypoglycaemic activity. P-7 was further purified by preparative TLC using a pet-ether/$CHCl_3$, (3: 7v/v) solvent system to produce 4 bands. The first band, P-7.1, the major component was purified 4 times until the final single spot was obtained on the analytical TLC plate. P-7.1 spot was recrystallised with hot petroleum ether and partially crystallised form upon cooling and evaporation of the solvent to obtain the yellowish compound.

Kandra *et al*., 2004 identified structure of tannin by using MALDI-TOF MS and $^{1}H$ and $^{13}C$ NMR. The molecular mass of the first [M + Na]þ peak (m/z 519) from MALDI-TOF MS precluded the presence of glucose and quinic acid. These data confirm that inhibition of salivary amylase by the commercial tannin is based on a galloylated quinic acid structure.

## 7. The role of α-amylase inhibitor in controling postprandial plasma glucose levels

Key enzymes involved in the breakdown of complex carbohydrates, salivary α-amylase, pancreatic α-amylase and intestinal α-glucosidase, are targeted for modulation of type 2 diabetes-associated post-prandial hyperglycemia. The inhibition of these enzyme activities leads to decrease meal-derived glucose absorption. A number of medicinal plant and herbal extracts have been found to inhibit the enzymatic activity of α-amylase and α-glucosidase which indicated the hypoglycemic activity (Kameswara Rao *et al*., 2001; Virdia *et al*., 2003; McCue *et al*., 2005).

Prolonged administration of an α -amylase inhibitor, isolated and purified from white kidney beans (*Phaseolus vulgaris*), can reduces blood glucose levels and body-weight gain in Wistar rats (Tormo *et al*., 2004).

Pine bark extract (PBE) showed its potency against salivary and pancreatin α-amylases and yeast *Saccharomyces cerevisiae* α-glucosidase with more than 90% inhibition. It was found that PBE effectively suppressed the increase of postprandial blood glucose level by delaying absorption of diet in diabetes mice. In addition, the body weights of the PBE-fed mice were significantly lower than in the control group. Thus, PBE can be used to suppress postprandial hyperglycemia of diabetic patients. It also can be applied for control of obesity by decreasing the food efficiency ratio, especially carbohydrates (Kim *et al*., 2005).

The ethanolic extract of *Annona squamosa* leaves can reduce blood glucose level by 17.1% in normal rats. The same dose of the ethanolic extract also reduced the fasting blood glucose by 38.5 and 40.6% at 1 and 2 hour, respectively, in alloxan-induced diabetic rabbits, and it reduced 37.2 and 60.6% at 1 and 2 hour, respectively, in streptozotocin (STZ)-induced diabetic rats (Gupta *et al*., 2005).

*Gentiana olivieri* Griseb. (Gentianaceae) is widely used in east and south-east Anatolia as bitter tonic, stomachic and to combat some mental disorders in the different regions of Turkey. Macerate of the dried flowering herb in water has been

used to lower the blood glucose in type-2 diabetic patients. Sezik *et al*. (2005) scientifically proved the claimed hyperglycaemic activity of the plant. Their study revealed that isoorientin, the main activity ingredient of the plant, exhibited significant hypoglycemic and antihyperlipidemic effects at 15 mg/kg body weight. This compound is known as c-glycosylflavone.

**8. The role of α-amylase inhibitor in insect resistance.**

The seeds of starchy grain legumes are an important staple food and a source of dietary protein in many countries. These seeds are rich in proteins, carbohydrates and lipids and therefore suffer extensive predation by bruchids (weevils) and other pests. The larvae of weevils burrow into the seedpods and seeds and the insects usually continue to multiply during seed storage. The damage causes extensive losses, especially if the seeds are stored for long periods. Many plants contain secondary metabolic compounds which are definitively associated with plant defence. These secondary metabolites include antibiotics, alkaloids, lectins and enzyme inhibitors. The enzyme inhibitors impede digestion through their action on insect gut digestive α-amylases which play a key role in the digestion of plant starch.

Insect α-amylases play a key role in carbohydrate metabolism of several insects, especially the seed weevils that feed on starchy seeds during larval and/or adult stages. Starch is digested by α-amylases for energy source, therefore α-amylases are one of a factor indicating the survival of several insects. Incorporation of amylase inhibitor gene in several crop plants by transgenic technology has been proposed for creating a natural defense of the plants against insects destruction.

A proteinaceous α-amylase inhibitor αAI-1 from common bean (*P. vulgaris* cv. Magna) was found to inhibit α-amylases of 30 species of insects, mites, gastropod, annelid worm, nematode and fungal phytopathogens. *In vitro* analysis showed a selective inhibition to α-amylase from three orders of insects (Coleoptera,

Hymenoptera and Diptera) and annelid worm. In addition, the αAI-1 can suppress the development of insect larvae, that expressed the sensitive digestive α-amylases (Kluh *et al*., 2005). Like αAI-1 from common bean, two α-amylase inhibitors, αAI-Pa1 and αAI-Pa2, from seeds of a cultivated tepary bean (*Phaseolus acutifolius* A. Gray, cv P1311897), purified by Yamada *et al*. (2001) were found to have inhibitory activity against five α-amylase pests. AI-Pa1 could inhibit the α-amylase activity of *Callosobruchus chinensis*, *Callosobruchus maculates*, *Tenebrio molitor* and *Tribolium conf.usum* but not *Zabrotes zubfasciatus*, while, αAI-Pa2 was significantly active against all insect α-amylases, but its inhibition of the *C. chinensis* and *C. naculatus* α-amylase was less than 50% (Yamada *et al*., 2001). Recently, cDNAs encoding αAI-Pa1 and αAI-Pa2 were isolated and transferred to azuki bean (*Vigna angularis* Willd. Ohwi & Ohashi). The expression of these cDNAs in azuki bean revealed the active forms of the inhibitor proteins accumulated in seeds and they also exhibited specificities for insect α-amylases (Yamada *et al*., 2005).

## 9. Sataw (*Parkia spesiosa* Hassk.)

Sataw (*Parkia speciosa* Hassk.) belongs to the family Leguminoceae, sub-family Mimosaceae (Figure 8). It is found growing naturally in the rainforests of southern Thailand. It is commonly found as village trees in many rural areas of southern Thailand, Malaysia and Java. In Malaysia, it is also known as "**Petai**" (Suranant, 2001).

### 9.1 Botanical descriptions

Sataw is grown for its edible seeds. It is a large, evergreen tree that can grow up to 15-35 m in height. The crown is variable in shape but is usually rather flat-topped or is umbrella-shaped. In a well-grown tree the shape can be oblong. The long, stalked leaves are bipinnate with 10-20 pairs of side branches bearing very small, dark green leaflets. Each leaflet is oblong with a blunt end and an asymmetric base. The

inflorescence resembles a drumstick as it has a long stalk carrying a large globular head of close-packed, cream-colored flowers at the end. The flowers produce a great deal of nectar and have a strong, somewhat sickly smell. They are pollinated by bats and only the apical flowers develop fruits. Six to ten fruits develop in each inflorescence. The pods are green at first, becoming dark brown or blackish brown when ripe. When the tree is fruiting, the groups of young, light green pods give it a distinctive appearance easily visible from a great distance. Pods are up to 50 cm long and 6 cm wide. They are usually collected when still green and are sold in the market (Suranant, 2001).

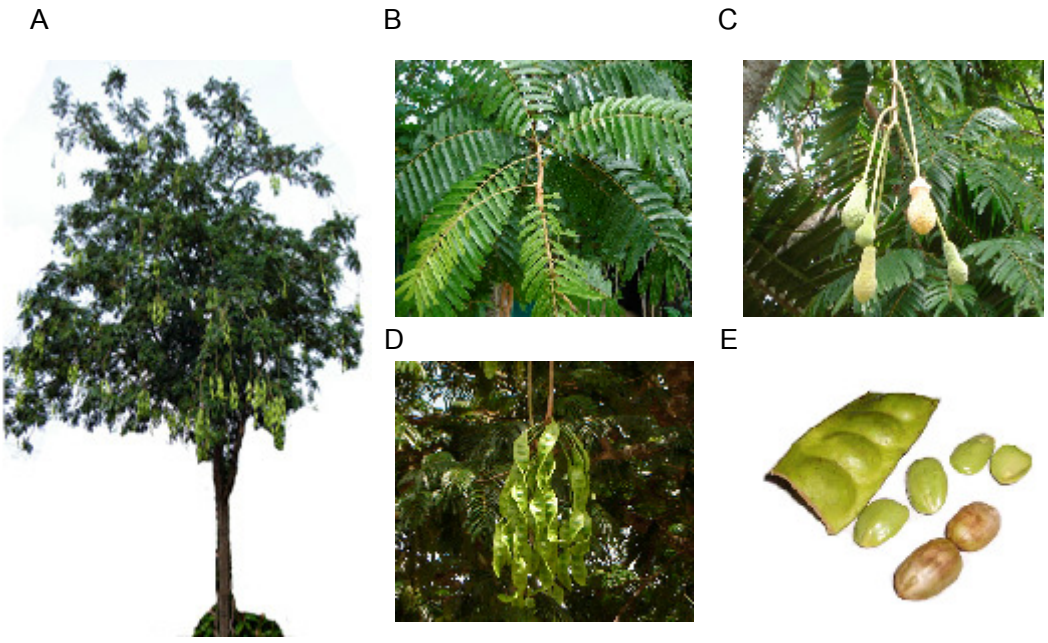


**Figure 8.** *Parkia spesiosa* Hassk. (Sataw) : (A) sataw tree, (B) leaves, (C) flowers, (D) green pods, (E) seeds
(Photographs by Miss Rattawan Poodproh)

### 9.2 Variety

There are many known varieties of sataw, but only three varieties are common in southern Thailand. Many other varieties are cultivated elsewhere, but they are poorly documented at present. The three varieties of southern Thailand are described by Banroongrungsa and Yaacob (1990) as follows:

i. Kow-sataw (or rice sataw): This is the most popular variety of sataw in the local markets. It has many small seeds in the pod. The seeds have a strong odour and are quite sweet. This variety is suitable for consumption. It can produce fruits at 4-5 years after planting and is also classified as an early maturing variety.

ii. Darn-sataw: This variety has larger pods and seeds than those of the kow-sataw, but it produces fewer pods per tree. In addition, its stem canopy is larger and taller than that of the kow-sataw. In this variety the first flowering can be seen at 6-7 years after planting. As the darn-sataw had harder seeds, a stronger odour, and better taste than kow-sataw, it is more popular.

iii. Tae-sataw: This variety has very hard pods and seeds, so it is not suitable for consumption (Suranant, 2001).

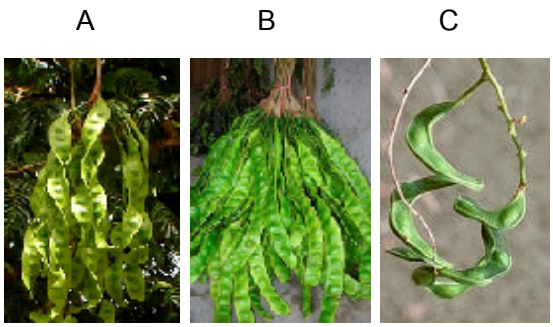


**Figure 9.** Variety of sataw (A) Kow-sataw, (B) Darn-sataw, (C) Tae-sataw (A: photograph by Miss. Rattawan Poodproh, B: from: http://www.ranong.doae.go.th /sator.html, and C: from http://www. kochconnect .com/ modulates. Php)

### 9.3 Uses

Sataw is grown for its edible seeds. The seeds contain high nutritional value and are served as a local vegetable in many dishes of southern Thailand. Composition per 100 g edible seeds is carbohydrates 11.4 g, protein 8.0 g, fat 8.1 g, fiber 0.5 g, ash 1.3 g, calcium 76 mg, phosphorus 83 mg, iron 0.7 mg, vitamin A 73.4 IU, vitamin $B_1$ 0.11 mg, vitamin $B_2$ 0.01 mg and niacin 1.0 mg. This rather high nutritional value makes sataw seed to be one of the most nutritious local vegetables of southern Thailand (Suranant, 2001).