CHAPTER III
LITERATURE REVIEW

3.1 Legumes and Their Characteristics

Legumes are plants in Leguminaceae family with high nutritive contents [1]. Legumes are widely known as the richest sources of macronutrients and micronutrients as well as protein, carbohydrate, lipid, vitamin, mineral and dietary fiber [2]. The structure of legume seed consists of three major components, including seed coat, embryo, and endosperm (Figure 3.1). Seed coat (testa) is grown from wall of embryo sack, which found in the outermost of seed. Seed coat protects embryo and prevents seed dehydration. Embryo is immature plant, consisting of cotyledon, epicoty, hypocoty and radical. Endosperm is food supply for embryo growth, composing of carbohydrate, protein and fat. However, most legumes possess little endosperm at the ripening stage, as the cotyledons making up a ripeness of the seed weight and containing the essential stores for growth [3].

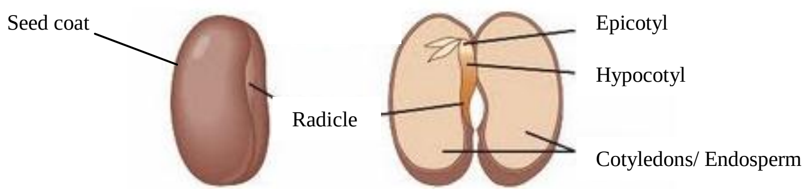


Figure 3.1 Structure of legume seed, which consists of seed coat (testa), embyo, and endosperm.

The global production of edible legumes was about 275 million tons in 2010 and has grown to 12–15% of the Earth’s arable surface [4]. China is rich in leguminous plants with the agriculture of more than 20 types of legumes throughout the country [5]. Legume consumption may play an important role in health maintaining through its well–recognized antioxidant, anti–proliferative and anti–genotoxic effects. The important health components of legumes are proteins and

bioactive compounds. Proteins in legume are located in the cotyledons and embryonic area [3] and referred to as enzymatic or catalytic proteins. These proteins are mostly functioned as protease inhibitors, lectins, lipoxygenases and amylase
inhibitors [6]. On the other hand, bioactive compounds that are mostly located in seed coat of legumes can function as antioxidants [7]. Epidemiological studies had reported the positive correlations between the consumption of foods with high contents of phenolics/antioxidants and the decrease in the incidence of several diseases such as cancer, ageing and cardiovascular disease [8]. All legumes including mung bean, black bean, red kidney bean, white bean, soy bean and peanut (Figure 3.2), which can be found in general supermarket and grown in every regions in Thailand, contain relatively high amount of phenolics and antioxidants. Thus, these legumes can be promoted regarding their health benefits against the oxidative stress related environments.

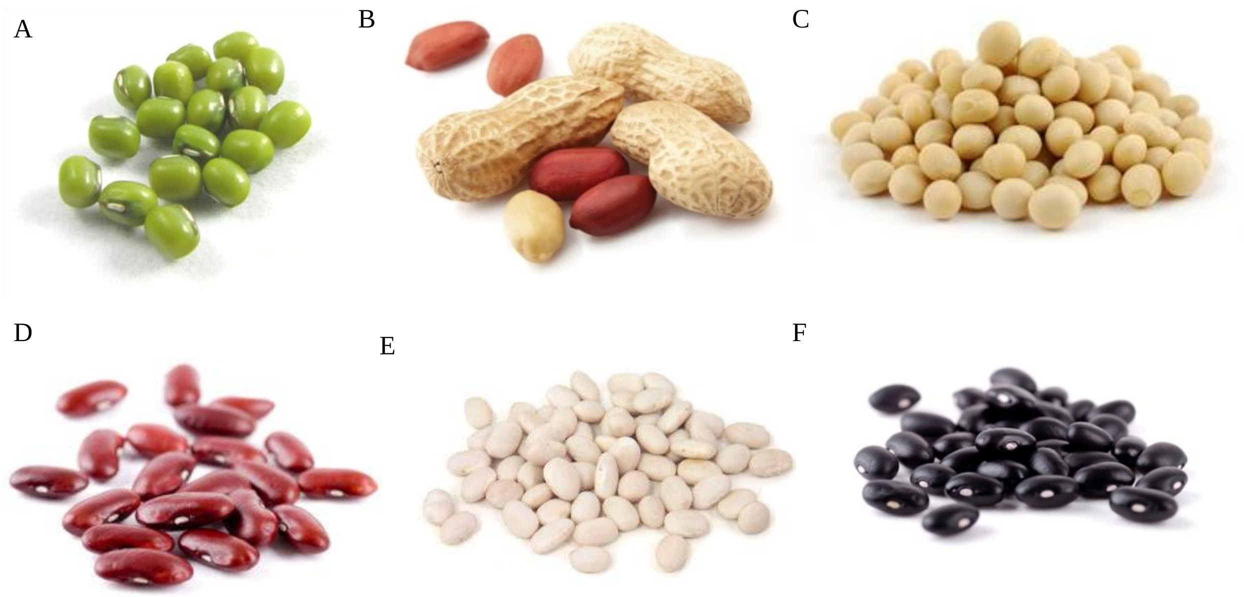


Figure 3.2 Different types of legumes including (A) mung bean, (B) peanut, (C) soybean, (D) red kidney bean, (E) white bean and (F) black bean.

3.1.1 Mung bean

Mung bean, *Vigna radiata* (L.) Wilczek, is an important crop in Thailand and can be grown throughout Asian regions such as India, Pakistan, Indonesia, Philippine and China [9]. It can be easily grown several times a year. A height of a trunk is about 40–60 cm with 4–6 branches. Seeds of mung bean are green, small, round and long [10, 11]. The chemical compositions per 100 g edible of mung bean consist of 21.7 g of protein, 1.5 g of fat, 26.1 g of fiber, 3.9 g of ash and 62.7 g of carbohydrate (Table 3.1) [12]. Previous study had indicated that consumption of mung bean could cause a minute increase in blood glycemic index in humans, making it an attractive food choice for diabetic patients [13].

3.1.2 Soybean

Soybean, *Glycine max* (L.) Merrill, is mostly found in America, Brazil and China. It is an annual crop with post–harvest time of about 90–130 days, depending on seed and environment. A height of a trunk is about 40–60 cm with 3–5 foliate leaves. Soybean pod is developed from the ovary and has long, curved shape with 2–3 yellow seeds within. Seeds in the pod are arranged horizontally and have ellipsoidal shape. Soybean has high protein content with nutritionally balanced amino acid profile [11, 14]. The chemical compositions per 100 g edible of soybean consist of 34.2 g of protein, 16.7 g of fat, 21.9 g of fiber, 5.9 g of ash and 33.9 g of carbohydrate (Table 3.1) [12]. Soybean contains various biologically active phytochemicals such as isoflavones, genistein, daidzein, coumestrol, phytate, saponins, phytate, lecthin, phytosterols and vitamin E. Genistein and daidzein are the major isoflavones found in soybeans, which counted for 50% and 40% of total isoflavone content, respectively [15]. These compounds provide several health benefits, including protection against oxidative stress and anti–carcinogenic properties [16, 17].

3.1.3 Red kidney bean

Red kidney bean, *Phaseolus vulgaris* L., is grown in the tropical and subtropical regions such as China, Indonesia, India, Turkey, Egypt, Morocco, Italy, Spain, Brazil, America, Tanzania, Mexico, Myanmar, Tanzania, Kenya, Argentina and Thailand [18]. It is the most widely produced edible legume in Africa, India, Latin

America and Mexico. Red kidney beans are good source of nutrients with 19.8 g of protein, 1.9 g of fat, 25.4 g of fiber, 4.3 g of ash and 62.5 g of total carbohydrates (Table 3.1) [12]. Previous study has been reported that anthocyanins, cyanidin 3–0–β–D–glucoside and cyaniding, from seed coat of red kidney bean may play biological function as antioxidants to prevent lipid peroxidation in cell membranes and protect oxidative damages in animal cells [19]. Red kidney bean is also reported to contain five major anthocyanins, including cyanidin–3,5–diglucoside, delphinidin–3–glucoside, cyanidin–3–glucoside, petunidin–3–glucoside and pelargonidin–3–glucoside with delphinidin–3–glucoside being the most abundantly found in seed coat [7].

3.1.4 White kidney bean

White bean, *Phasecolus vulgaris* L., is originated in highlands such as Mexico and Guatemala, where provide suitable weather for growth. In Thailand, white bean is mostly grown in the north, but the crop is not widespread populated. A shape of seed is similar to that of red kidney bean. Both seed coat and seeds are white. The chemical compositions per 100 g edible of white kidney beans are 22.5 g of protein, 2.3 g of fat, 4 g of ash and 60.3 g of carbohydrates (Table 3.1) [12]. Previous study had indicated that crude extract of white kidney beans exhibited α–amylase inhibitory activity, suggesting that white kidney bean could affect blood glucose, starch digestion and glucose absorption in mammals [20].

3.1.5 Black bean

Black bean, *Phaseolus mungo* Linn or *Vigna mungo*, is an annual crop, which is normally populated in India and Thailand. A height of a trunk is about 25–90 cm with greenish yellow leaves and tap root system. Black bean seed has an ellipsoidal shape and white helium at a curve of the seed [11]. The chemical compositions per 100 g edible of black bean consist of 22.9 g of protein, 2.2 g of fat, 19.7 g of fiber, 4.2 g of ash and 60.9 g of carbohydrate (Table 3.1) [12]. It was previously suggested that anthocyanin, a constituent of black bean seed coat, might be one of the agents responsible for protection against DNA damage, which is caused by highly reactive free radicals [19, 21]

3.1.6 Peanut

Peanut, *Arachis hypogaea* L., is a native legume found in South America such as Brazil, Paraguay, Peru, Uruguay and Argentina. It is also an economic crop in Thailand due to its easy growth in all soil types. Peanut is an annual, softwood type and short harvested crop. A height of a trunk is about 15–70 cm with 4–6 branches. Seed of peanut has cylindrical shape, which is bigger than that of mung bean. The seed skin has a pink–red color, which is rich in phenolics and other potential health promoting compounds [22]. The chemical compositions per 100 g edible of peanut consist of 24.4 g of protein, 44.1 g of fat, 20 g of fiber, 2.6 g of ash and 24.1 g of carbohydrate (Table 3.1) [12]. Peanut has been identified as a source of phytochemicals such as trans–resveratrol, phytosterols, isoflavones, genistein and daidzein. These biologically active compounds have been reported to exhibit anti–bacterial, anti–viral, anti–inflammatory, anti–carcinogenic and anti–estrogenic properties [23].

Table 3.1 Nutritional compositions of legumes including protein, carbohydrate, fat, ash and fiber (per 100 g edible portion)

Type of legumes	*Composition per 100 g edible*						
	Protein (g)	*Carbohydrate (g)*	*Fat (g)*	*Fiber (g)*	*Ash (g)*	*Water (g)*	*Energy (kcal)*
Mung bean	21.7	62.7	1.5	26.1	3.9	10.2	351
Black bean	22.9	60.9	2.2	19.7	4.2	9.8	355
Red kidney bean	19.8	62.5	1.9	25.4	4.3	11.5	346
White kidney bean	22.5	60.3	2.3	–	4.0	10.9	352
Soybean	34.2	33.9	16.7	21.9	5.9	9.3	423
Peanut	24.4	24.1	44.1	20.0	2.6	4.9	591

Source: Thai Food Composition Table [12].

3.2 Legume and Health Promotion

Chinese believes that legumes can protect internal organs according to their colors. Black bean, a good source of isoflavone and anthocyanin, encourages the improvement of the kidney through elimination of toxin, protection against DNA damage and invigoration of blood circulation [24]. Red kidney bean with high dietary fibers is believed to strengthen heart, relax bowel, control blood pressure and adjust blood sugar level [25]. Mung bean (green bean) with sweet taste and cold nature can tonify liver, decrease cholesterol level, act as anti–allergic food and show anti–inflammatory property [13, 26]. Soybean (yellow bean), a saponin rich source, can reinforce the spleen through secretion of cholic acid that helps digesting fat [27]. White bean contains saponin, thus inhibiting digestive enzyme of carbohydrate and reducing triglyceride [28]. Lastly, peanut, a procyanidins rich source, can decrease total blood lipid and plasma fatty acid profile [29]. Interestingly, previous studies had been suggested that high antioxidant activities and total phenolic contents (TPCs) were found in pigmented legumes such as black and red beans [30]. These properties of legumes are correlated to inhibitory activity of some key enzymes that control diabetes, obesity, Alzheimer's disease and hypertension. In this study, biochemical properties of legumes regarding antioxidants and inhibition of lipase, α–glucosidase, α–amylase, cholinesterases, β–secretase and angiotensin–converting enzyme were focussed.

3.2.1 Chemical compositions and antioxidant properties

Legumes have been reported as a good source of phenolics and antioxidants, which provide health benefits against oxidative stress and chronic diseases. Consumption of antioxidant–rich foods is related to reduction in risk of oxidative stress related diseases. Legumes contain many bioactive phytochemicals such as phenolic compounds (i.e. phenolic acid, tannin and flavonoids), tocopherol, vitamin C, phytic acid and saponin [30-32]. Different types of legumes have been reported to show different phenolics regarding their biological properties and quantities [33]. Most phenolics can act as antioxidants as well as biological agents against some key enzymes that control NCDs.

Phenolic acids

Phenolic acids are derivatives of benzoic and cinnamic acids (Figure 3.3), which can be divided into two classes, including hydroxybenzoic acid (i.e. gallic, vanillic, syringic, protocatechuic and *p*–hydroxybenzoic acids) and hydroxycinnamic acid (i.e. coumaric, caffeic, ferulic and sinapic acids) [34]. Previous study suggested that phenolic contents and antioxidant activities in legumes are varied and can be divided into three major groups, including low (0.325–0.966 mg GAE/g), moderate (1.014–1.878 mg GAE/g) and high (2.086–6.378 mg GAE/g) phenolic contents (Table 3.2) [33]. Lablab bean with white seed coat provided the lowest phenolic content and antioxidant activity, while brown seed coat of lablab bean showed moderate phenolic content and antioxidant activity. Cow pea with red seed coat exhibited the highest phenolic content. Almost legumes with darker seed coats showed higher antioxidant activities than those of the ones with lighter seed coats, except for moth bean, black pea, black gram and lentils. Likewise, among legumes being investigated in India, legume seed of *T. indica* (dark brown color) exhibited the highest content of phenolics and antioxidant activities as being determined by ferric reducing antioxidant power (FRAP) and 1,1–diphenyl–2–picryl–hydrazyl (DPPH) assays (Table 3.3) [32]. On the other hand, legume seeds from *B. variegate* and *A. leucophloea* (light brown color) exhibited the lowest phenolic contents and antioxidant activities [35], suggesting that seed coated color is correlated to the quantity of phenolic contents and antioxidant activities. Nevertheless, the variation in phenolic content is also suggested to be depended on genetic factors, level of maturity and environmental conditions. The last involves extraction conditions (type of extraction solvent (polarity), extraction time and extraction temperature), degree of polymerization of phenolics and interaction of phenolics with other food constituents [8, 32, 36, 37].

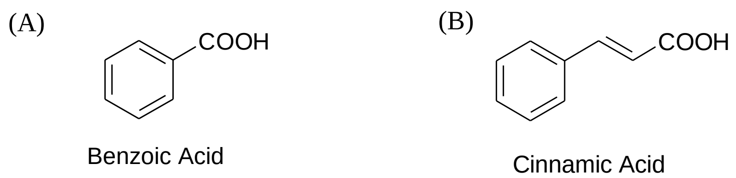


Figure 3.3 Basic chemical structures of phenolics including (A) benzoic acid and (B) cinnamic acid.

Table 3.2 Phenolics contents and antioxidant activities as being analyzed by DPPH radical scavenging, ABTS and FRAP assays of different types of legumes [33].

Legumes	***Antioxidant activities***			
	Phenolics (mg GAE/g)	***DPPH***	***ABTS***	***FRAP***
A. Low phenolic content (<1.0 mg GAE/g)				
Lablab bean (white)	0.325 ± 0.002	L	L	L
Chick pea (cream)	0.460 ± 0.053	L	L	L
Moth bean (moderate brown)	0.480 ± 0.001	L	M	L
Chick pea (green)	0.491 ± 0.002	L	L	L
Chick pea (big brown)	0.514 ± 0.009	L	L	L
Butter bean (white)	0.648 ± 0.036	L	L	L
Lablab bean (cream)	0.693 ± 0.002	L	L	L
Lentil (red–orange)	0.914 ± 0.115	M	M	M
Pea (white)	0.938 ± 0.039	L	L	L
Pea (green)	0.966 ± 0.040	L	L	L
B. Moderate phenolic content (>1.0 and < 2.0 mg GAE/g)				
Common bean (white)	1.014 ± 0.020	L	M	M
Lablab bean (brown)	1.017 ± 0.038	M	M	M
Pea (black)	1.040 ± 0.040	M	M	M
Pigeon pea (light brown)	1.054 ± 0.011	M	M	M
Green gram (yellow)	1.217 ± 0.030	M	M	M
Chickpea (small brown)	1.359 ± 0.046	M	M	L
Cowpea (white)	1.480 ± 0.028	M	M	M
Common bean (maroon)	1.600 ± 0.037	M	M	M
Green gram (green)	1.834 ± 0.016	M	M	M
Black gram (black)	1.878 ± 0.029	M	M	M
C. High phenolic content (>2.0 mg GAE/g)				
Cow pea (red)	2.086 ± 0.058	H	H	H
Soybean (yellow)	2.170 ± 0.062	M	H	H
Common bean (black)	2.184 ± 0.036	H	H	H
Common bean (brown)	2.406 ± 0.022	H	H	H
Peanut (brown)	2.448 ± 0.055	H	M	M
Common bean (beige)	3.235 ± 0.061	H	H	H
Horse gram (light brown)	3.579 ± 0.072	H	H	H
Common bean (red)	3.583 ± 0.059	H	H	H
Fenugreek(light brown)	4.298 ± 0.054	H	H	H
Cowpea (brown)	6.378 ± 0.054	H	H	H

DPPH activity: L<6.0 units/g legume; M 125–400 units/g legume; H>400 unit/g legume
ABTS activity: L<6.0 µmol Trolox equivalent antioxidant capacity (TEAC)/g legume; M 6.0–12 µmol TEAC/g legume; H >12.0 µmol TEAC/g legume
FRAP activity: L<8.5 µmol Ferric reducing antioxidant power (FRAP)/g legume; M 8.5–16.5 µmol FRAP/g legume; H >16.5 µmol FRAP/g legume

Table 3.3 Phenolic contents and antioxidant activities as being analyzed by FRAP and DPPH radical scavenging assays in wild legumes [32].

Legumes	***Analyses***		
	Phenolic (μg/mg)	***FRAP Assay (mM $FeSO_4$/mg)***	***DPPH assay (%)***
A. leucophloea (dark brown)	148.7 ± 35.1 [b]	32.3 ± 0.9 [d]	52.1 ± 5.9 [a]
B. variegate (dark brown)	120.0 ± 80.0 [c]	39.4 ± 0.2 [d]	49.0 ± 2.4 [a]
C. gladiate (red)	128.6 ± 55.1 [c]	121.1 ± 4.2 [b]	63.5 ± 4.8 [a]
E. scandens (dark brown)	148.0 ± 3.0 [b]	53.4 ± 1.6 [c]	49.0 ± 5.9 [a]
M. pruriens (black)	136.0 ± 70.0 [b]	53.3 ± 1.5 [c]	57.3 ± 3.3 [a]
S. bispinosa (yellow)	202.7 ± 46.2 [a]	52.9 ± 1.2 [c]	58.3 ± 1.8 [a]
T. indica (brown)	168.0 ± 36.1 [a,b]	145.0 ± 2.9 [a]	63.0 ± 5.0 [a]

Values are mean ± SD of three determinations.
Different superscripts within a row indicate significant difference of wild legume seeds (p<0.05)

Tannins

Tannins are phenolic compounds with intermediate to high molecular weight ranges (0.5–3 kDa). Tannins can be classified into two groups, including condensed tannins (i.e. proanthrocyanin and flavan–3–ols) and hydrolyzable tannins (i.e. glycosylated gallic acids, catechin, gallocatechin, epicatechin, epigallocatechin, kaempferol and quercetin). In nature, proanthocyanin that is mostly found in bark and heartwood is more abundant than hydrolyzable tannins. Likewise, tannins in legumes are mostly found in a form of condense tannins (Table 3.4). Previous research had suggested that darker pigmented legumes such as black bean, lentil and red kidney bean contain high contents of tannins, which are varied within a range of 0.12–8.78 mg catechin equivalent/g [38, 39]. Lighter colored legumes (i.e. yellow pea, chick pea) possess lower content of condensed tannins (black bean and red kidney bean). *Acacia mearnsii*, a good source of catechin–like flavan–3–ols (such as robinetinidol and fisetinidol, which are the complex tannins), are able to inhibit several enzymes such as α–amylases, α–glucosidases and lipases [40].

Table 3.4 Contents of condensed tannins in leguminous seeds [38, 39].

Legumes	***Condensed tannins (mg catechin equivalent/g)***	***Ref.***
Green pea	0.03–1.71	[38]
Yellow pea	0.00–1.52	[38]
Chick pea	0.00–1.85	[38]
Lentil	0.12–8.78	[38]
Red kidney bean	0.12–5.53	[38]
Black bean	0.37–6.74	[38]
Common bean	0.00–0.384	[39]

Flavonoids

Flavonoids are natural compounds that are mainly found in all parts of plants, especially photosynthesized plant cells. Flavonoid is consisted of two aromatic rings linked by three–carbon moiety. More than 5,000 flavonoids have been found in nature, including anthocyanins, flavanols, flavones, flavanones, flavonol and isoflavone (Figure 3.4) [41]. Flavonoids have been reported to exhibit particular biological activities such as anti–allergic, anti–inflammatory, anti–viral, anti–proliferative and anti–carcinogenic functions [42]. The flavonoids in legumes seeds are mainly anthocyanidins, flavanols, flavan–3–ols and flavones, which are present as glycosides in the seeds (Table 3.4) [39, 43, 44]. Besides, legumes have been reported as natural sources of isoflavone [41]. The content of total flavonoids ranges from 0.08–3.21 mg catechin equivalent/g in all legumes being investigated (Table 3.5) [39, 43, 44]. Like other bioactive compounds, darker colored legumes tend to possess more total flavonoids than lighter colored legumes.

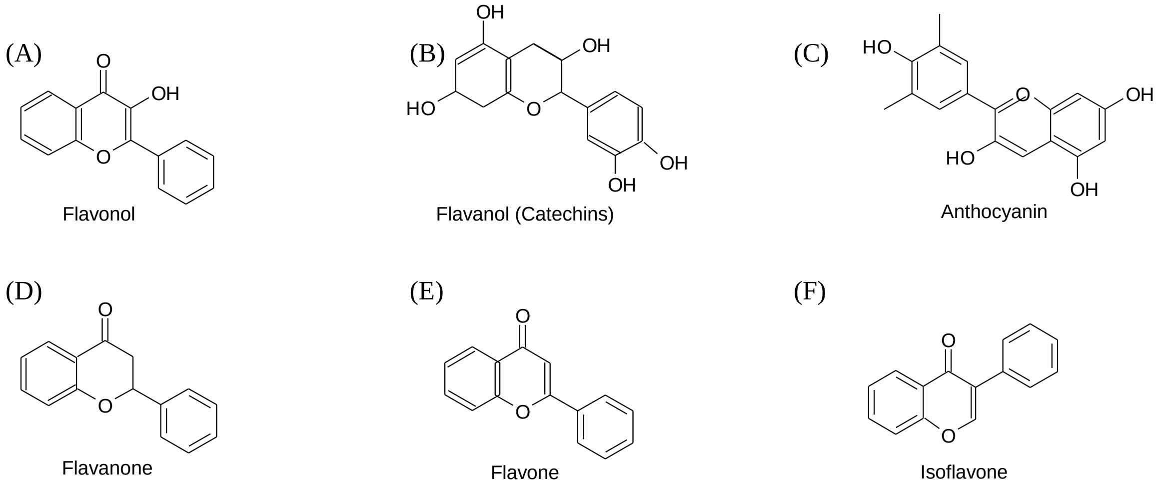


Figure 3.4 Chemical structures of different types of flavonoids including (A) flavonol, (B) flavanol (catechins), (C) antrocyanins, (D) flavanone, (E) flavone and (F) isoflavone.

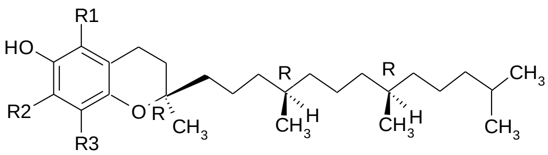
Table 3.5 Contents and types of flavonoids in leguminous seeds.

Legumes	***Compounds***	***Content***	***Ref***
Green pea	Total flavonoid	0.08–0.39 mg catechin equivalent/g	36
Yellow pea	Total flavonoid	0.18–0.32 mg catechin equivalent/g	36
Chickpea	Total flavonoid	0.18–3.16 mg catechin equivalent/g	36
Lentil	Total flavonoid	0.72–2.21 mg catechin equivalent/g	36
Red kidney bean	Total flavonoid	0.85–2.93 mg catechin equivalent/g	36
Black bean	Total flavonoid	1.19–3.21 mg catechin equivalent/g	36
Kidney bean seed coat	Cyanidin 3,5–diglycoside	4.3 µg/g	40
	Delphinidin 3–glucoside	182 µg/g	40
	cyanidin 3– glucoside	125 µg/g	40
	petrunidin 3– glucoside	167 µg/g	40
	pelargonidin 3– glucoside	588 µg/g	40
Cowpea	Quercetin 3–O–galactoside	3.6 µg/g	41
	Quercetin 3–O–glucoside	11.5 µg/g	41
	Quercetin diglycoside	1.8 µg/g	41
	Myricetin 3–O–glucoside	9.6 µg/g	41

Vitamin E

Vitamin E, a fat soluble vitamin in the forms of tocopherol and tocotrienol (Figure 3.5), is a powerful antioxidant. The physiological property of vitamin E depends on its aptitude to eliminate free radicals in cell membranes and other lipid environment, thus preventing the oxidation of polyunsaturated fatty acids [43]. The health benefits of vitamin E are also reported to affect smooth muscle proliferation and platelet adhesion [45]. Besides, higher vitamin E intake could reduce coronary heart disease and prostrate cancer risks and mortality [44].

Unlike other bioactive compounds as being mentioned earlier in legumes, the content of vitamin E is not correlated to the color of seed coat. It was previously suggested that γ–tocopherol are mainly found in all legumes, especially soybean, which contains up to 237.6 mg/kg (Table 3.6) [46].



Isomer	R_1	R_2	R_3
Alpha	CH_3	CH_3	CH_3
Beta	CH_3	H	CH_3
Gamma	H	CH_3	CH_3
Delta	H	H	CH_3

Figure 3.5 The chemical stucture of four isomers of tocopherols family inlcuding α–, β–, γ– and δ–tocopherols.

Table 3.6 Concentrations of different forms of tocopherols (vitamin E) in different types of legumes seeds [46].

Legumes	***Tocopherols (mg/kg)***		
	Alpha	***Gamma***	***Delta***
Broad bean (*Vicia faba var.maior*)	16.6	53.2	–
Common bean (*Phaseolus vulgaris*)	–	34.1	6.3
Field pea (*Pisum arvense*)	7.8	87.2	–
Flat pea (*Lathyrus sativus*)	7.2	79.2	6.2
Garden pea (*Pisum sativum*)	7.1	85.3	–
Horse(field) bean (*Vicia faba* var. Minor)	15.3	50.3	5.4
Kidney bean (*Phaseolus coccineus*)	–	54.5	7.2
Lentil (*Lens culinaris*)	10.1	66.5	–
Soya bean (*Glycine max*)	65.5	237.6	62.4

Vitamin C

Vitamin C (ascorbic acid) is an essential micronutrient required for metabolic function of the body. Another form of vitamin C is dehydroascobic acid, which occurred though degradation of ascorbic acid in the presence of oxygen (Figure 3.6). Vitamin C is a cofactor for several enzymes involving in the biosynthesis of collagen, carnitine and neurotransmitters.

Leguminous seeds are not good sources of vitamin C [30]. However, limited vitamin C content could efficiently support antioxidant capacity of seeds. In legumes, vitamin C is found in both ascorbic acid and dehydroascorbic acid forms. The content of vitamin C in legumes seeds are varied with mung bean containing the highest quantity of ascorbic acid (Table 3.7) [30, 47]. Like vitamin E, the quantity of vitamin C in legumes is not correlated to the color of seed coat.

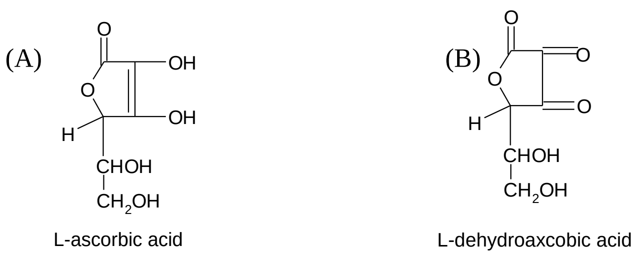


Figure 3.6 The chemical structures of vitamin C from leguminous seeds that are found in two forms, including (A) L–ascorbic acid and (B) L–dehydroaxcobic acid.

Table 3.7 Content of different forms of vitamin C found in leguminous seeds.

Legumes	***Ascorbic acid***	***Dehydroascobic acid***	***Both form***	***Unit***	***Ref***
Mung bean	6.27 ± 1.09	0.23 ± 0.05	–	mg/100g fresh weight	[47]
Adzuki bean	2.59 ± 0.28	0.02 ± 0.01	–	mg/100g fresh weight	[47]
Black gram	0.45 ± 0.04	–	–	mg/100g fresh weight	[47]
Kintoki bean	0.79 ± 0.12	–	0.06±0.00	mg/100g fresh weight	[47]
Tora bean	0.05 ± 0.01	0.44 ± 0.02	–	mg/100g fresh weight	[47]
Broad bean	0.21 – 1.94	0.00 – 0.06	–	mg/100g fresh weight	[47]
Green pea	0.00 – 1.74	0.32 – 6.24	–	mg/100g fresh weight	[47]
Pea	0.48 – 1.48	–	–	mg/100g dry weight	[48]

3.2.2 Anti–obesity property

Overweight and obesity are simply defined as abnormal or excessive fat accumulation associating with health consequences that vary considerably [49].

Obesity is a consequence of a positive energy balance that occurs when energy intake is greater than energy expenditure over a considerable period of time. Main causes of the disease are increased sedentary lifestyle, reduced physical activity and diet change such as increased sugar consumption [50]. Besides, the causes of obesity may be genetic, metabolic, physiologic, environmental, hormonal and social factors. However, energy intake and physical activity play a major role in developing overweight and obesity. Numerous options are available for treatment of obesity, including lifestyle therapy, pharmacological interventions and surgical operation. The change in life style for reducing calorie imbalance takes a major role in obesity treatment. Anti–obesity drug and surgery will be recommended in combination with life style therapy, once the later choice alone cannot maintain weight loss. The selection of treatment is depended on each individual’s profile.

Lipid is the major source of energy (9 kcal/g), and over–consumption of lipid can cause energy imbalance, which eventually lead to development of obesity. Pancreatic lipase (EC 3.1.1.3) is a major enzyme that hydrolyzes lipid in the form of triglyceride into free fatty acids (FAs) and monoacylglycerol (MGs). Free FAs and MGs then form micelles by mixing with bile salts, cholesterol and lysophosphatidic acid (LPA) before being absorbed into enterocytes. Triglycerides are re–formed and stored in adipocytes. Thus, lipase inhibition would reduce rate of fat accumulation.

Currently, there are only few commercially available anti–obesity drugs that approved by the Food and Drug Administration (FDA). These drugs are phentermine, diethylpropion, topiramate, zonisamide, lorcaserin and orlistat. However, numerous drugs are withdrawn because of their adverse effects. For examples, rimonabant can cause serious psychiatric disorders, while sibutramine can increase risk of heart attack and stroke in high–risk cardiac patients [51]. So far, orlistat is the only available drug that has been approved by FDA for the treatment of long term weight management through inhibition of lipase. Nevertheless, orlistat also has disturbing side effects, including oily stool, flatulence, constipation nausea, vomiting, diarrhea and lost appetite [52]. Thus, anti–lipase agents from natural sources that can prevent obesity without causing side effects are of interest.

Previous researches had been reported that phytochemicals might be associated with enzyme inhibition for anti–obesity, especially flavonoids that are effective lipase inhibitors [53]. The pancreatic lipase inhibitory effect is found in phytochemicals such as saponin, terpene and phenolic compounds. Previous studies had reported that extracts from plants rich in phenolics could provide pancreatic lipase inhibitory effect, for examples, pennywort, sweetleaf, Beijing grass, mulberry, safflower and ginkgo [54]. Legumes contain high phenolics and antioxidants such as isoflavone isoforms [55], thus legumes might be promising sources for prevention and treatment of obesity due to their lipase inhibitory activities. It was found that moth bean (light brown), Adzuki bean (black), Adzuki bean (red) could provide stronger anti–lipase activities than mung bean (green), suggesting that dark pigmented legumes might contain higher level of anti–lipase agents (Table 3.8) [56-58]. Anthocyanins that are mainly found in dark colored legumes might be responsible for this property. Anthocyanins from black chokeberry *(Aronia melanocarpa L.)*, cyanidin–3–glucoside, provide lipase inhibitory activity with IC_{50} (half maximal inhibitory concentration) value of 1.17 ± 0.05 [59]. Cyanidin and cyanidin–3,5–diglucoside can inhibit lipase with the IC_{50} values of 0.17 and 0.89 mg/mL, respectively [60]. Nevertheless, it was found that moth bean contains low quantity of anthocyanins; therefore, it is worthy to mention other phenolic compounds that might as well contribute the lipase inhibitory activity. However, lipase inhibition of legume in Thailand has not yet been reported.

Table 3.8 Summaries of lipase inhibitory activities from different legumes, bioactive compounds and anti–obesity drug, orlistat.

Samples	*Species*	*Solvent*	*IC_{50} (mg/ml) of Lipase inhibitory activity*	*Ref*
Legumes				
Mung bean	Vigna Species	Methanol	17.74	[56]
Moth bean	Vigna Species	Methanol	7.32	[56]
Adzuki bean (black)	Vigna Species	Methanol	7.92	[56]
Adzuki bean (red)	Vigna Species	Methanol	9.85	[56]
Faba bean	cv. Nura	Acetone	81.43	[57]
	cv. Rossa	Acetone	43.28	[57]
	TF(Ic *As) *483/13	Acetone	105.6	[57]
Bioactive compounds				
EGCG		DI	0.0004	[58]
Kaempferol		DI	0.0038	[58]
Quercetin		DI	0.0072	[58]
Curcumin		DI	0.0162	[58]
Resveratrol		DI	0.0207	[58]
Ferulic acid		DI	0.0241	[58]
Medication				
Orlistat		–	0.0160	[58]

3.2.3 Anti–diabetic property

Prevalence of diabetes in adults was estimated to increase up to 5.4% worldwide in year 2025. The occurrence is higher in developed countries with preference in female patients than in male [61, 62]. Main causes of the disease are associated with irregular insulin action, insulin secretion and endogenous glucose output [63]. Numerous treatment options are available including 1) non–pharmacological therapy such as appropriate diet, exercise, weight loss and lifestyle modification and 2) pharmacological interventions, which involve inhibition of enzymes that control carbohydrate degradation [64].

The carbohydrate digestive enzyme, α–amylase (EC 3.2.1.1), is a metalloenzyme belonging to glycosyl hydrolase, that randomly catalyzes endohydrolysis of α–1,4 glycosidic bonds in starch [65]. Starch digestion is begun in oral cavity by salivary α–amylase and continued to a small intestine by pancreatic α–amylase. Similarly, the intestinal α–glucosidase (EC 3.2.1.20), a membrane–bound enzyme located in the epithelium of the small intestine, can hydrolyze carbohydrate into disaccharide and monosaccharide before uptaking glucose to blood circulation

[66]. Thus, inhibition of α–glucosidase and α–amylase would prevent carbohydrate digestion and glucose absorption, which, in turn, could retard and deduce the level of postprandial hyperglycemia after meal as well as decrease triglyceride synthesis in adipose tissue, liver and intestinal wall [67].

Currently, acarbose, an anti–diabetic drug, is used to treat diabetes by inhibiting α–amylase and α–glucosidase activities. Acarbose is composed of acarviosin moiety, which is an unsaturated cyclitol unit, and 4,6–dideoxy 4–amino–D–glucose at the terminal reducing end. The drug possesses the IC_{50} of 80 and 36 ug/mL for α–amylase and α–glucosidase inhibitions, respectively [68, 69]. However, acarbose has side effects, including bloating, diarrhea and flatulence [70]. Thus, natural plant extracts that can inhibit α–amylase or α–glucosidase without or less side effects are of interest.

Previous study has been reported that phenolic compounds regarding their quantities and types are in directed correlation with inhibition of key enzymes that control diabetes [71]. Latin–America fruits including açaí fruits (*Euterpe oleracea* L.), Maqui (edible berry) (*Aristotelia chilensis* L.), gooseberry (*Physalis peruviana* L.) and papaya (*Carica papaya* L.) as well as three native Australian herbs including anise myrtle (*Syzygium anisatum*), lemon myrtle (*Backhousia citriodora*) and tasmannia pepper leaf (*Tasmannia lanceolata* R.) are found as the dominant sources of anthocyanins, ellagic acid, ellagic acid–derivatives and flavonoids [53]. These compounds could effectively act against α–glucosidase, suggesting that enzyme inhibitory activities are depended on the levels and type of total phenolics contents [53, 66]. Other study has indicated that anthocyanin could inhibit α–glucosidase, while soluble tannin could effectively inhibit α–amylase [72].

Legumes have been reported as excellent sources of diverse phytochemicals with potential efficacy for prevention or treatment of various diseases. Previous research has been reported that legume consumption is related to incidence of the disease. It was found that legumes with high phenolic contents could reduce risk of some diseases such as cancer, aging, diabetes and cardiovascular disease [30]. For example, acetone extracted legumes in Indian such as *T. indica* and *A. leucophloea* have been reported to contain high phenolic contents, which can inhibit α–amylase with approx. 30–35% inhibition (Table 3.9) [32]. Under the same concentration of extracts, methanolic extracted Jack bean and Karanja seed showed high inhibitory activities against α–amylase (approx. 77–78% inhibition) and α–glucosidase (75–97% inhibition) (Table 3.9) [73, 74]. These data might suggest that types of extraction solvent and legume species could greatly affect enzyme inhibitory activity. It was also found that under the same species (Vigna species), Adzuki bean (black) exhibited the most effective IC_{50} against α–glucosidase (26 mg/mL), suggesting that seed coated color might play a significant role towards enzyme inhibitory activity (Table 3.9) [56].

From these previously data, it was highly possible that flavonoids, the largest plant polyphenolics in food and legumes, are responsible for effectiveness of enzyme inhibitory activity (Table 3.9) [75-77]. It was found that anthocyanin, a main component in seed coat of red kidney bean and black bean, could reduce risk of type 2 diabetes *in vivo* [78, 79]. Several experiments have indicated that anthrocyanin could control diabetes by reducing blood glucose, preventing free radical production, increasing insulin secretion and improving insulin resistance [80]. Previous research also reported that anthocyanin could inhibit α–glucosidase with the IC_{50} of 6.2 µg/mL (0.3 mM), while cyanidine showed α–amylase inhibitory activity with the IC_{50} of 170 µg/mL (0.38 mM) [77]. These IC_{50} values are in the same range of acarbose, suggesting that natural bioactive compounds from foods could effectively act against α–amylase in the same level as the commercial available anti–diabetic drug. The data also suggested that the enzyme inhibitions were in directed correlation with TPCs and antioxidant activities. Since α–amylase and α–glucosidase can be inactivated by several phytochemicals such as flavonoids, phenolics and tannin, the compounds that are rich in legumes, it is highly possible that legumes can provide negative effect toward α–amylase and α–glucosidase activity. Nevertheless, α–amylase and α–glucosidase from legumes in Thailand has not yet been reported.

Table 3.9 Summaries of α–amylase and α–glucosidase inhibitory activities from different legume extracts, bioactive compounds and synthesized anti–diabetic drug, acarbose.

Legumes	***Species***	***Solvent***	***Inhibition (%)***		***IC$_{50}$ (mg/mL)***		***Ref***
			α–amylase	*α–glucosidase*	*α–amylase*	*α–glucosidase*	
Soybean							
Free phenolic		acetone	–	–	0.54	0. 37	[81]
Bound phenolic		acetone	–	–	0.32	0.46	[81]
*Jack bean	*C. ensiformis* (L.)	methanol	77.56	75.45	–	–	[73]
*Abrus precatorius		methanol	11.36	39.41	–	–	[82]
*Acacia leucopholea		methanol	14.25	46.13	–	–	[82]
*Bauhinia variegate		methanol	10.56	44.79	–	–	[82]
*Canavalia gladiate		methanol	17.11	41.46	–	–	[82]
*Cassia floribunda		methanol	18.45	38.71	–	–	[82]
*Entada scandens		methanol	16.84	49.93	–	–	*[82]*
*Indigofera linifolia		methanol	13.72	35.82	–	–	[82]
*Mucuna pruriens		methanol	16.25	37.17	–	–	[82]
*Sesbania bispinosa		methanol	18.09	36.39	–	–	[82]
*Tamarindus indica		methanol	14.68	47.28	–	–	[82]
$^{\$}$Black gram	*Vigna mungo* L.	ethanol	–	80	–	–	[83]
Azuki bean	Vigna Angularis	ethanol	–	–	–	53.74	[84]
Mung bean	Vigna Species	methanol	–	–	–	41.21	[56]
Moth bean	Vigna Species	methanol	–	–	–	50.42	[56]
Adzuki bean (black)	Vigna Species	methanol	–	–	–	26.28	[56]
Adzuki bean (red)	Vigna Species	methanol	–	–	–	319.22	[56]
*Karanja seed	*Pongamia pinnata* L.	methanol	77.92	86.5	–	–	[74]

Legumes	*Species*	*Solvent*	***Inhibition (%)***		***IC_{50} (mg/mL)***		***Ref***
			α–amylase	*α–glucosidase*	*α–amylase*	*α–glucosidase*	
Indian legumes							
**A. leucophloea*		acetone	31.11	–	–	–	[32]
**B. variegate*		acetone	34.03	–	–	–	[32]
**C. gladiata*		acetone	29.63	–	–	–	[32]
**E. scandens*		acetone	28.89	–	–	–	[32]
**M. pruriens*		acetone	29.63	–	–	–	[32]
**S. bispinosa*		acetone	28.37	–	–	–	[32]
**T. indica*		acetone	34.01	–	–	–	[32]
Bioactive compounds					–		
Catechin		methanol	–	–	–	1.003	[75]
Quercetin		methanol	–	–	–	0.015	[75]
Ellagic acid		methanol	–	–	–	0.002	[75]
Chlorogenic acid		methanol	–	–	–	0.300	[76]
Cyanidin 3–O–glucoside		methanol	–	–	–	0.205	[76]
Luteolin		DI	–	–	0.103	–	[77]
Myricetin		DI	–	–	0.121	–	[77]
Quercitin		DI	–	–	0.169	–	[77]
(–)–3–O–galloylepicatechin		DI	–	–	0.327	–	[77]
(–) – 3–O –galloylcatechin		DI	–	–	0.292	–	[77]
Cyanidine		DI	–	–	0.170	–	[77]
Anthocyanins		DI	–	–	0.006	–	[77]
Medication							
Acarbose	–	–	–	85.96	0.080	0.036	[57, 68, 69]

*****Concentration of extracts = 1g/10 mL**,** [$] Concentration of extracts = 1g/30 mL

3.2.4 Anti–hypertentive properties

Hypertension is caused by high systolic blood pressure (≥140 mmHg) and/or diastolic blood pressure (≥90 mmHg). Hypertension is major of public health issue with prevalence of adult's population in year 2000 being occurred in developing countries (639 million) more than developed countries (333 million) [85]. Hypertension was accounted for 26.4% of the world population with 26.6% being male and 26.1% being female [86]. The disease will be increased to 60% (1.56 billion of the world population) in year 2025 [85]. Main causes of the disease are devised into 2 groups: primary hypertension (essential hypertension) and secondary hypertension. The causes of primary hypertension are still not clear; however, it is believed to be related to high salt intake, sedentary lifestyle, obesity and genetic factor (related to high secretion of rennin–angiotensin–aldosterone system (RAAS) and the sympathetic nervous system) [87]. Secondary hypertension involves renal artery stenosis, chronic kidney disease, high aldosterone secretion, sleep apnea and pheochoromocytoma [88]. In addition, hypertension is a major risk factor to cardiovascular disease, coronary heart disease, cerebrovascular stroke and renal disease [85].

Hypertension is related to RAAS, a group of hormone homeostasis that controls blood pressure and water balance. When blood volume is low, renin, a kidney hormone, is secreted into blood circulation and conversed liver angiotensinogen, a precursor hormone, into angiotensin I (AngI). This decapeptide is then hydrolyzed into the octapeptide, angiotensin II (AngII), by angiotensin–converting enzyme (ACE) found in lungs. AngII directly promotes high blood pressure by increasing the secretion of aldosterone from adrenal cortex. Aldosterone causes blood vessels to retract and increase kidney tubules to reabsorb sodium and water into blood circulation.

ACE (EC 3.4.15.1) is a metallo–glycoprotein with catalytic zinc atom or so called dipeptidyl carboxypeptidase [89]. ACE is responsible for hydrolysis of AngI into AngII. Thus, ACE inhibition would reduce blood pressure and maintain electrolyte balance [90]. Even though the primary treatments of hypertension are exercise, diet control and lifestyle changes, in some case, medical treatment will be used to control blood pressure. These medicinal treatments include diuretics, calcium channel blockers (CCBs), angiotensin receptor blockers (ARBs), and a group of drugs, which are commonly used to treat hypertension such as angiotensin–converting enzyme inhibitors (ACE inhibitors). These ACE inhibitors are captopril, enalapril, lisinopril, ramipril and perindopril [87]. However, the side effects of these drugs are cough, dizziness and agioneuretic edema [91].

Previous studies had showed that natural product with anti–hypertension property were coffee (rich in chlorogenic acid), garlic (rich in polyphenols, flavonoids, flavanols, tannins, ascorbic acid and anthocyanin), gingko leaves (consist of kaempferol, quercetin, and isorhamnetin), hawthorn (main compounds including flavonoids, proanthocyanidins, triterpenes, chlorogenic acid), onion, tea and ginger [92]. Moreover, natural compounds found mainly in fruits and vegetable could, as well, inhibit ACE, for examples, vitamin A, vitamin C, vitamin E and coenzyme Q10 (delivered form animal sources) [92]. Besides, proanthocyanidin from grape seed and skin (*Vitis vinífera* L. cv. País) could act against ACE [93]. In Thai edible plants, *Apium graveolens* (kuen chai) extract could significantly inhibit ACE with the IC_{50} of 1.7 mg/mL [94]. It was found that the bioactive compound responsible for this inhibition was junipediol A 8–*O*–β–D–glucoside (1–β–D–glucosyloxy–2–(3–methoxy–4–hydroxyphenyl)–propane–1,3–diol), which inhibited ACE with the IC_{50} of 76 µg/mL [94]. Some phenolics also reported to be associated with ACE inhibition, for examples, chlorogenic acid, isoquercitrin, vitexin, isovitexin, isovitexin and orientin (Table 3.10) [95]. Flavonoids such as anthocyanins, flavones, flavonols, flavan–3–ols and flavonoid metabolites could, as well, retard ACE reactions (Table 3.11) [96]. However, these natural compounds (IC_{50} of 8–400 µM) are not comparable with anti–ACE drugs with very low IC_{50} values (IC_{50} of 0.02–1.8 µM) (Table 3.11) [96].

The only available report of legumes and anti–ACE activity was focused on soybean [81]. Free phenolics and bound phenolics isolated from soybean exhibited the IC_{50} values of 159.74 ± 6.0 and 143.27 ± 6.1 µg/mL, respectively, against ACE [81]. This report had showed great possibility of legumes that might be employed as sources of anti–ACE agents. However, no scientific evident of other legumes with their anti–ACE properties is available at present.

Table 3.10 Angiotensin–converting enzyme inhibitory activities of some phenolics (0.33 mg/mL) [95].

Compounds	*Inhibition (%)*
Chlorogenic acid	4 ± 1
Isoquercitrin	32 ± 2
Vitexin	21 ± 1
Isovitexin	46 ± 1
Orientin	20 ± 2
Isoorientin	48 ± 1
(+)–Catechin	16 ± 3
(–)–Epicatechin	34 ± 1
Procyanidin B_2	25 ± 5
Procyanidin C_1	45 ± 2

Table 3.11 The IC_{50} values of angiotensin–converting enzyme inhibitory activities from different flavonoids groups in comparison to those of ACE drugs [96].

Group	***Compounds***	***IC_{50} (μM)***
Anthocyanins	Delphinidin–3–*O*–sambubioside	142
	Cyanidin–3–*O*–sambubioside	118
	Cyanidin–3–*O*–β–glucoside	139
Flavones	Apigenin	280
	Luteonin	290
	Luteolin–7–*O*–glucopyranoside	280
Flavonols	Quercetin glucuronide	200
	Quercetin–3–*O*–(6´´–galoyl)–galactoside	160
	Quercetin–3–*O*–α—(6´´´–caffeoylglucosyl–β–1,2–rhamnoside)	159
	Quercetin–3–*O*–α—(6´´´–*p*–coumaroylglucosyl–β–1,2– rhamnoside)	352
	Isorhamnetin–3–β–glucopyranoside	409
	Quercetin–3–β–glucopyranoside	709
	Quercetin–3–α–arabinopyranoside	310
	Kaempferol–3–α–arabinopyranoside	393
Flavan–3–ols	Epicatechin – dimer	97
	Epicatechin – tetramer	8
	Epicatechin – hexamer	8
Medication	Captopril	0.02
	Lisinopril	1.8

3.2.5 Anti–Alzheimer property

Prevalent of Alzheimer's disease (AD) is approximately 0.6% in individuals with ages between 65–69 years, 1.0% of ages between 70–74 years, 2.0% of ages between 75–79 years, 3.3% of ages between 80–84 years, and 8.4% of ages 85 years and older [97]. AD is mainly found in older female adult than in male. AD is the neurodegenerative disorder, characterized by progressive loss of memory and other cognitive functions. Risk factors of AD including age, family history, genetics as well as history of head trauma, obesity, diabetes, midlife hypertention and hypercholesterolemia have been investigated regarding their correlations [98]. However, causes of AD are still not clear. Nevertheless, four recent pathways consisting of termination of physiological role of cholinergic synapse, β–amyloid formation, abnormality of tau protein and oxidative stress induction have been hypothesized.

Currently, the medicinal treatments of AD have been focused on cholinergic hypothesis and β–amyloid formation. The former is emphasized on cholinesterase enzymes including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) [99]. Both enzymes can degrade neurotransmitters, causing a decrease in cognitive functions. The later is focused on β–secretase, the enzyme that degrades amyloid precursor protein (APP), leading to the formation of toxic β–amyloid plaques. A medication for AD includes many medicines such as donepezil, galantamine, physostigmine (or eserine) and rivastigmine. These drugs are in the categories of anti–cholinesterase inhibitors, which can increase acetylcholine in brain. Memantine is a drug used for treatment of moderate to severe AD by controlling *N*–methyl–daspartate receptor (NMDA), a receptor antagonist. All drugs are approved by FDA [97]; however, these drugs have side effects such as nausea, tired, diarrhea, headache, dizziness, confusion, vomiting, insomnia, heart attack and stroke [97].

Epidemiological studies suggested that dietary of antioxidants involves with the negative effect on incidence of neurodegenerative disease. Phenolics from fruits and vegetables are related to anti–AD property (Table 3.12). For example, (–)–epigallocatechin–3–gallate (EGCG), a major phenolic compound in green tea, can reduce the production of amyloid–β peptides and have potential treatment in AD induced mice [100]. Some phenolic acids (chlorogenic acid, caffeic acid, gallic acid and quinic acid) and flavonoid–derivertives (apigenin, quercetin, genistein, biochanin A, luteolin–7–*O*–rutinoside, kaempferol–3–*O*–galactoside, diosmin, naringin, silibinin and silymarin) can, as well, inhibit AChE and BChE (Table 3.13) [101]. Previous study had suggested that natural compounds in category of alkaloids, which are found as main compounds in China medical herbs for anti–AD, can act as anti–

cholinesterase agents [102]. Besides, flavonoids and anthocyanins, which are predominant phenolic compounds in blueberry extract, can act against amyloid–β peptide–induced neurotoxicity and anti–inflammatory property [103]. It was also found that consumption of vitamin C and vitamin E rich foods was correlated with low risk of developing AD [104].

Legumes contain many bioactive compounds such as phenolic acids, flavonoids, anthocyanins, saponins and tannins that could possibly prevent AD occurrence (Table 3.12). It was found that high consumption of tempe, a fermented soybean product, was associated with better memory in participants over 68 years old [105]. Previous study also showed that isoflavones in soybean including daidzein, daidzin, genistein, genistin and equol can inhibit amyloid–β peptide fibril formation [106]. However, the detailed research of legumes on AD has been limited. Thus, the study of biochemical properties in different legumes against AD is of interest.

Table 3.12 Summary of phenolic compounds that could act against Alzheimer's disease from different plant sources.

Phenolic compounds	***Plants***	***Ref***
(–)–Epigallocatechin–3–gallate (EGCG)	Green tea	[107]
4–O–methyl honokiol	*Magnolia officinalis*	[107]
Resveratrol	grape	[107]
Ginkgolide A	*Ginkgo biloba*	[107]
Isoflavone	soybean	[106]

Table 3.13 Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of phenolic compounds (1 mg/mL) [101].

Compounds	***Inhibition (%)***	
	AChE	***BChE***
Chlorogenic acid	–	30.8 ± 0.81
Caffeic acid	–	–
Gallic acid	15.7 ± 1.02	48.8 ± 0.88
Quercetin	76.2 ± 0.99	46.8 ± 1.35
Quinic acid	–	–
Apigenin	–	–
Genistein	–	65.7 ± 1.24
Biochanin A	–	–
Luteolin–7–O–rutinoside	24.7 ± 0.34	54.9 ± 0.98
Kaempferol–3–O–galactoside	–	–
Naringin	–	13.7 ± 0.56
Diosmin	–	–
Silibinin	–	51.4 ± 1.05
Sillymarin	–	43.2 ± 0.78
Galanthamine	99.8 ± 0.31	80.3± 1.14