Chapter

Nutraceutical Properties of Legume Seeds: Phytochemical Compounds

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Abstract

Legume seeds have an important role as nutraceuticals in human health (providing protein, carbohydrates, fiber, amino acids, and micronutrients) and act as sustainable food sources in livestock farming and aquaculture. Legume seeds contain a wide range of bioactive compounds that have significant health benefits, mainly classified under phenolic compounds, phytosterols, oligosaccharides, carbohydrates, and saponins. Some of these compounds play an important role in plant defense mechanisms against predators and environmental conditions. Heat-labile antinutritional factors (protease inhibitors and lectins) and heat-stable antinutritional factors (tannins and phytic acid) can be reduced by thermal treatment or postharvest to eliminate any potential negative effects from consumption. Substantial studies have demonstrated that these bioactive compounds possess multiple biological activities, including antioxidant properties, antibacterial, anticancer, anti-inflammatory, antidiabetic, cardiovascular protective. They also have various values for aquaculture, such as fishmeal alternative. In this review, the main bioactive compounds and important biological functions of legume seeds are summarized, and the mechanism of action is discussed.

Keywords: phytochemicals, bioactivities, antioxidant, antibacterial, anticancer, legume seeds

1. Introduction

Legumes, including pulses (dried seed legumes), belong to the Leguminosae family (also called Fabaceae), as shown in the Food and Agricultural Organization (FAO) of the United Nations. Leguminosae is an extensive family of plants with over 18,000 species of various types, and only a limited number are used as human resources or as animal feed. Seeds such as dry beans, broad beans, dry peas, chickpeas, cowpeas, lentils, and mung beans are listed by FAO as being consumed for their high nutrition source of proteins, minerals, vitamins, and bioactive compounds. In general, legumes are known for their high levels of bioactive compounds, such as phenolic compounds, phytosterols, bioactive carbohydrates, and saponins, which aid in the reduction in the risk of oxidant properties, bacteria, cancer, inflammation, and diabetes. Legumes have recently gained popularity as excellent sources of high nutrition and can be vital sources of ingredients for use in functional foods and other applications. Legumes are also a rich source of amino acids such as lysine and tryptophan but are low in sulfur-containing amino acids and may be a cost-effective ally in the fight against malnutrition. They are now regarded as a future superfood, capable of achieving zero hunger at a time when one in every five children under the age of five is chronically malnourished [1]. As a result, people consume legume seeds as a major source of protein worldwide. Legumes are more affordable, especially to low-income families, where consumption of animal protein may be restricted due to economic, social, cultural, or religious factors. In addition, the consumption of legumes has also been demonstrated to be connected with outstanding beneficial health, including hypocholesterolemic, antiatherogenic, anticarcinogenic, cardiovascular protection, and hypoglycemic properties [2].

The nutritional demand for legumes is increasing globally as consumers have become more aware of their nutritional and health benefits. Furthermore, in recent years, more people have substituted vegetable protein for animal protein, increasing demand for legumes, which are the primary source of plant proteins. Therefore, developing good extraction and isolation techniques to obtain a high content of bioactive compounds is critical for legumes to become a competitive source of phytonutrients.

The aim of this review is to concentrate on the phytochemicals and bioactivities of legume seeds on mechanisms of action. Furthermore, the quantities and compositions of these phytochemicals in a variety of legumes are shown. The information demonstrated in this study is helpful for the ingredient selection of legumes for the application of functional foods [1].

2. Bioactive compounds

In a conventional method for extracting bioactive compounds in legume seeds, the first step is to soak the dry seeds in water, followed by heat treatment such as boiling. It is effective in that it increases the nutritional value of legumes to some extent and diminishes the levels of phytates and tannins, leading to a higher starch digestibility [3]. However, phenolic acid and saponins can be destroyed in boiling water.

The isolation and purification of biomolecules, especially phenolic compounds, different types of solvents, such as methanol, hexane, and ethanol in a liquid–solid extraction method, are used based on the polarity of the solute of interest. In a study carried out by Xu BJ et al. [3, 4], 50% acetone can be used to extract bioactive constituents in chickpeas and soybeans, whereas black beans are treated with 70% acetone.

In recent years, new extraction techniques have been developed to provide a significant reduction in extraction time, solvent needed, and energy consumption, as well as to improve compounds recovery (**Table 1**). Microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) have attracted the attention of researchers for isolating bioactive compounds. MAE is an efficient method used in extracting bioactivities from legume seeds [22]. On the other side, UAE has also been used for the extraction by using ultrasound to disrupt legume seed cell walls. This method is regarded to be one of the simplest extraction techniques because it makes use of common laboratory equipment such as an ultrasonic bath [15].

2.1 Phenolic compounds

Phenolic compounds can be easily found in legume seeds. This is a vast group of bioactive compounds, chemically containing at least one benzene ring with one or more hydroxyl substituents, and ranges in complexity from simple phenolic

No.	Compound	Extracts and techniques	Content	Pulse	Unit	References
1	Total phenolic content	70% ethanol	21.9	Lentil	mg GAE/g	[5]
			18.8	Red beans		
			18.7	Soybeans		
			17.0	Mung beans		
2	Gallic acid	70% acetone and HPLC	479.26	Velvet beans	μg/g	[6–8]
			28.64	Black beans		
			24.55	Broad beans		
			12.26	Red kidney beans		
3	*p*-hydroxybenzoic acid	HPLC-DAD	19.2 - 60.5	Chickpea varieties	mg/kg	[9]
	Syringic acid		45.9			
	Gentisic acid		8.1–26.0			
	Protocatechuic acid		12.1–163.5	Pea varieties		
	p-hydroxybenzoic acid		45.5–101.7			
4	Kaempferol	—	6% of TPC	Raw and germination of dark common beans	—	[10]
	Quercetin		26% of TPC			
5	v	Methanol extraction and pressure cooking	Between 50 and 300	Kidney, pinto, black and borlotti beans	μg/g	[11]
			372.2–287.87	Black beans		
	Genistein		1166.15	Soybeans		
	Daidzein		1064.56			
6	Proanthocyanidins	T-25 ULTRA-TURRAX homogenizer	4.09–5.73	Black beans	mg CAE/g	[4, 9]
			13.8	Adzuki bean coats		
			3.73–10.20	Lentil cultivars		
7	Catechin and procyanidins	80% HCl-methanol and HPLC-MS	74.48	Lentil coats	μg/g	[12]
8	Soyasaponin I	Solid phase extraction and HPLC	630–900	Different raw legume samples	mg/kg	[13]
	Dehydrosoyasaponin I		650–1300			
9	Saponins	Ultrasound-assisted extraction (ethanol solvent)	4.55	Lupins	g/100 g	[14–17]
			10.63	Lentils		
			2.97	Chickpeas		
			4.08	Soybeans		
			12.90	Fenugreeks		
10	Total saponin content	70% acetone (0.5% acetic acid)	24.29 (bean hull) 2.20 (whole bean)	Mung beans	mg SbaE/g	[18]
			73.60 (bean hull) 10.82 (whole bean)	Adzuki beans		

No.	Compound	Extracts and techniques	Content	Pulse	Unit	References
11	β-sitosterol	Hexane/diethyl-ether (1:1), saponification and HPLC	15.4–24.2 (cooked)	Lentils	mg/100 g	[19, 20]
			123.4			
	Campesterol		2.18–2.58 (cooked)			
			15.0			
	Stigmasterol		2.60–2.63 (cooked)			
			20.0			
12	Resistant starch	Incubated with enzymes	0.6	Cowpeas	g/100 g	[7]
			3.4	Lentils		
			2.5	Peas		
			2	Kidney beans		
			4.2	White beans		
13	Raffinose	HPLC-HRMS analysis	3.3 (kernel)	Adzuki beans	g/kg	[21]
			2.9 (coat)			
			13.2 (kernel)	Peas		
			11.1 (coat)			
			4.8 (kernel)	Broad beans		
			4.3 (coat)			
			9.2 (kernel)	Green soybeans		
			10.1 (coat)			
			4.0 (kernel)	Mung beans		
			10.3 (coat)			
14	Lectins	—	2.4–5	Total protein in kidney bean seeds	%	[16]
			0.8	Total protein in soybean and lime bean protein		
			0.6	Total protein in pea seeds		

Table 1.
Content of bioactive compounds in legume seeds.

molecules to highly polymerized compounds [23]. The primary phenolic compounds found in legume seeds are phenolic acids, flavonoids, and condensed tannins (**Figure 1**). The distribution of these compounds differs in the cotyledon (mainly containing non-flavonoids, such phenolic acid and hydroxycinnamic) and primarily concentrates on the seed coats (mainly flavonoids) [3, 24, 25]. The phenolic compound may exist in a free, solubilized conjugated form or in an insoluble-bound form. Some free and conjugated phenolic compounds are thought to be absorbed in the small and large intestines; otherwise, the bound forms with associated non-digestive sugars are made bioavailable by the digestion of enzymes or microorganisms present in the intestine lumen [26].

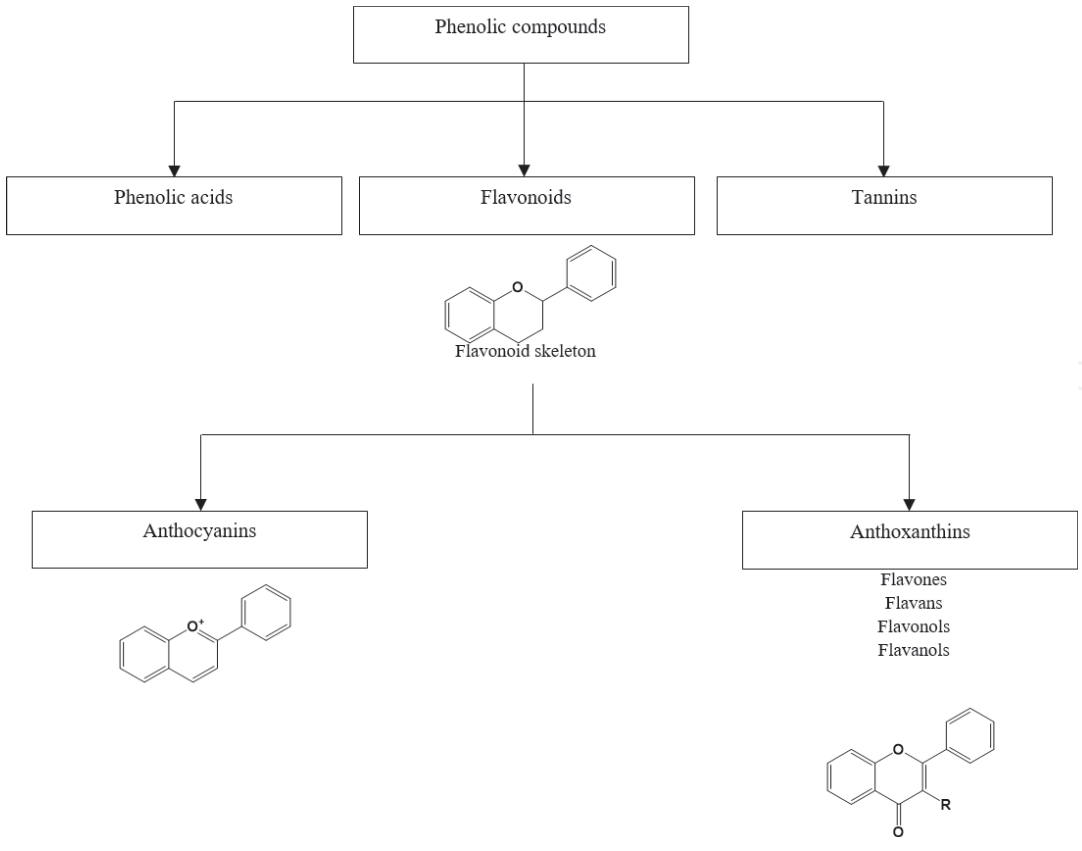

Figure 1.
Phenolic compounds in legume seeds.

Phenolic compounds found in important legumes, including lentil, pea, bean, and chickpea, are flavonoids, such as glycosides of flavonols, flavones, and isoflavonoids (primarily daidzein and genistein), and some hydroxybenzoic and hydroxycinnamic compounds [25]. Lentils were reported to have the highest amount of total phenolic content (TPC), which had 21.9 mg gallic acid equivalents (GAE)/g, compared with soybean, bean, and peas [5], and a slight decrease in red bean and soybean (18.8 and 18.7 mg GAE/g, respectively) and the lowest in mung bean (17.0 mg GAE/g).

In general, processing of legumes (including thermal processing, soaking, and roasting) usually affects the number of phenolic compounds. The study by Lafarga et al. [26] revealed that boiling methods retained more polyphenol than that of cooking broth. This explained that high temperature and the destruction of tissue structures of the cooking process caused the diffusion of phenolics and their leaching into water. By contrast, the germination process in legumes generally improved the nutritional quality, including phenolic compounds [27].

2.1.1 Phenolic acid

According to chemical structure, phenolic acids in legume seeds can be divided into hydroxybenzoic acids and hydroxycinnamic acids (**Figure 2**). Gallic, *p*-hydroxybenzoic, protocatechuic, vanillic, and syringic acids are the most common hydroxybenzoic acids in common beans and are mainly present in foods as glycosides. In addition, caffeic, ferulic, *p*-coumaric, and sinapic acids are the most frequently occurring hydroxycinnamic acids in legumes. The level of gallic acid in velvet beans was the highest (479.26 μg/g), followed by black, broad, and red kidney beans (28.64, 24.55, and 12.26 μg/g, respectively) [3, 28, 29]. Lopez et al. [20] documented that ferulic acid derivatives contained the highest percentage of TPC in

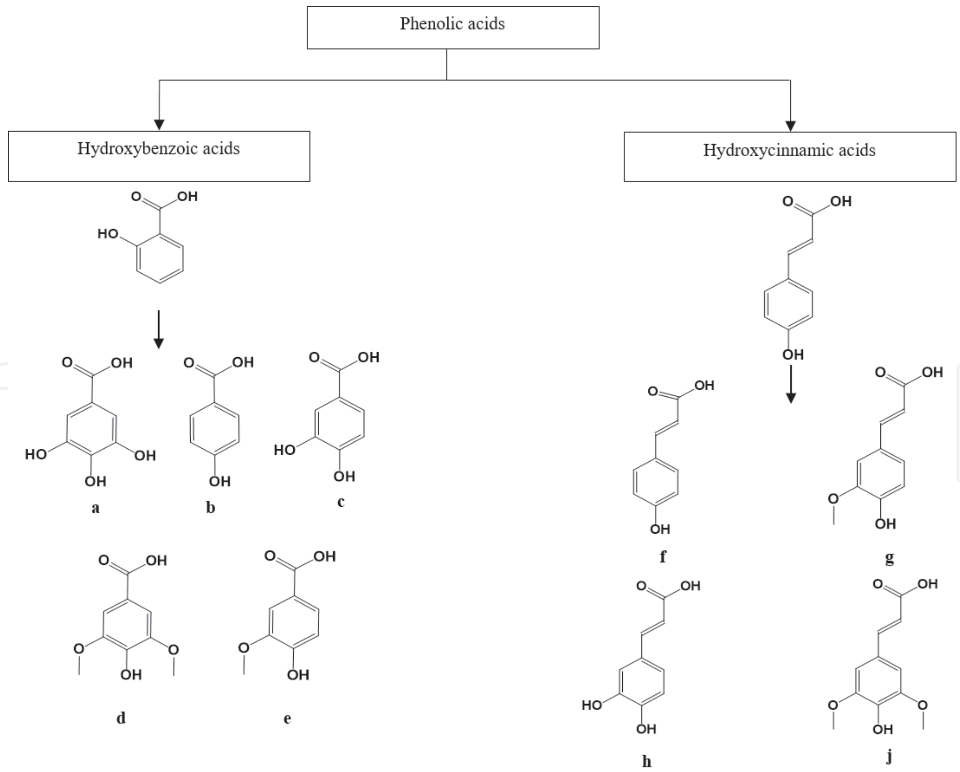

Figure 2.
Chemical structure of major phenolic acid compounds present in legume seeds: Gallic acid (a), p-hydroxybenzoic acid (b), protocatechuic acid (c), syringic acid (d), vanillic acid (e), ferulic acid (f), p-coumaric acid (g), caffeic acid (h), sinapic acid (i).

both raw and cooked dark beans (19 and 24%, respectively). The *p*-hydroxybenzoic acid (19.2 to 60.5 mg/kg), syringic acid (45.9 mg/kg), and gentisic acid (8.1 to 26.0 mg/kg) were presented in significant amounts in seeds of six chickpea varieties, while the six field pea seeds were found to contain protocatechuic acid of between 12.1 and 163.5 mg/kg, and *p*-hydroxybenzoic acid, which ranged from 45.5 to 101.7 mg/kg [7]. Yihan Liu et al. [30] reported that the differences among four types of cooking methods (traditional or boiling, pressure, microwave, and slow) and heating solution can affect the percentage of phenolic acids. Gallic acid content increased after processing in soybean (79.81 from 54.96 μg/g dry weight), while on the contrary, it decreased in black beans (36.02 from 67.88 μg/g dry weight) [31].

2.1.2 Flavonoids

Flavonoids are the main phenolic compound found in legumes, and their presence affects the flavor and color of common beans [7, 20]. They are low-molecular-weight compounds (approximately 300 g/mol), and their general structures are formed with two aromatic rings, joined by a three-carbon bridge, usually in the form of a heterocyclic ring C. Flavonoids are divided into two groups: anthocyanins (colored compounds) and anthoxanthins (colorless compounds) (**Figure 3**). According to the study by Amarowicz and Pegg [32], flavonols, flavan-3-ols (flavanols), flavones, and anthocyanins are the main flavonoids present in leguminous seeds. The presence of flavonols and flavones can impact the color of anthocyanins group [33]. Catechins are called flavan-3-ols, which are primarily identified in legumes as having colored seed coats, such as kidney, navy, and pinto beans. Catechins, along with procyanidins, are common in raw lentil coats and represent 69% of TPC (74.48 μg/g) [12], while other flavonoid glycosides, such as quercetin, myricetin, apigenin, and luteolin, are only found in trace amounts. Duenas et al. [20] also showed that

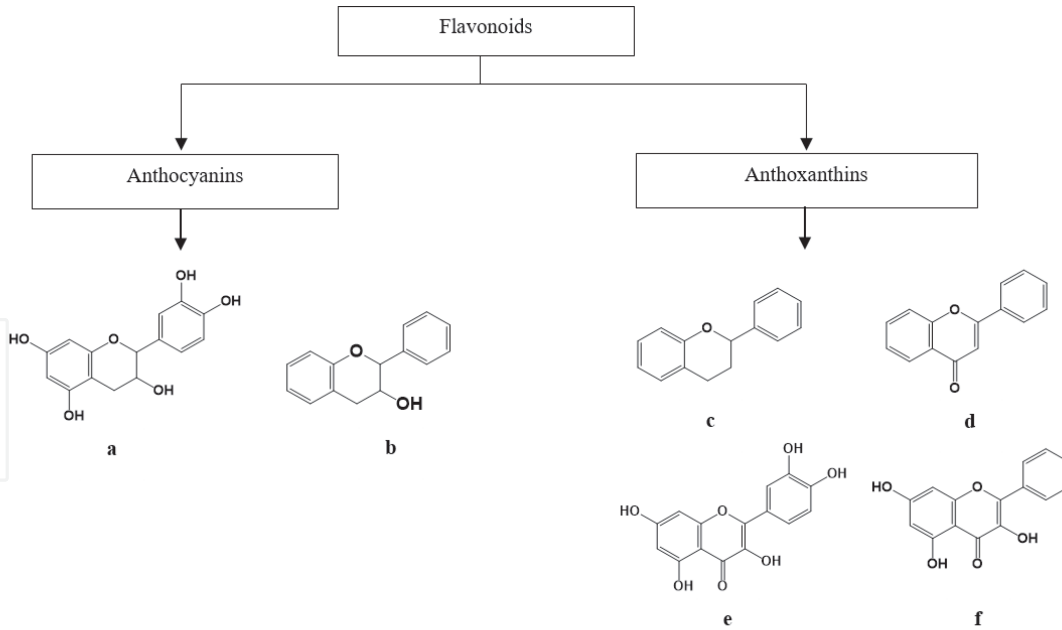

Figure 3.
Chemical structure of major flavonoid compounds: Flavanols (a), catechin (b), flavonols (c), flavones (d), quercetin (e), kaempferol (f).

kaempferol and quercetin in raw and germination of dark common beans contained approximately 6 and 26% of TPC, respectively.

A study by Teixeira-Guedes et al. [31] showed the effect of cooking methods on flavonoid profiles of different varieties of common beans, such as kidney, pinto, black, and borlotti bean. Pressure cooking increased the levels of catechins for all bean varieties, which ranged approximately from 50 to 300 μg/g, except in black beans, where the levels decreased from 372.2 to 287.87 μg/g. The catechin levels in genistein and daidzein content were also increased by this processing and were detected only in soybeans (go up to about 906 and 988 μg/g, respectively).

2.1.3 Condensed tannins

Tannins are polyphenols that are high in molecular weight with large numbers of hydroxyl groups in their structure, which have the ability to bind with carbohydrates and protein, but only to a limited extent. They are classified as hydrolyzable or condensed (non-hydrolyzable tannins), with flavonoids present at various levels of condensation [3]. Condensed tannins, also known as proanthocyanidins (PACs), are chemically oligomeric and polymeric flavonoids, which at high temperature release into catechins and anthocyanidins [7]. Mostly in the same class as flavonoids, PACs mainly distribute in common bean seed coats and play a crucial function in plant defenses that are susceptible to oxidative damage by many environmental factors. Lentils, black beans, and red beans were recorded to contain a high concentration of condensed tannins [34]. In the case of common beans varieties, PACs' content of black beans was in the range of 4.09 to 5.73 mg catechin equivalents (CAE)/g, for adzuki bean coats the content was 13.8 mg CAE/g, and for lentil cultivars, 3.73 to 10.20 mg CAE/g [9, 26].

2.2 Saponins

Saponins are bioactive compounds found in legumes, consisting of a triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties. They

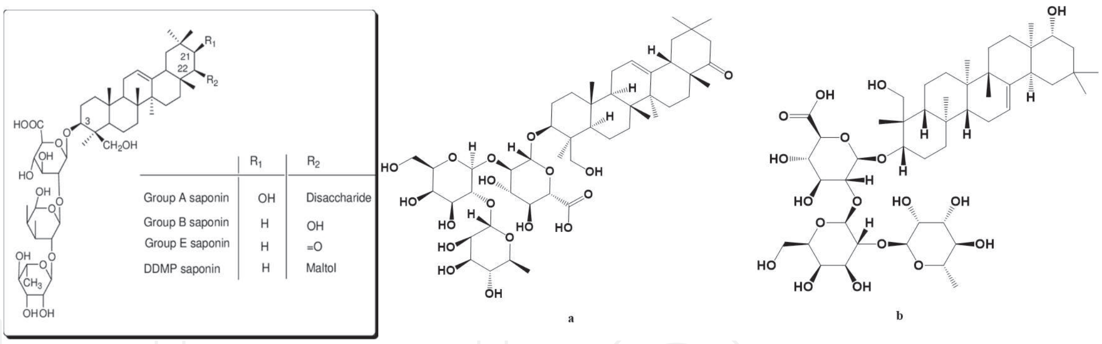

Figure 4.
Chemical structure of major saponins: Dehydrosoyasaponin I (a), soyasaponin I (b) [36].

have the ability to absorb free radicals and activate antioxidant enzymes. The most common saponins include the soyasaponins, which are divided into three groups, A, B, and E saponins, based on the chemical structure of aglycone. Saponins from the B group, which have been studied to be the primary compound in legume seeds [3, 35], contain soyasaponin I (approximately from 630 to 900 mg/kg) and dehydrosoyasaponin I (approximately from 650 to 1300 mg/kg) (**Figure 4**). In contrast to the seed coat or cotyledon part, the hilum portion of the seed has the highest concentration of saponins [37].

Saponins have been investigated in a variety of edible legumes, as well as the effect of solvent during UAE, which has been evaluated from lupins (4.55 g/100 g), lentils (10.63 g/100 g), chickpeas (2.97 g/100 g), soybeans (4.08 g/100 g), fenugreek (12.90 g/100 g), and various beans [14, 15, 17, 38]. The research by Wu et al. also showed that mung beans and adzuki beans contained the highest total saponins content in bean hull (24.29 mg saponins Ba equivalent (SbaE)/g and 73.60 mg SbaE/g, respectively) and whole bean (2.20 mg SbaE/g and 10.82 mg SbaE/g, respectively) [6].

2.3 Phytosterols

Phytosterols, primarily β-sitosterol, campesterol, and stigmasterol, are structurally similar to cholesterol and occur in a variety of plant types (**Figure 5**) [8, 10]. Phytosterols have a double bond at carbon-5 that can be saturated by enzymatic hydrogenation in plants or during food processing to form plant stanols. They are assumed to have a wide variety of biological potentials and are a rich source of grain legumes, vegetable oils, cereal grains, and nuts. β-sitosterol was identified in lentils to be a common component in plants, of which the level was 123.4 mg/100 g, followed by 20.0 mg/100 g of stigmasterol and 15.0 mg/100 g of campesterol [13]. Additionally, β-sitosterol was found as the predominant compound in cooked lentils, ranging from 15.4 to 24.2 mg/100 g [8].

2.4 Carbohydrates

Carbohydrates are an essential component of legume seeds and possess a bioactive property against chronic diseases. Chickpeas have been shown to be a good source of carbohydrates, such as dietary fiber, starch, and oligosaccharides [39, 40]. Pigeon peas have a high content of carbohydrates (57.6%), which is the same in cotyledons. Black gram beans have also been documented to possess soluble mucilaginous polysaccharides along with dietary fiber.

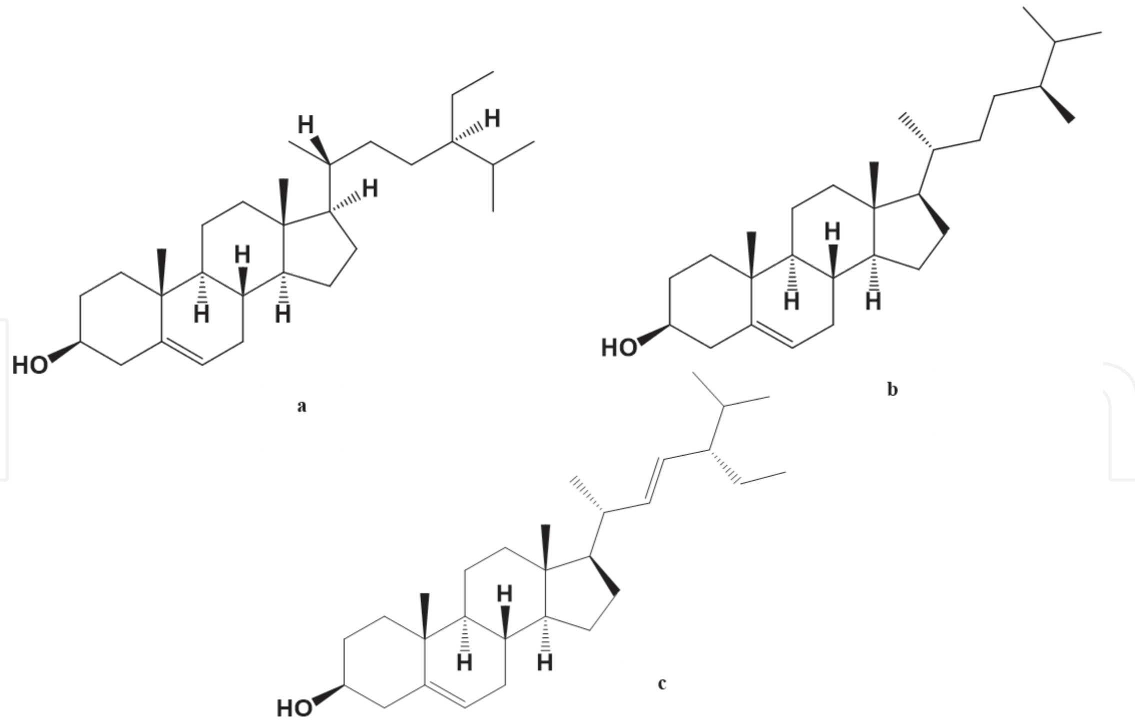

Figure 5.
Chemical structure of major phytosterols: β-sitosterol (a), campesterol (b), stigmasterol (c).

2.4.1 Dietary fiber

Dietary fiber has been demonstrated to be a beneficial food component and is made up of a combination of polymeric non-starch substances (such as cellulose, hemicellulose, and pectin) that are resistant to enzymatic digestion in the human gastrointestinal tract [41]. The dietary fiber contents of legume seeds vary according to the species, variety, and processing method. Dietary fiber, also called cell wall material, is of a lower level in cotyledon than testa [42]. Fiber concentration ranges from 8 to 27.5% and is between 3 and 14% of soluble fiber from almost gain legumes consumption. The gut bacteria metabolize and convert the solute fiber into fatty acids, which aid in the health of colonic cells. Guar beans have been identified to have the richest amounts of fiber, as well as soluble fiber (12.5%), among other legumes [41].

Some research projects have shown that dietary fiber can interact with other bioactive compounds such as phenolic compounds, which play an important role in health advantages. The interaction is accomplished through the formation of hydrogen, hydrophobic and covalent linkages between phenolic compounds, and components of legume cell wall fibers [43].

2.4.2 Resistant starch

Legumes are one of the best sources of resistant starch, and this component is not digested by humans. Resistant starch (RS) concentrations in legume seeds have been found to be higher than in cereals and many tubers (**Figure 6**) [45]. Cowpeas, lentils, peas, kidney beans, and white beans displayed the RS content of 0.6, 3.4, 2.5, 2, and 4.2 g/100 g of total seed material, respectively [3]. Legume seed processing has an effect on the RS content. Alonso et al. [46] showed that the formation of RS increased after refrigeration of legumes, which corresponded to the fact that cooling after gelatinization can develop the formation of RS.

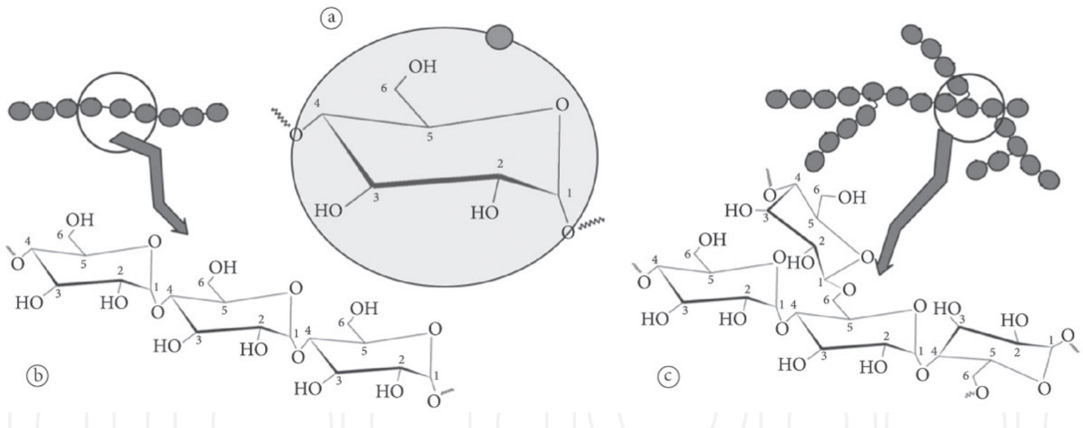

Figure 6.
Chemical structure of resistant starch: Glucose units (a), amylose (b), amylopectin (c) [44].

2.4.3 Oligosaccharides

Oligosaccharides, found in legumes, such as raffinose, stachyose, ciceritol, and verbascose (**Figure 7**), frequently cause flatulence in humans consuming legume seeds. They also induced discomfort and diarrhea. Nonetheless, oligosaccharides have recently been reported to have bioactive activities, particularly in small amounts. Raffinose was found in all parts of the legume plants, but it is built up in the seeds and roots during development. There were detectable amounts of these oligosaccharides in chickpeas, lentils, lupins, beans, peas, and faba beans (from 0.4 to 16.1% dry matter), with significant differences between the pulses studied [38]. In another study, Fan et al. [21] discovered that raffinose, stachyose, and verbascose were more concentrated in the seed kernels than in the seed coats (ratio of content in kernel/coat >1); the same was true for adzuki bean, pea, and broad bean, which showed the distribution ratio of oligosaccharides between 1 and 2

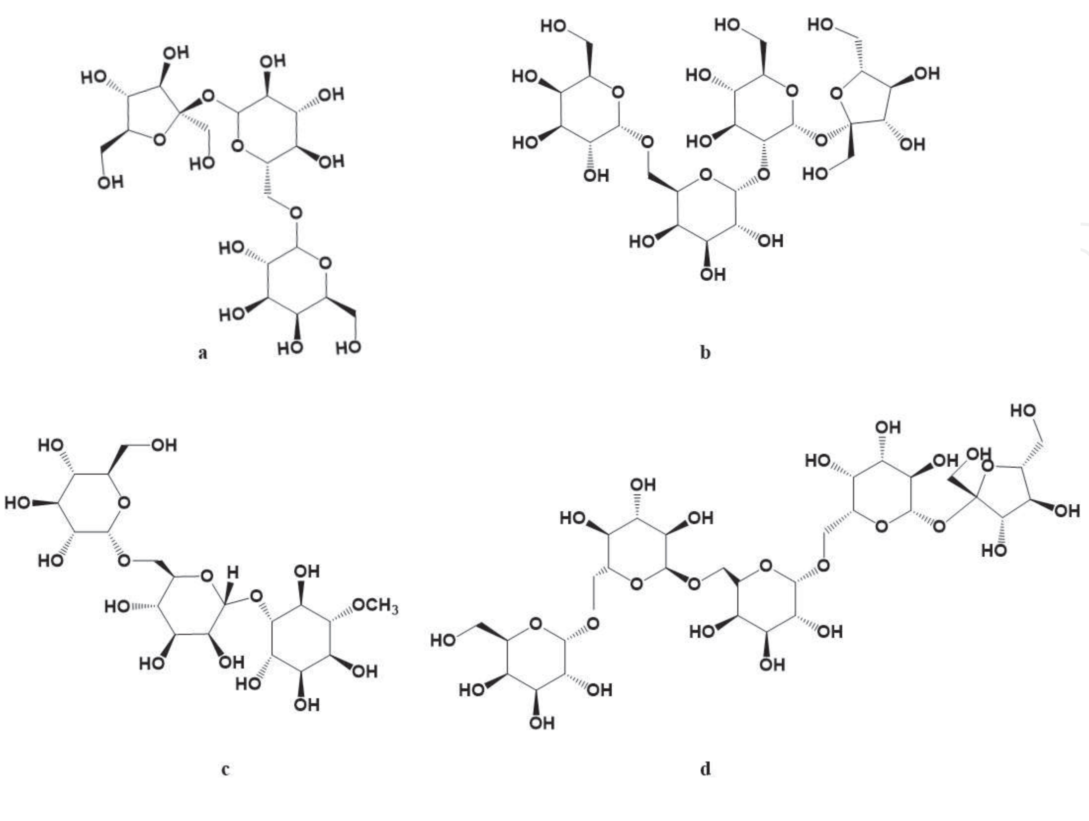

Figure 7.
Chemical structure of oligosaccharides: Raffinose (a), stachyose (b), ciceritol (c), verbascose (d).

in kernel and coat. Green soybean and mung bean, however, had higher levels of stachyose and raffinose in their seed coats than kernels (ratio of content in kernel/coat <1). Legumes generally have a decrease in total oligosaccharide content after soaking, which is most likely due to oligosaccharides leaching into the soaking water [43]. In chickpeas, lentils, yellow peas, green peas, and soybeans, it is observed that the oligosaccharide content is reduced after soaking in water with different factors (ultrasound and high hydrostatic pressure).

2.5 Antinutritional factors

Besides the nutritional compounds, legume seeds also contain some antinutritional factors (ANFs) that have been identified as bioactive constituents, which are lectins, phytic acid, alkaloids, amines, cyanogens, and other factors (**Figure 8**). Some of the ANFs that have unfavorable, undigested, or toxicological properties can be eliminated through plant genotype selection, postharvest, or thermal processing such as dehulling, soaking, germination, extraction, boiling, leaching, and/or fermentation [47].

Phytic acid is known widely as myo-inositol hexaphosphate (IP6), which is majorly stored in plants along with the salts (called phytates) [16, 38]. It is mostly considered an antinutrient due to its strong mineral, protein, and starch binding properties, which reduces bioavailability. Therefore, phytates affect enzyme activity, such as pepsin and trypsin; they also change the solubility, as well as digestibility. It has, however, been recognized for its antioxidant activity due to its ability to inhibit the formation of hydroxyl iron radicals. Another phytochemical of interest in legume seeds are lectins. Lectins are proteins or glycoproteins that are widely present in pulse and have the unique property of binding to carbohydrate-containing molecules. Lectins are hardly protein that does not degrade easily, and they can withstand stomach acid and digestive enzymes. Legumes have a wide range of lectin concentrations. They have been reported to contribute between 2.4 and 5% of total protein (17–23%) in kidney bean seeds, 0.8% of total protein in soybean and lime bean (34% and 21%, respectively), and approximately 0.6% of total protein (24–25%) in pea seeds [16].

Some of alkaloids are neurotoxins or neuromodulators. Quinolizidine alkaloids (QAs) are neurotoxin-secondary metabolites found in some Fabaceae, particularly in the genus Lupinus, including *Lupinus albus, L. mutabilis, L. angustifolius*, and others. QAs protect plants from insect pests; however, QA levels in food must be less than 0.02% when lupin is used as an ingredient. Pyrrolizidine alkaloids have the potential to cause mutations and even cancer in both animals and humans.

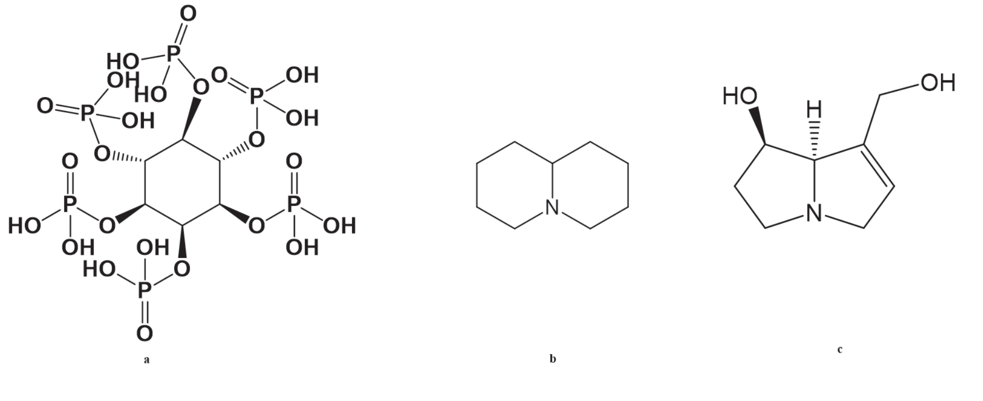

Figure 8.
Chemical structure of some ANFs: Phytic acid (a), quinolizidine alkaloids (b), pyrrolizidine alkaloids (c).

Furthermore, some toxins have long-term consequences by affecting species survival and reproductive fitness. The toxicity of alkaloids varies with concentration and is nontoxic at lower levels. Lupanine is the most toxic and higher in *L. albus* (700 mg/g total alkaloids), while sparteine (300 mg/g total alkaloids in *L. luteus*) and lupinine are the least toxic. After enzymatic hydrolysis, cyanogenic glucosides release HCN after wounding. HCN is a respiratory poison because it inhibits the mitochondrial respiratory chain and is lethal to most animals [48].

The endophytic fungus *Phomopsis leptostromiformis* is frequently found in *L. angustifolius*, an Australian forage plant used to feed sheep. Because of its antimitotic activity, this microorganism produces fomopsins and hepatocarcinogenic toxins that affect sheep [49].

Aside from lectins, protease inhibitors isolated from legume are divided into two main categories: the Kunitz inhibitor, which has a specificity aimed primarily against trypsin, and the Bowman-Birk inhibitor, which has the ability to inhibit chymotrypsin and trypsin at separate binding sites. They are found in common beans, lima beans, cowpeas, and lentils [16]. These hydrolyzed or modified proteins will become bioactive peptides (BPs), be commercialized as a nutraceutical product, and be involved in several body functions. BPs can be liberated from food proteins and exhibit bioactivity in both the small and large bowels [50].

3. Biological activities

3.1 Antioxidant activities

Natural products, such as mushrooms, vegetables, cereal, flowers, and wild fruits, have been extensively studied for their antioxidant properties. Antioxidant bioactive compounds have the ability to slow the oxidation process of important biomolecules found in human tissues and cells. It has been known that overproduction of free radicals plays a significant role in the onset of many chronic diseases such as Alzheimer's disease, various types of cancer, and diabetes. As can be seen from a review of some literature, the production of bioactive compounds is often less than 1% of the dry weight of the legume. Therefore, based on this consideration, even a technique such as chemical synthesis cannot yield large quantities of bioactive compounds.

Antioxidant activities of various legume species have been identified in numerous studies, with a positive association between antioxidant activities and total phenolic content. The chemical composition of phenolic compounds impacts their antioxidant function. The position and degree of hydroxylation on the B ring are the most significant factors in the activity of flavonoids, which are considered primary antioxidants [51]. Natural precursors of flavones and flavonols are chalcones, which have antioxidant potential (**Figure 9**) [25]. Several methods have been developed and utilized to evaluate the antioxidant activities in legumes, including *in vitro* assays for ferric-reducing antioxidant potential assay (FRAP),

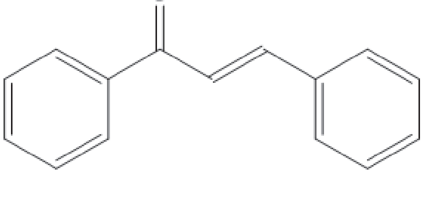

Figure 9.
Chemical structure of chalcone.

Trolox-equivalent antioxidant capacity (TEAC), 1-diphenyl-2-picrylhydrazyl free radical-scavenging assay (DPPH), the tests to measure values of oxygen radical absorbance capacity (ORAC), and total radical-trapping antioxidant parameter (TRAP). Xu and colleagues showed that dark-colored pulses had higher phenolic content and antioxidant activity than pale-colored pulses [19]; the same was true for anthocyanins, which attracted interest for their high antioxidant effects. Their study also reported that lentils had the highest DPPH and ORAC activity than green pea, yellow pea, and chickpea [52]. Similarly, lentils were observed to have the highest total antioxidant potential measured by FRAP and TRAP, among test pulses, but came in the second place by TEAC to broad beans [8], because seed coats contained mainly flavonoids, hydroxycinnamic, and hydroxybenzoic acids [25]. Red, brown, and black beans have been reported to have strong antioxidant activities in comparison with white beans [3]. More importantly, the antioxidant activity of the common bean seed coat was found to be higher than that of the cotyledon in several studies [20]. M. Dueñ et al. [25] demonstrated that the seed coats of lentils (with EC_{50} values were between 0.05 and 0.07 mg of sample) had a higher free radical-scavenging capacity than in cotyledon (with values from 21 to 29 mg of sample). Another research found that red kidney beans had the most antioxidant activity (15-μmol Trolox equivalents (TE)/g seed dry weight), while brown-eyed bean varieties had the least (6.22-μmol TE/g seed dry weight) [3].

In different circumstances, processes such as thermal processing, fermentation, and germination have a major impact on the antioxidant activities of common beans. Because of the increased level of total phenolic content, germination and fermentation may enhance the antioxidant properties of legume seeds even further. In some studies, it was noticeable that the antioxidant activities significantly increased in peas 4 days after germination and in the presence of light [27].

3.2 Antibacterial activities

Antimicrobial resistance has made the spread of bacterial, fungal, and viral infectious diseases a major public health concern. Natural compounds from plants are nowadays excellent candidates for use as alternative sources of antimicrobial substitutes. Legume seeds, which are high in phytochemical varieties, used these chemicals to defend themselves against microbes, pathogens, etc. The aforementioned antinutritional compounds, protease inhibitors, and polyphenols have been shown to be highly biologically antimicrobial agents [40]. Besides antioxidant agents, phenolics are also demonstrated in antibacterial potential against a wide spectrum of microorganisms. Polyphenols deplete critical essential mineral micronutrients (iron and zinc), disrupt the cytoplasmic membrane, inhibit microbial metabolism, and cause permeabilization of the cell membrane, resulting in microbe death [40]. Flavol-3-ols, flavonoids, and tannins (**Figure 10**) have received the most attention, because of their efficiency in resisting a variety of microbial virulence factors such as inhibition of biofilm formation, ligand adhesion reduction, and bacterial toxin neutralization [53]. Moreover, prenylated phenolics derived from legume seedlings indicated potent antibacterial activity against *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus*; this compound has also served multiple goals, including providing health benefits and natural food preservation [54]. Protease inhibitors are also thought to have antimicrobial properties, and their mechanisms of action involve suppressing enzyme activities in response to attack by phytopathogenic microorganism-produced proteases. Methyl esterification of protein by methanol, which is isolated from broad bean, chickpea, and soybean, revealed efficient antibacterial activity against *Escherichia coli, S. aureus, Bacillus subtilis,* and *Pseudomonas aeruginosa* [40]. Methylate subunits interact with cell

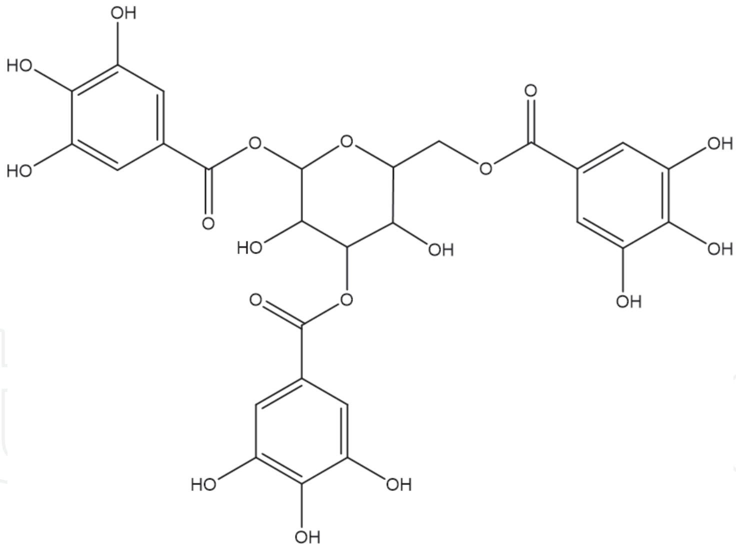

Figure 10.
Chemical structure of tannin.

walls and cell membrane, produce channels and pores and affect the integrity of bacteria cells, and finally achieve the lysis and death of the microorganism [55]. Antimicrobial peptides (AMPs) from natural sources of plants are generally effective against a wide range of microorganisms by interaction or disruption of the bacterial cell wall. Lectins are carbohydrate-binding proteins involved in plant's defense through the growth inhibition of bacteria, or disruption of the microbial cell wall by interacting with components on them such as teichoic and teichuronic acids, peptidoglycans, and lipopolysaccharides [53]. The seed extracts of lentils, fava beans, and peas show antibacterial activity (*P. aeruginosa* and *S. aureus*) [56]. AMPs derived from chickpeas, such as cicerin and arietin, have shown antifungal activity against *Botrytis cinerea, Mycosphaerella arachidicola, and Fusarium oxysporum*; and serine proteinase inhibitors that are found in chickpea seed extracts display antimetabolic activity against *Helicoverpa armigera* [40]. The effect of water extracts of colored azuki beans (such as green, black, and red) has been revealed to be more effective against *S. aureus, Aeromonas hydrophila,* and *Vibrio parahaemolyticus,* due to higher concentrations of polyphenols including proanthocyanidins, compared with the extracts of white azuki beans, which indicated no inhibition toward any of the bacteria examined [55].

In summary, the antibacterial properties of legumes are related to their variety and processing methods. These effects can be attributed primarily to the suppression of bacterial biofilm formation, cell wall disruption, and inhibition of microbial metabolism.

3.3 Anticancer activities

Cancer is recognized as the leading cause of death worldwide, and several research works have indicated that plant-derived secondary metabolites possess properties that fight against types of cancer varieties. According to the American Institute for Cancer Research (AICR), legumes contain a variety of compounds that may protect the human body against cancer, including lignans, saponins, resistant starch, and polyphenolic compounds [8]. Phenolic compounds, bioactive protein, and short-chain fatty acids extracted from legume seeds have several bioactive activities related to anticancer potentials, such as anti-inflammation, anti-proliferation, and pro-apoptotic effect [19]. Many studies reported that phenolic and flavonoids that are derived from plants exhibit potent anti-inflammatory activity by regulating the concentration of various inflammatory cytokines or mediators such as cyclooxygenase-2 (COX-2), tumor necrosis factor (TNF-α), and nuclear factor-kappa (NF-κB), interleukin 1, interleukin 6, interleukin 10, nitric oxide (NO), lipoxygenase (LOX), and iNOS [8]. Flavonoids isolated from black bean hulls can affect cell cycle by inducing cell cycle arrest at the S-phase and preventing progression to G2/M stages, as well as causing activation of apoptosis on OCI-Ly7 lymphoma cells in mouse [57]. In another experiment, phytosterol treatment reduces the development of production of carcinogens, inhibits cell growth, and also promotes apoptosis in cancer cells [10]. Saponins similar to those present in soybeans have been shown to have anticancer activity. Ginsenosides, a form of saponins isolated from ginseng, have been indicated to inhibit tumor cell proliferation and induce tumor cell differentiation and apoptosis in an *in vitro* assay, as well as *in vivo* to inhibit tumor invasion and metastasis [51]. In fact, several polypeptides have recently been researched and found to have powerful anticancer potential. Anticancer peptides derived from legumes can be found in the form of an intact long polypeptide chain, or they can be synthesized from their protein precursors through enzymatic hydrolysis [18]. Lunasin, a leader anticancer peptide derived from soybeans and other legumes, inhibits the chemical carcinogen-induced transformation of murine fibroblast cells to cancerous foci and induces selective apoptosis (**Figure 11**) [50].

Experimental studies have demonstrated that legume seeds and their active components can prevent and treat several types of cancers. These anticancer mechanisms mainly involve the regulation of carcinogen metabolism, inhibition of cell growth and proliferation, and induction of apoptosis.

3.4 Cardiovascular protection

Cardiovascular diseases have been considered to be a leading cause of premature death, of about 17.9 million people die per year. Dyslipidemia, hypertension, and type 2 diabetes are known to be risk factors for cardiovascular diseases, including stroke and coronary heart disease. A series of studies has shown that legume seeds can decrease the levels of blood lipids and blood pressure, contributing to protection from cardiovascular protection [11].

Legumes are rich in phytosterols, which have been demonstrated to inhibit the absorption of cholesterol in the intestine, followed by decreasing levels of low-density lipoprotein-cholesterol (LDL-C) and TAG, and enhanced the concentration of serum high-density lipoprotein-cholesterol (HDL-C) in the blood, a protective factor against coronary heart disease (CHD) [40, 58]. Furthermore, β-sterols, which are abundant in chickpeas, help to lower serum cholesterol, blood pressure, and

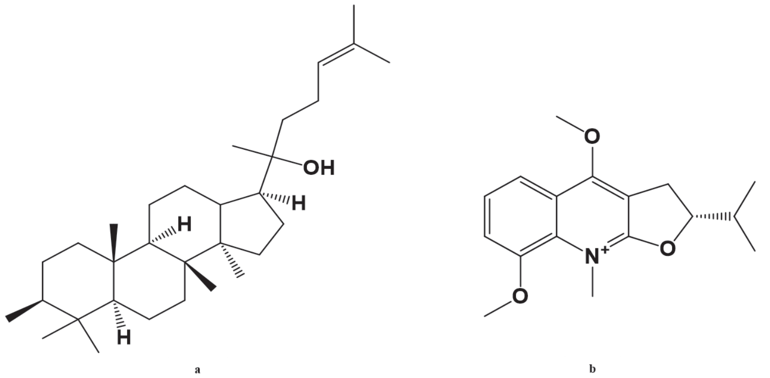

Figure 11.
Chemical structure of some anticancer constituents: Ginsenosides of Panax ginseng root (a), lunasin (b).

the risk of coronary heart disease [40]. Chickpeas also are high in dietary fiber, which help to lower total plasma cholesterol levels and can aid in weight loss and obesity reduction. These compounds are also thought to improve body metabolism and reduce chronic inflammation, serum lipid levels, blood pressure, and insulin resistance, as well as to affect fibrinolysis and coagulation, which may be essential in the plaque formation of existing atherosclerotic plaques [59].

Generally, legume has exhibited cardiovascular protective effects by attenuating hypertension and ameliorating dyslipidemia, such as in the improvement of HDL-C, reduction of LDL-C, TAG, and blood pressure.

3.5 Other bioactivities of legume

Apart from the bioactivities mentioned earlier, legume has other beneficial effects, such as anti-obesity and antidiabetic effects.

Diabetes mellitus is known as a severe metabolic disorder caused by insulin deficiency and/or insulin resistance, resulting in an abnormal increase in blood glucose. Legumes have been shown to regulate the levels of blood glucose and, in turn, provide protection against diabetes by resistant starch (NSPs). Moreover, short-chain fatty acids and the inhibition of α-amylase and α-glucosidase have been reported to induce hypoglycemic and hypocholesterolemic effects by suppressing glucose release and cholesterol production [17, 60]. Adzuki bean extracts reduce the final body weight of mice and adipose tissue accumulation and enhance lipolysis. This treatment also considerably decreases the serum triglyceride levels, total cholesterol, LDL-C, and liver lipids [61].

3.6 Benefits of legume seeds in aquaculture

Legumes contain large amounts of valuable protein. These proteins are not only abundant but also have a well-balanced amino acid profile, and may be used to substitute fish meal, which is an unsustainable resource. The substitution of plant protein sources for fish meals without compromising fish growth and physiology is a strategy for lowering feed costs and reducing aquafeed reliance on fish meals. Some studies reported that commercial hexane-extracted soybean meal with methionine supplement could replace 67% of the fish meal in the diet without negatively impacting milkfish growth and feed conversion ratio [62]. Another experiment showed that the substitution of up to 20% of fish meal protein with soybean meal protein in realistic diets for spotted rose snapper was an essential move for this high-value species [9]. Green mung bean in which the ANFs were inactivated by thermal processing was used as a replacement for fish meal in Asian sea bass and milkfish diets, with no negative effects on the fish's development [62, 63], and these studies were carried out on a 15-week feeding trial and were evaluated to measure growth, survival, FCR, PER, HSI, and liver and gut histology. Overall, legume is a promising alternative protein source for the aquaculture feed industry.

4. Conclusions

In conclusion, the utilization of legumes as food ingredients is of tremendous interest, not only for increasing the functionality of food items but also for developing functional foods with health advantages. The content, composition, and distribution of legumes, as well as their biological functions, are systemically outlined and analyzed in this review. However, the following aspects require additional investigation to fill knowledge gaps.

The extraction solvent has a significant impact on the extraction efficiency of bioactive substances. Mathematical modeling, such as response surface methodology, which is an ideal candidate for predicting the interactions between the target compound and solvent, has been successfully applied in the selection of a specific solvent for higher compound extraction yield in many plants. However, no study has used these modeling tools to optimize the extraction conditions of legume chemicals yet. As a result, future research can use these methods to reduce the time and effort required to identify solvents for common bean polyphenols in different kinds.

Furthermore, the research should seek more bioactive substances and investigate their benefits, as well as biologically active metabolites. Thus, future study can focus on extracting and purifying novel active chemicals from legumes, and clinical trials are also required to confirm the medicinal advantages of legumes. Moreover, legumes have the high potential to be a valuable substitute source of feed for the aquaculture industry.

Conflicts of interest

The authors declare that there is no conflict of interest.

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