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# Anti Nutritive Bioactive Compounds Present In Unconventional Pulses and Legumes

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## ABSTRACT

Unconventional legumes are promising in terms of nutrition, providing food security, agricultural development and in crop rotation in developing countries. The wild legumes are in great demand as food; livestock feed and pharmaceutically valued products. The anti-nutritive factors (ANFs) may be defined as those substances generated in natural feed stuffs by the normal metabolism of species and by different mechanisms e.g inactivation of some nutrients, interference with the digestive process or metabolic utilization of feed which exert effects contrary to optimum nutrition. Being an ANF is not an intrinsic characteristic of a compound but depends upon the digestive process of the ingesting animal. Some of the phytochemiclas such as phytic acid, isoflavenoids, total phenolics, alkaloids, cyanogens and tannins play an important role as showing therapeutic effect in living body.
**Keywords:** unconventional legumes, anti nutritive factor, phytochemicals, therapeutic effect

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## INTRODUCTION

Legumes also known as dried beans and pulses are the edible seeds that grow in pods of annuals, biennials and perennials which are modified in many ways to facilitate their dispersal by animals, wind, and water. Legumes are simple, dry dehiscent fruit bearing pods containing one or more seeds, which split open by the two longitudinal lines into two halves at maturity [1]. The terms legume and pulse are used interchangeably in this study to refer to the seeds of plants of the legume family. Pulse is an English word that is derived from the Greek Poltos that means porridge of meal, from the Latin word Puls or Pultis, which means pottage or porridge, Old French pols, and Middle English Puls. Legume, from the Latin Legumen, is an alternative term for the edible seeds of pulse, which means any leguminous plants. Legume plants belong to the family variously referred to as Fabaceae or Leguminosae within the order Fabales which is one of the largest and economically important families of the flowering plants. Cereals and pulses are the two main groups of grain crops that are grown around the world [2]. These two groups are the staple foods of most of the world population. Some part of the world might favour one over the other, but they are often consumed in a combination. Pulses are also known as "the poor man's meat", as they regularly replace meat, cheese, eggs and fish in the diet, especially during the harsh time of poverty. Besides being a source of nutrients, foods, particularly plant foods, are a rich source of bioactive phytochemicals or bionutrients or anti nutritive factors. Trypsin inhibitors, which are studied during the past 2–3 decades, have shown that these phytochemicals have an important role in preventing chronic diseases like cancer, diabetes, coronary heart disease and hyper-cholesterolaemia. The major classes of phytochemicals with disease-preventing functions are dietary fibre, antioxidants, detoxifying agents, immunity-potentiating agents and neuropharmacological agents. Each class of these functional agents consists of a wide range of chemicals with differing potency. For example, antioxidant function is exhibited by some nutrients, such as vitamin E, vitamin C and provitamin A [3]. Other phytochemicals that have antioxidant properties are carotenoids, phenolic compounds, flavonoids and isothiocyanates. Some of these phytochemicals have more than one function. Foods rich in these chemicals and exhibiting disease-protecting potential are called functional foods. Indian habitual diets, which are based predominantly on plant foods like cereals, pulses, oils and spices, are all good sources of these classes of phytochemicals [4, 5].

**Phytic acid**: It is found in dried beans of Rhynchosia bracteata Benth. Phytic acid (inositol hexakisphosphate (IP6), or phytate when in salt form) is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phytate is not digestible to humans or nonruminant animals, however, so it is not a source of either inositol or phosphate if eaten directly. Morever, it chelates and thus makes unabsorbable certain important minor minerals such as zinc and iron, and to a lesser extent, also macro minerals such as calcium and magnesium. Catabolites of phytic acid are called lower inositol polyphosphates. Examples are inositol penta- (IP5), tetra- (IP4), and triphosphate (IP3). In seeds and grains, phytic acid and its metabolites have several important roles. Most notably, phytic acid functions as a phosphorus store, as an energy store, as a source of cations and as a source of myoinositol (a cell wall precursor). Phytic acid is the principal storage forms of phosphorus in plant seeds. In animal cells, myoinositol polyphosphates are ubiquitous, and phytic acid (myoinositol hexakisphosphate) is the most abundant, with its concentration ranging from 10 to 100 µM in mammalian cells, depending on cell type and developmental stage. The interaction of phytic acid with specific intracellular proteins has been investigated in vitro, and these interactions have been found to result in the inhibition or potentiation of the physiological activities of those proteins. The best evidence from these studies suggests an intracellular role for phytic acid as a cofactor in DNA repair by nonhomologous end-joining. Other studies using yeast mutants have also suggested intracellular phytic acid may be involved in mRNA export from the nucleus to the cytosol. There are still major gaps in the understanding of this molecule, and the exact pathways of phytic acid and lower inositol posphate metabolism are still unknown. As such, the exact physiological roles of intracellular phytic acid are still a matter of debate. Phytic acid may be considered a phytonutrient, providing an antioxidant effect. Phytic acid's mineral binding properties may also prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract. Researchers now believe phytic acid, found in the fiber of legumes and grains, is the major ingredient responsible for preventing colon cancer and other cancers. Phytates also have the potential to be used in soil remediation, to immobilize uranium, nickel and other inorganic contaminants. Phytic acid is also protective against bowel cancer which is why a diet high in unprocessed grains is recommended to prevent it. As with all things a balance is required. Unless you have evidence that you are deficient in calcium, iron, magnesium and zinc, maybe you shouldn't worry about the impaired absorption of these minerals present in the grains. There are plenty of other sources for these minerals than grain [6].

**Phenolic compounds**: Pulses contain a range of phenolic compounds, with the darker grains such as black beans and red kidney beans generally having higher amounts. The phenolic compounds in pulses are generally polyphenols and include tannins, phenolic acids, and flavonoids. Antioxidant activity is related to total phenolic content. It is found in dry beans of Canavalia ensiformis (L.) DC. (jack bean), Canvalia gladiata (Jacq.) DC. (Sword bean), Canavalia maritima Thouars (betch bean) and Canavalia cathartica Thouars (maunaloa bean), Rhynchosia minima, Caucalis platycarpos L (burr parsely). Their potential role in weight management is unclear, although studies indicate that certain phenolics interfere with enterocyte glucose absorption through interference with the glucose transporters. Over the past 15 years there has been an exponential increase in publications related to plant phenolics. Epidemiologic studies showing that plant based diets lead to less chronic diseases) fruits, vegetables, and whole grains are rich in phenolic compounds many of the phenolic compounds in fruits, vegetables, and whole grains are excellent antioxidants in vitro. Researchers began to suggest oxidative stress was a strong contributing factor in the development of cancer, cardiovascular diseases, and neurodegenerative diseases. Relatively little is known about the phenolic compounds in dry beans compared to fruit, vegetables, chocolate, wine, and tea. More recently, several groups have identified phenolic compounds in dry beans. Anthocyanins are present in black and blue-violet colored beans. A black colored Italian bean contained 170 mg of anthocyanins/kg of flour which is equivalent to 6.5 mg/serving. It was reported that only flavonol present in 2 lines of pinto beans was glycosides of kaempferol. The amount of kaempferol that would be consumed in one serving was 26.6 mg and 64 mg for the 2 lines. Studies reported the only significant flavonol in yellow, brown, and black colored beans was kaempferol glycosides, although a trace of quercetin was detected in the brown and black variety. The amounts of kaempferol glycosides that would be consumed per serving were 23.5 and 20 mg for the 2 yellow varieties, 25.2 mg for the brown variety and 4.2 mg for the black bean. Aparicio-Fernandez identified glycosides of kaempferol, quercetin, and myriceten in a black bean as well as oligomers containing (epi) catechin, (epi) afzelechin and (epi) gallocatechin in the proanthocyanidin fraction (i.e., condensed tannins). No quantitative data was available from this report, but estimates based on other published studies indicate that one serving of beans would provide 300 – 1300 mg of proanthocyanidins depending on seed coat color, storage time, etc. Luthria and Pastor-Corrales identified ferulic acid, p-coumaric acid, and sinapic acid in 15 varieties of raw dry beans. Caffeic acid was found only in 2 black bean varieties. The average phenolic acid that would be consumed was 11.1 mg/serving with a range of 6.8 to 17.2 mg/serving. For comparative purposes, dark blue-black berries and grapes provide 100 – 1500 mg of anthycyanins/serving. One serving of fruit provides 10 – 200 mg of phenolic acids and the amount of procyanidins from beans is equal or greater than that per serving of chocolate or green tea. The studies reported above for phenolics all used raw beans which is not what we eat. Therefore, we cooked navy beans, black beans, pinto beans and small red beans and extracted the phenolic compounds. It is identified protocatechuric acid, p-hydroxybenzoic acid, (+)-catechin, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid and isovanillic acid in all 4 types of beans. Ferulic acid was the predominant phenolic compound present in all beans. The flavonol, quercetin was detected in the black and red beans, while kaempherol was identified in pinto and red beans. We have not attempted to identify anthocyanins that have been identified in raw black beans and we do not have quantitative data for phenolic content at this time. In general, polyphenolics are poorly absorbed. Of the phenolic compounds in beans, the phenolic acids would be absorbed the best and maximum plasma concentrations should occur one to three hours after beans are consumed (based on data reviewed by Manach. One would not expect plasma concentrations to ever be greater than 0.5 µmol/l and clearance from the blood is rapid (half-life for clearance in urine is < 2 hours). We can predict the amount of quercetin and kaempherol that would absorb would be 1 – 2% of what is in beans. Blood concentrations would not exceed 0.5 µmol/l. Even though the blood concentrations would be low, the clearance of quercetin (and maybe kaempherol) from the blood is slow compared to the other phenolic compounds (clearance half-life 15- 20 hr). Therefore, what little reaches the blood would be there for a longer period. Very little anthocyanins and essentially no proanthocyanidins would be absorbed. Part of the poor absorption is due to the very active glucuronidation system in intestinal cells which results in the export of the glucuronidated phenolic compound back into the gut. Even if the phenolic compound escapes glucuronidation in the enterocytes, much of the absorbed phenolic compound is glucuronidated and/or sulfated in the liver. Following glucuronidation and/or sulfation, much of the absorbed polyphenolic compound may be excreted into the bile and never appear in the blood. Rarely are glucuronidated and/or sulfated polyphenolic compounds in the blood in more than 1 umol/l concentrations when polyphenolic containing foods are consumed. The steady-state concentrations of polyphenolics in blood are often in the 100 – 900 nanomolar range and the concentration of unesterified polyphenolics in blood rarely exceeds picomolar concentrations. Almost all of the research that demonstrates anti-cancer activity and biological end points that are purported to indicate a reduction in cardiovascular diseases, cancer, or untoward consequences of diabetes are done with concentrations that are 1,000 to 10,000 or more times greater than the concentrations of unesterified polyphenolics found in humans or laboratory animals. To have physiological relevance, the experiments need to be conducted with no more than 1 or 2 µmol/l concentrations of the glucuronidated and or sulphated polyphenolic compound. Even though polyphenolics are poorly absorbed, have low concentrations in blood, are present as conjugates with greatly reduced bioactivity, and are eliminated fairly quickly, beneficial effects have been attributed to the consumption of plant phenolics by humans and laboratory animals. Much additional research is required to determine if the phenolic compounds found in beans are protective against chronic diseases [7].

**Flavonoids**: It is found in dry beans of Phaseolus vulgaris, Glycin max (L.), Phaseolus acutifolius, Phaseolus coccineus, Phaseolus lunatus and Phaseolus vulgaris Caucalis platycarpos L. Flavonoids are one of the most important groups of bioactive compounds in plants, which exist in the free aglycones and the glycoside forms showing a diverse structure and a broad range of biological activities. Flavonoids include several classes of compounds with similar structure having a $C_6$-$C_3$-$C_6$ flavone skeleton. They are differentiated on the degree of unsaturation and oxidation of the three carbon segment. Within different subclasses further differentiation is based on the number and nature of substituent groups attached on the rings [8]. Mostly they occur in O-glycosidic forms with a number of sugars such as glucose, galactose, rhamnose, arabinose, xylose and rutinose but they are also present as C-glycosides. Flavonoid glycosides have many isomers with the same molecular weight but different aglycone and sugar component at different positions attaching on the aglycone ring. Naturally occurring phenolic acids are phenylpropanoids with an aromatic ring and attached three carbon side chains. Caffeic, ferulic and p-coumaric acid, as hydroxycinnamic acids, are almost ubiquitous. Phenolic acids are distributed in nature in their free and bound forms, as esters and glycosides. Chlorogenic acids are a family of esters formed between trans cinnamic acids and (-) - quinic acid (1L-1(OH), 3, 4/5-tetrahydroxycyclohexanecarboxylic acid). A subgroup of chlorogenic acid is defined by the number and identity of the constituent cinnamic acids, and there are usually several isomers within each subgroup. Many plants produce chlorogenic acids in which esterification occurs at positions 3, 4 and 5 of the quinic acid moiety [10]. Esterification at position 1 is less frequent, but 1-acyl chlorogenic acids are found in some Asteraceae. Flavonoids and phenolic acids have protective role in carcinogenesis, inflammation, atherosclerosis, thrombosis and have high antioxidant capacity. Furthermore, flavonoids have been reported as aldose reductase inhibitors blocking the sorbitol pathway that is linked to many problems associated with diabetes. Flavonoids interact with various enzymatic systems [10]. Their inhibition of the enzymes cyclooxygenase and lipooxygenase results in a decrease of platelet activation and aggregation, protection against cardiovascular diseases, cancer chemoprevention and their anti-inflammatory activity [11]. Many other biological activities are attributed to flavonoids and phenolic acids: antiviral, antimicrobial, antihepatotoxic, antiosteoporotic, antiulcer, immunomodulatory, antiproliferative and apoptotic activity.

**Isoflavonoids**: Isoflavonoids are a unique subgroup of the flavonoids, one of the largest classes of plant phenolics with approximately 5000 member compounds. Isoflavonoids are found mainly in Glysin max (soybean), Phaseolus acutifolius (runner bean), Phaseolus lunatus (lima bean or butter bean), Phaseolus vulgaris (rajmah, kidney bean) and possess a chemical structure that is similar to the hormone estrogen. The major isoflavonoids found in soybean are genistein and daidzein. Because their structures resemble estrogen and they can interact with the estrogen receptor, soybean isoflavonoids are sometimes referred to as phyto-estrogens [12]. Isoflavonoids are flavonoid variants in which the location of one of the phenolic rings is shifted. As diphenolic secondary metabolites, isoflavonoids are synthesized from products of the shikimic acid and malonyl pathways in the fusion of a phenylpropanoid with three malonyl CoA residues. Isoflavonoid content in soybean ranges from 0.14– 1.53 mg/g and in soy flour from 1.3–1.98 mg/g. The Japanese are estimated to consume 25–100 mg of isoflavonoids/day. Chinese women are estimated to consume 39 mg of isoflavonoids/day. Phenolic compounds, such as isoflavonoids, normally occur as glucoside-bound moieties called glycones. However, it is the aglycone (glucoside-free) form of isoflavonoids that is metabolically active. After consumption, probiotic bacterial enzymes in the intestine cleave the glycoside moieties from glycone isoflavonoids and release the biologicallyactive health-promoting aglycone isoflavonoids. Aglycone phenolic compounds have been found to possess higher antioxidant activity and to absorb faster in the intestines than their glucoside bound forms [13]. Interestingly, fermented soy foods are potentially rich in isoflavonoid aglycones due to microbial bioprocessing during fermentation. Once inside the blood stream, biologicallyactive aglycone genistein travels to the liver, where it is converted into an inactive β-glucuronide. Cellular glucuronidases must remove the glucuronide moiety before genistein can exert its biological activity [14]. Isoflavonoids have been well-studied and possess numerous biological activities. For example, genistein possesses inhibitory activity against topoisomerase II, tyrosine kinase, NF-κB, cancer cell proliferation, and non-oxidative pentosephosphate pathway ribose synthesis in cancer cells. Many of the health-promoting benefits of isoflavonoids have been linked to the ability of phenolics to serve as antioxidants [15]. Soybean isoflavonoids possess various biological activities that may help to explain the chemopreventive properties associated with the consumption of soybean foods. In in vitro studies, daidzein has been reported to activate the catalase promoter to stimulate caspase-3 and apoptosis and to down-regulate the activities of Bcl-2 and Bcl-xL. Genistein can stimulate p53 antioxidant enzyme activities, BRCA2, caspase-3 and apoptosis, and chloride efflux. Genistein has also been reported to suppress the activation of NF-κB, matrix metalloproteinases, lipogenesis, and COX-2. As soybean isoflavonoids also show chemopreventive activity in cells that lack estrogen receptors [16, 17].

**Melanin**: Melanin in seeds is responsible for negative health effects. It has been predicted that melanin may be present in Mucuna seeds even after processing. For instance, cooking or soaking in water with sodium bicarbonate resulted in darkening, which is presumed to be due to conversion of L-DOPA into melanin. Hence, future studies may be directed towards alkaline additives in minimizing and understanding the conversion of L-DOPA into melanin [18].

**Protease and amylase inhibitors:** Those compounds, which suppress the proteolytic activity of digestive enzymes, are considered as protease inhibitors (e.g. trypsin/chymotrypsin). Proteases inhibitors are from edible legumes (canned chick-peas, canned kidney beans and bean curd) were capable of blocking the superoxide response of human polymorphonudear leukocytes produced by the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Presence of protease inhibitors in diet cause considerable decrease in the digestibility of dietary protein due to the formation of irreversible trypsin and trypsin inhibitor complexes. They are found in comparably high amounts in the pulses compared with other plant foods and negatively affect digestibility of food proteins if not processed (i.e. mainly cooked) properly. They do not appear to have an important role in weight management. On the other hand, α-amylase inhibitors found in pulses, specifically dry beans (up to 2–4 g/kg), reduce starch digestibility and thus energy availability. Isolated α-amylase inhibitor lowers postprandial glycemic responses, although it may be inactivated through cooking or other processing methods. α-amylase inhibitors are naturally present in a variety of plants, but are particularly high in common beans. They are large glycoprotein molecules (38-60 kDa) that are inhibitory towards mammalian α-amylases. It was screened that 150 common beans and isolated two types of inhibitors, I-1 and I-2. The α-amylase inhibitors varied to some degree in thermal stability, subunit composition, molecular weight, and ratios of I-1: I-2 between bean varieties. Inhibitor activity was not correlated with seed color. Normal cooking methods destroy most if not all of the α-amylase inhibitor activity. However, there are occasional public health problems resulting from consuming active α-amylase inhibitors. For example, a raw white bean preparation was sold in Japan to promote weight loss. Many people became sick and some required hospitalization due to dehydration. Protease inhibitors have been examined in several different model systems for the ability to suppress carcinogenesis. The most effective are those that inhibit chymotrypsin, and within this group, the Bowman-Birk inhibitor (BBI) from soybeans has been most extensively studied. Purified BBI or a concentrate enriched in BBI (BBIC) incorporated into the diet at 0.5-1% has been repeatedly shown to reduce colon carcinogenesis in both mice and rats without producing adverse effects on pancreatic lesions or body weight gain. The effects on colon carcinogenesis are most pronounced when low doses of carcinogen are administered. BBI has also been demonstrated to inhibit DMBA-induced oral carcinogenesis in hamsters, dimethylhydrazidine (DMH)-induced liver angiosarcomas and 3-methylcholanthrene (MC) induced lung tumorigenesis in mice, and in vitro, suppress radiation or chemically induced malignant transformation [19].

**Lectins or Pytohemaggutinins**: Lectins are large glycoprotein molecules that bind to glycoconjugates on cell membranes and can agglutinate red blood cells in vitro. Following ingestion, they can survive passage through the acid environment of the stomach and proteolytic activity in the duodenum. It is found in dry bean Phaseolus acutifolius [20]. Lectins bind to epithelial cells in the small intestine thereby affecting nutrient absorption. Binding of lectin causes jejunal villi hypoplasia and crypt cell hyperplasia resulting in shorter, thicker microvilli and a reduction in brush border enzymatic activity. In the 1980s, there were several reported outbreaks of lectin poisoning in Britain as the result of consumption of incompletely cooked beans, causing severe nausea, vomiting, and diarrhea. Growth depression has also been observed in animals fed either purified lectins or raw beans. Proper cooking methods are therefore important to reduce the possibility of illness. Moist heat is more effective in eliminating lectin activity than dry heat [21]. The lectins from the common bean Phaseolus vulgaris have been identified and characterized in cultivars from all over the world. The most extensively characterized lectins from this species have been purified from the “red kidney” variety. The lectin fraction from this bean is composed of five kinds of isolectins, each consisting of non-covalently bound tetramers made up of different combinations of subunits, which are known as E (erythroagglutinating) and L (leukoagglutinating). Each of these subunits differs from the other slightly in their amino acid sequences and possesses differential affinities for erythrocytes and lymphocytes. Phytohemaggluttins proteins are also referred as lectins which agglutinate red blood cells and are capable of damaging the intestinal mucosa. They have been shown to occur in some important fodder trees. The highest concentrations of lectins are found in seeds but, in the leaves, their concentration is low due to translocation [22]. The biological effects of lectins probably result from their affinity for sugars. They may bind to the carbohydrate moieties of cells of the intestinal wall and cause a non-specific interference with nutrient absorption. In fodder trees, the lectins of interest are robin and ricin. In contrast to most other proteins, lectins resist digestive breakdown and substantial quantities of ingested lectins may be recovered intact from the faeces of animals fed diets containing one of a legume seeds. Robin, a lectin from Robinia psedoacacia, has been reported to cause poisoning in all class of livestock. Due to ricin, de-oiled castor seed cake is seldom used as a livestock feed. However, the mature leaves of R. communis have been found suitable for feeding to sheep; hence precautions against bean contamination are necessary. The tepary bean Phaseolus acutifolius A. Gray is an annual legume adapted to arid and semi-arid regions extending from North America to Costa Rica, including Puerto Rico and Mexico. Beans thrive under adverse agronomic conditions such as high salt concentrations and low water levels. Additionally, this species possesses high resistance to microbial pathogens and other predators. Like other legume beans of the genus Phaseolus, tepary beans produce lectins and other antinutritional factors. The tepary bean is quite toxic to man and animals in its raw form due to the presence of lectins in its seeds [23, 24].

**Cyanogens**: Cyanogens occur widely in plants and in diverse forms and they are glycosides of a sugar and cyanide containing aglycone. Cyanogens can be hydrolyzed by enzymes. It is found in dry beans of Vicia sativa (vetch) and Vicia faba (Broad bean) [25, 26]. Cyanide compounds are present in lima beans and they should therefore not be eaten raw unless they are among the low-cyanogen varieties.

**Alkaloids**: Dry beans of Cajanus cajan L (red gram, pigeon pea), Rhynchosia minima L (turvel) and visia faba (board bean) contain alkaloids. Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and more rarely other elements such as chlorine, bromine, and phosphorus. Pyrrolizidine alkaloids are toxins found naturally in a wide variety of plant species. Pyrrolizidine are probably the most widely distributed natural toxins and affect wildlife, livestock and humans. Poisoning caused by these toxins is associated with acute and chronic liver damage and is commonly fatal. Poisoning in animals has been reported from all of the sources listed above with known outbreaks attributed to Heliotropium, Trichodesma, Senecio, and Crotalaria species. In general, grazing animals will avoid Pyrrolizidine alkaloids bearing plants but may have little choice in conditions of drought or when searching for food on over-grazed or otherwise depleted pastures. If weedy crops are used for the production of hay or silage the animals can no longer exercise discrimination when feeding because the toxins survive storage processes and are completely intermingled with the fodder. Mortality is reported to be high. Poisoning by Pyrrolizidine alkaloids is due to in acute and chronic liver damage. In affected animals and humans, Pyrrolizidine alkaloids demonstrate marked toxicity to the liver, resulting in hepatocellular injury, cirrhosis and veno-occlusive disease. In extreme cases neighbouring organs such as the heart and lungs may be affected. Symptoms are those of liver failure and cirrhosis. In humans, it is reported that following a poisoning outbreak in which sub-acute toxicity is observed, some 50% of patients will recover completely and 20% will die rapidly. Of the survivors about 20% will appear to recover clinically but may go on to develop cirrhosis and liver damage in later years. In animals, symptoms have been described as “depression, photosensitization, and scouring, straining, depraved appetite, staggering gait, circling and death”. Although metabolites of PAs have been shown to have mutagenic activity, mainly in Drosophila and many have been shown to be carcinogenic, mainly in the rat, there is no evidence of pyrrolizidine alkaloid-induced cancer in humans [27, 28].

**Saponins**: Saponins are found in species of canavalia ensiformis (L) and Canvalia gladiata (Jacq.), Medicago sativa (L) (alfalfa seed). They are amphiphilic compounds present in a wide variety of plants and herbs. Saponins are divided into two groups: Steroidal saponins, which occur as glycosides in certain pastures plants and triterpenoid saponins, which occur in soybean and alfalfa. Saponins are glycosides containing a polycyclic aglcone molecule of either C27 steroid or C triterpenoid (collectively termed as 30 sapogenins) attached to a carbohydrate. Structurally saponins in food exist as glycosides, with a hydrophobic triterpenoid or steroid (sapogenin) group linked to water-soluble sugar residues. The main types of steroid aglycones include the spirostan, furostan, and nautigenin derivatives whereas oleanan derivatives comprise the more common triterpenoid aglycones. The amount and type of sugar residues vary between saponin species, the most common being glucose, glucuronic acid, arabinose, rhamnose, xylose, and fucose attached at either the C-3 position (monodesmoside saponins) or on both the C-3 and C-22 position (bidesmoside saponins). The major saponins present in Phaseolous vulgarus were identified as soyasaponin I, V, and phaseoleamide. Saponins are characterized by a bitter taste and foaming properties. Erythrocytes lyse in saponin solution and these compounds are toxic when injected intravenously. The anti-nutritional effects of saponins have been mainly studied using alfalfa saponins. Sharma et al. (1969) observed that 4-7 weeks of ad lib feeding of Albiza gave rise to toxic manifestation in sheep having symptoms listlessness, anorexia, weight loss and gastroenteritis. The adverse effects of saponins can be overcome by repeated washing with water which makes the feed more palatable by reducing the bitterness. Saponins are categorized as surfactants, and were initially thought to be harmful due to their strong hemolytic activity in vitro. It was founded that after feeding mice, rats, and chicks a 20% soy flour diet, neither saponins nor sapogenins were detectable in blood. Saponins were the major form present in the small intestine and sapogenins were primarily detected in the cecum and colon after hydrolysis by microflora. Since the saponins found in dry beans are the same triterpenoid type of saponins found in soy, it is unlikely that dry bean saponins would be absorbed. Saponins have been shown to have anticarcinogenic and antimutagenic properties in a variety of in vitro approaches (cell culture). The saponins used in these studies were from soy beans. Since dry bean saponins are similar to soy saponins, it would be expected that dry bean saponins would produce similar results. Soya saponins reduced the growth of HCT-15 and HT-29 colon carcinoma cells and also significantly decreased TPA-associated protein kinase C activation. Because saponegins are presumably the major form of saponins present in the colon, Gurfinkel and Rao looked at the effect of the chemical structure of soya saponins on anticarcinogenic activity. Only one study with saponins on carcinogenesis has been conducted in vivo. Researchers founded that incorporation of soyasaponins into the diet of mice (3%) reduced the incidence of mice with ACF, and significantly decreased the number of ACF/colon and the number of AC/foci. A number of publications extol the health benefits of the steroid type of saponins. Steriod saponins are absorbable and apparently elicit numerous biological responses following systemic distribution of the saponin. However, the triterpenoid saponins are not absorbed and presumably provide benefits only in the intestine. Saponins were found to show inhibitory effects on plant germination and fungal growth. These compounds also act as antifeedants to animals and cause hemolysis of red blood cells. Saponins were found to be present in the soil, and their allelochemical function was shown. Alfalfa contains biologically active saponins of medicagenic acid which, among other effects, causes blood hemolysis and fungal growth inhibition [29].

**Plant sterols:** β-sitosterol, campesterol, and stigmasterol are the most common types of phytosterols found in food, including beans (Rhynchosia minima L), and are structurally similar to cholesterol [30]. The absorption of phytosterols by humans is low relative to that of cholesterol (20-50%) with only about 5% of ingested phytosterols being absorbed and the remaining 95% excreted from the colon. Phytosterols have been documented to decrease plasma cholesterol in humans and animals. The cholesterol reducing activity of bean sterols should help reduce cardiovascular diseases. The cholesterol lowering effect is likely due to a reduction in cholesterol solubilization into bile salt micelles resulting in a reduction in cholesterol absorption. The relatively low absorption of ingested phytosterols from the intestine suggests that they can potentially affect colon carcinogenesis either directly or indirectly. Only a few animal studies, however, have been conducted to date. Phytosterols have been shown to reduce the rate of colonic epithelial proliferation and the proliferation zone in animals either induced with a carcinogen or administered 0.1-0.2% cholic acid. Addition of phytosterols (0.2-0.3%) to the diet also caused a reduction in both preneoplastic colon lesions and colon tumorigenesis in rodents [31]. Lastly, although phytosterols were not examined specifically in this study, wheat bran oil (2%) decreased colon tumor incidence, multiplicity, and tumor size and reduced tumor expression of iNOS and COX-2 in rats injected with azoxymethane (AOM). The mechanisms of chemoprevention by phytosterols have been suggested to include (a) alterations in membrane phospholipid composition (b) decreased formation of secondary bile acids, and (c) an increase in apoptosis [32].

**Tannins:** Tannins are found in chief concentration in dry beans of Canvalia gladiata (Jacq.) (Sword bean), Canavalia ensiformis (jack bean) Canavalia cathartica Thouars (maunaloa) and Mucuna prusriens (velvate bean), Rhynchosia bracteata, Rhynchosia minima. Tannins are water soluble phenolic compounds with a molecular weight greater than 500 Dalton and with the ability to precipitate proteins from aqueous solution. They occur almost in all vascular plants. Hydrolyzable tannins and condensed tannins are two different groups of these compounds. Generally tree and shrub leaves contain both types of tannins. The two types differ in their nutritional and toxic effects. Condensed tannins are widely distributed in leguminous forages and seeds.

**Trypsin/chymotrypsin inhibitors:** Trypsin and chymotrypsin (protease) inhibitors are present in many legumes including dry beans such as Mucuna prusriens DC.(velvate bean) and Vicia faba (broad bean). Trypsin inhibitors have generally been considered as anti-nutritional due to the long-standing observations that feeding animals raw beans causes growth depression and reduces nitrogen retention. In rats, chickens, and growing guinea pigs, long-term feeding of raw legume flour or purified trypsin inhibitor stimulated pancreatic hypertrophy and, in rats, pancreatic adenoma development. This has raised some concern whether chronic consumption of legumes and other foods containing protease inhibitors may also produce adverse effects in humans. However, commonly employed cooking methods reduce the trypsin inhibitor activity in beans by 80-95%. Based on animal feeding studies, only 55-69% of the trypsin inhibitor activity needs to be destroyed to reduce pancreatic hypertrophy in susceptible animals and 79-87% destruction insufficient to allow maximum weight gain [34, 35].

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