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ETHNOMEDICAL AND PHARMACOLOGICAL PROPERTIES OF Phaseolus vulgaris L. AN OVERVIEW

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Abstract: Phaseolus vulgaris L. (Leguminosae), commonly known as kidney bean, is a food item of mass consumption in Asian and Eastern countries. A large variability exists in common bean seeds; color and size are two important quality characteristics for the consumers. Seeds size and weight depends on genetic variation, cultivar and environmental conditions. The seeds of Phaseolus vulgaris contain alkaloids, flavonoids, glycosides, polyphenols, saponins, tannins and terpenoids which are the main phytochemical groups with biological activities. The P.vulgaris seeds have anti-hyperglycemic potential and may use as complementary medicine to treat the diabetic population by significantly reducing dose of standard drugs. The presence of tannins also showed that the seeds could be used as purgative, cough, asthma and hay fever. Different families of proteins are known to be associated with a plants response to stresses by being newly synthesized, accumulating or decreasing. Among other things, these proteins are involved in signaling, translation, host-defense mechanisms, carbohydrate metabolism and amino acids metabolism. Changes in protein profile of common bean could modify the biological activity of peptides released by enzymatic hydrolysis.

Keywords: Phaseolus vulgaris, Alkaloids, Diabetes, Antioxidants, Asthma.

Introduction: Since the beginning of human civilization, medicinal plants have been used by humanity for its therapeutic value. According to the world Health Organization in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs. Plant compounds are of interest as asource of safer or more effective substitutes then synthetically produced bioactive components. Phytochemical aided enormously bv progress was the development of rapid and accurate methods of screening plants for particular chemicals. These procedures have shown that many substances originally thought to be rather rare in occurrence are of almost universal distribution in the plant kingdom. The drugs contained in medicinal plants are known as active principles. The bioactive principle are divided chemicallyinto a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleoresins, steroids, tannins and terpenoids. ^[1] Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of various affections. In either way the bioactive compounds from plants play a determining role in treatment of the various diseases. Therefore, the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extract by various analytical method is important. The genus *Phaseolus vulgaris* includes all species of legume seeds normally known as common beans. Archeological investigations showed that common beans originated on the American continent, specifically in sourthern United States, Mexico, central America, and The northern part of South America.^[2] A large variability exists in common bean seeds; color and size are two

important quality characteristics for the consumers. Seed size and weight depend on genetic variations, cultivar and environmental conditions.^[3] The seed color of beans is determined by the presence and concentration of flavonol glycosides, anthocyanins, and tannins.^[4] Recently, P. vulgaris is gaining increasing attention as a functional or nutraceutical food, due to its rich variety of phytochemicals with potential health benefits such as proteins, amino acids, complex carbohydrates, diatary fibers, oligosaccharides, phenols, flavonoids, alkaloids, tannins, among others.^[5] Important biological activities have been described for fibers phenolic compounds lectins inhibitors and phytic acid from common beans like enhancement of the effect,^[6] anticarcinogenic effects. bifidogenic P.vulgaris seeds have a notable place in the folklore throughout the world and in the traditions of many cultures such as pharmaco therapeutic effects. Phaseolus vulgaris L. (Leguminosae), commonly known as kidney bean, is a food item of mass consumption in Asian and Estern countries.

Taxonomical Classification

Kingdom: Plantae Subkingdom: Tracheobionta Superdivision: Spermatophyta Division: Mangnoliophyta Class: Mangnoliopsida Subclass: Rosidae Order: Fabales Family: Fabaceae Genus: Phaseolus Species: Phaseolus vulgaris

Morphology and Anatomy of Plant

Morphology of the Bean Plant: Phaseolus vulgaris

A. Leaves: The leaves are broad at the blade and are attached to the stem by means of a stalk-like petiole. The leaf may be simple (have only one blade per petiole) or compound (usually three blades per petiole). There may be two simple leaves or one compound leaf attached at a spot on the stem called a node. The veins of each leaf blade are arranged into a complicated network. Where the stem and leaf join, there is a swollen area of the petiole (pulvinus) that is responsible for leaf movements. At night the bean leaves fold together and down toward the soil; at dawn the leaves unfold and are lifted into the sun. Two special leaves may still be attached to your bean plant. These are the cotyledons or seed leaves.

These leaves are very fleshy and are used by the plant to store starch and other complex molecules in the seed for the later nourishment of the growing plant. On your plant, the cotyledons may be withering due to loss of starch and may have turned green to help produce more nutrients through photosynthesis. The cotyledons may have been completely used up and abscised (fallen off). The portion of the stem below the cotyledons is called the hypocotyl.

B. Stem: The bean stem is quite long and the internodes between leaf attachments (nodes) are quite obvious. The lowest portion of the stem is below the cotyledons and is called the hypocotyl. The stem terminates at the top of the plant in the apical bud. Lateral buds are found in the axils of each leaf just above the node. This bean plant is a "bush" variety that has shorter stems than the wildtype "pole" beans. When the stem touches an object, changes in the production of plant hormones cause the stem to twist tightly around and grow up the object. This twining habit is called circumnutation and is common among vines like pole beans. How did we get bush beans from wild pole beans? They resulted from a plant with a chance mutation in a gene coding for a plant hormone involved with stem growth. They cannot produce enough gibberellic acid for extensive stem growth.

C. Root: The roots of the bean plant are mostly fibrous, although a single main root (the taproot) is larger than the others. The taproot forms many fine lateral roots that make up the bulk of the mineral absorption area. Some plant roots are contractile; they shorten to pull the plant around in the soil. In one case (Oxalis) the roots can pull the plant 60 cm through the soil in one year. While they operate very slowly, these roots prove that plant locomotion is possible. Many plant species have contractile roots, but they usually serve only to pull the stem deeper into the soil, not across the soil.

II. Plant Anatomy (Internal Structure): Now that you have some idea of the external parts of a plant, you will examine some internal parts. You will briefly examine the internal anatomy of a typical vascular plant. The parts inside of a leaf, stem, or root are very small, so the examination would normally require the use of a microscope. This optical device is used to examine structures that are in the range of one millimeter or smaller in diameter. It can, for example, easily detect the

individual fibers that make up the paper of this page. The period at the end of this sentence could easily cover the complete field of view through a microscope. In the absence of suitable microscopes, your instructor will show you some photographic slides taken through a microscope. If microscopes are available, remember the microscope is a delicate instrument and should be handled carefully. Your instructor will demonstrate the parts and use of the microscope for you. Please do not manipulate the microscope until you are sufficiently familiar with it. Vascular plants have an advanced form of vascular (conductive) tissue consisting of xylem and phloem tissues. These two tissues are arranged in a characteristic pattern that we shall soon examine. These tissues are typically surrounded by a tissue known as ground parenchyma. Each plant organ is covered by a single layer of cells known as the epidermal tissue. The cells of these plant tissues typically have walls, true nuclei. numerous cellulosic chloroplasts, prominent vacuoles, and store starch. You should be able to observe these cellular structures in some of the cells you will observe today with your microscope or from the photomicrographs. The colors you will observe in specimens are artificial. The thin sections of plant organs have been dyed with a series of dyes (green, red, and purple) that are absorbed by structures containing particular chemicals. The red dye, for instance, stains areas rich in fatty, oily, or waxy chemicals, whereas the green dye stains cellulose (a polysaccharide). Sections of living plant tissues would typically not have any color except yellow or green in the chloroplasts (chlorophyll is a green pigment, carotene is a yellow pigment) or red colors in the vacuole (anthocyanin pigments found typically only in flower or fruit tissues). In making your drawings, do NOT draw in every cell you observe; if a region is composed of many cells, outline the region and draw only a few cells (5 or 6) in detail. DO NOT draw in hundreds of tiny circles!! imperfect Your instructor will demonstrate a good drawing early in the laboratory period. Every important structure should be labeled. In this exercise, the structures to be labeled are indicated in bold type. Before handing your drawings in for examination, BE SURE that you have labeled all of the structures indicated. Spend no more than 20 minutes making each drawing.

A. Leaf: Obtain a prepared slide of a leaf cross section and examine it carefully with your microscope. Locate the three major layers of the leaf.

1. The epidermis surrounds the inner tissues. Notice how the upper epidermis has very few stomata compared to the lower epidermis. The epidermis is covered with a waxy material called cutin which prevents evaporation and water loss. The cutin picks up the red dye and should appear as a thin pinkish layer on the outer surface of the leaf. Thus, the only meaningful openings for gas exchange are the stomata surrounded by guard cells. The guard cell pairs work in a special manner involving light, hormones, and ion pumps to fill up with water by osmosis and open the stoma, or to lose water by osmosis and close the stomata.

The mesophyll consists of large cells 2. (parenchyma) filling up the bulk of the leaf mass. This is subdivided into the upper palisade mesophyll and the lower spongy mesophyll layers. The palisade layer is a parallel array of columnar cells each containing many chloroplasts. The spongy layer has nearly isodiametric cells arranged in a loose network. Both areas of mesophyll carry out photosynthesis for the plant and need good gas exchange to do this. You will notice that each cell in Page 3 the mesophyll is largely surrounded by an apparent gas space. The gases produced as waste in the cell (e.g.: oxygen) can be exchanged for essential gases (e.g.: carbon dioxide) in the gas space. The gas space, in turn, might exchange gases with the external atmosphere through the stomata.

B. Stem: The stem is largely a supporting structure and it holds a display of leaves to the sun. It is also a conductive structure. The stem transfers water and minerals from the soil to the upper parts of the plant. The stem also transfers water and photosynthetic products from the leaves to the rest of the plant. Its structure is very similar to a leaf and has three fundamental parts. Obtain a slide of a stem cross section and examine it carefully using your microscope.

1. The epidermis. You will notice that there is an outer layer of epidermis. Of course the stem is usually round, so there is no inner and outer or upper and lower distinction. The epidermis, like that in the leaf, is responsible for preventing water loss except through stomata. The stem epidermis

will also have guard cells that regulate loss through the stomata, they are rare, however.

2. The cortical parenchyma filling up the stem volume is roughly equivalent to the mesophyll parenchyma of a leaf. In many species the stem is green and the outer layers of the cortex contain the chloroplasts necessary for photosynthesis. The outer cortex area may also contain some cortical collenchyma. These cells have unevenly thickened walls and are responsible for mechanical support. Embedded in the cortex are veins or vascular bundles as discussed below. Near the center of the stem cross section is more parenchyma. This inner area is called the pith region.

3. The vascular bundles are equivalent to the veins of a leaf. Since these are oriented primarily up and down the length of the stem, the stem cross section shows only slices of these elongated cells. Each bundle consists of two major tissues as in the leaf. The cells with green cell walls located toward the epidermis are the phloem cells. The cells with red cell walls grouped toward the center of the stem are the xylem cells. The tissues are separated by the cambium and surrounded by fibers

C. Root: The root has an even more primitive structure than the stem. There is still a single-cell layer of epidermis and a cortical region of ground parenchyma, but the vascular bundles are coalesced into a single solid cylinder.^[7]

Cultivation and Collection: Common bean (Phaseolus vulgaris L.) was domesticated by Native Americans during pre-Colombian times. Archeological data suggest that bean was independently domesticated in different regions of the Americas including the Andean region of South America, Argentina, and maxico.^[8] The oldest domesticated beans found at archeological sites in each of these regions were estimated to have been cultivated between 7000 to 9600 years ago. Wild or putatively wild relatives of P. vulgaris grow currently from northern Mexico to Argentina, often in the same regions as cultivated forms. Domestication has altered the morphology and phenology of the plant, especially growth habit, size, seed retention and maturity.^[9] Selection toward smaller, denser plants resulted in shorter internodes, suppressed climbing ability, fewer and thicker stems, and larger leaves. And upright in terminate bean cultivars that were more suitable for mechanized crop production.^[10]

Extraction Methods for Seeds of Plant: Six procedures for the extraction of total phenolic compounds were used. In the first four, only one solvent, methanol-water 80:20 v/v acidified with 0.1% HCl was used, and different extraction techniques were used. The two remaining procedures consisted of sequential extractions with different solvents. The extraction techniques were:

Plate Stirring: A sample (1g) was suspended in 25ml methanol-water 80:20 v/v acidified with 0.1% HCl, and stirred on a plate (Corning, model PC 420, USA) for 2h at room temperature. Later, the mixture was centrifuged at 1800g for 15min, the methanol was decanted and the residue was reextracted with 25ml of fresh methanol. It was centrifuged again and the extracts were combined.

Wrist-action Shaking: A sample (1g) was suspended in 25ml of methanol-water 80:20 v/v acidified with 0.1% HCl and shaken for 2h in a wrist-action shaker (Burrel, USA) at maximum speed. Subsequently, the same procedure described above was followed.

Sonication: 1 g of sample was suspended in 25ml of methanol-water 80:20 v/v acidified with 0.1% HCl and sonicated (Bransonic, Branson 2510, USA) for 15min at room temperature.

Homogenisation-Sonication: A sample (1g) was suspended in 25ml of methanol-water 80:20 v/v acidified with 0.1% HCl and homogenised for 60s at 15rpm with an homogenizer (Kinematica, Polytron 3100, USA) and later sonicated for 15min. at room temperature. Subsequently, the same procedure described above was followed.

Sequential Extractions with Water, NaOH 0,2N and Methanol 50%: According to the method of Singleton and Rossi (1965) modified, 2g of sample were extracted with 100ml of N₂ saturated water, plate stirred and centrifuged. In this step, three extraction times were tested: 1, 14 and 20h. To the residue, 50ml of NaOH 0.2N were added, platestirred for 30min and centrifuged. Again, to this residue, 50ml of 50% methanol were added; it was plate-stirred for 30min and centrifuged. This last step was repeated once more, and then the supernatants were combined.All centrifugations were performed at 1800g for 15min, and the supernatants were set aside for the quantification of the total phenolic compounds.

Methanol Extraction followed by Alkaline Hydrolysis and Extraction with Ethyl Acetate: This was performed according to the method of Luthria and Pastor-Corrales (2006) modified. The methanol extraction described above was performed, and the resulting residue was hydrolyzed with 25ml of NaOH 2N, stirring on a plate for 1h. The reaction mixture was then acidified with 7ml HCl 7.2N. The phenolic compounds released were extracted with ethyl acetate (2'32ml). The organic layers were combined and evaporated at 45°C under vacuum. The residue was dissolved in 25ml methanol-water 80:20 v/v with 0.1% HCl.

Quantification Method: Gallic and tannic acids were used as standards for the quantification. The calibration curves had a concentration ranging 0.01-0.6mg·ml⁻¹ and the results were expressed as mg GAE (Gallic acid equivalent)/100g of sample and as mg TAE (tannic acid equivalent)/100g of sample. The quantification of the total phenolic compounds was based on the Folin-Ciocalteu reaction, according to the method of Singleton and Rossi (1965), measuring the absorbance at 765nm. [11]

Chemical Constituents: The Phytochemical Screening indicated that the seeds of Phaseolus vulgaris contains alkaloids, flavonoids, glycosides, polyphenols, saponins, tannins and terpenoids which are the main phytochemical groups with biological activities. Alkaloids, comprising large groups of nitrogenous compounds are widely used as cancer chemotherapeutic agents. Alkaloids also interfere with cell division, hence the presence of alkaloids on the plants makes it is possible remedy in the treatment of cancer. Glycosides have been found effective in congestive heart failure, regardless of the cardiac rhythm and that the beneficial effect is brought about by its direct action to increase the force of myocardial contraction. It is also acts directly on the smooth muscles of the vascular system. They exert a number of effects on neural tissues and this indirectly influence the mechanism and electrical activities of the heart, and modify vascular resistance and capacitance.^[12] Saponins are glycosides of both terpenes and sterols having hypertensives and cardiac depressant properties, ^[13] hence the presence of these metabolites in P. vulgarisseeds tends to support its medical uses. The result of the phytochemical screening revealed that tannins, flavonoids are presents in the seeds. This could be responsible for their antibacterial properties, as purgative, cough, asthma and hay fever. ^[14] Furthermore reported the antioxidant activity of extract from *P.vulgaris*.

Chemistry of Plant: The seed color of beans is determined by the presence and concentration of flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins).

Polyphenols: The seed color of beans is determined by the presence of polyphenolic compounds. The main polyphenolic compounds are flavonoids such as flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins), however the most widely distributed group of flavonoids in beans includes proanthocyanidins.

Lectins: Lectins are part of a major group of bioactive proteins found in almost all organisms, vertebrates, including plants, invertebrates, bacteria and virus. ^[15] Lectins are glycoproteins and constitute a heterogeneous group of proteins, often resistant to proteolysis in the gastrointestinal tract and with the ability to agglutinate erythrocytes of some blood types in vitro. ^[16] Lectin content in legumes is in the range of 5% to 20% (26-30). Lectin activity (LA) is commonly expressed as the maximal geometric dilution in which visible macroscopic hemaglutination is observed (HAU/g sample) and reported lectin content for four varieties of dry beans Phaseolus vulgaris L. cultivated in different regions, with hemagglutinin activity units (HAU) from 0.42 to 8.89 HAU/g (average 2.15 HAU/g). The authors suggested that variability in hemagglutinin activity can be influence by the variety as well as locality growing conditions.^[17]

Trypsin Inhibitors: Although trypsin inhibitors (TI) in common beans (Phaseolus vulgaris) are protease inhibitors that are considered as antinutritional factors, to our knowledge there is no scientific evidence about their role in human health benefits.

Carbohydrates: Carbohydrates constitute the main fraction of grain legumes, accounting up to 55-65% of the dry matter. Of these, starch and non- starch polysaccharides (dietary fiber) are the major constituents, with smaller but significant amounts of mono, di and oligosaccharides. ^[18] These leguminous contain slowdigested carbohydrates and high proportion of non-digested carbohydrates (NDC) that might be fermented in the large intestine. Non-digested carbohydrates reaching the colon include mainly resistant starch

(RS), soluble and insoluble dietary fiber, and non-digestible oligosaccharides (NDO).^[19]

Ethnomedical Properties and Uses: Common beans (Phaseolus vulgaris L.) seeds have some bioactive components related with health benefits. such as alkaloids, steroids, fibersanthocynin, carbohydrate, catechin, flavonoids, phasine, phytic acid, quercetin, saponins, steroids, tannins, and terpenoids and trypsin inhibitors; however, there is still more to learn about the mechanism of those bioactive compounds on chronic degenerative diseases. Together, these data suggest that seeds of P. vulgaris may constitute potentially interesting, novel remedies for the treatment of overweight and syndrome such diabetes. metabolic as Undoubtedly, this area of research holds considerable potential on nutraceutical foods. Further studies, designed to confirm and extend those currently available in literature, are needed. Likewise, the need to axploit the potentials of Phaseolus vulgaris seeds especially in areas of traditional medicine and pharmaceutical industries arises.

Pharmacology of Plant Phaseolus Vulgaris

Antidiabetic Activity: From the study, it may be concluded that the P.vulgaris seeds have antihyperglycemic potential and may use as complementary medicine to treat the diabetic population by significantly reducing dose of standard drugs. They may suggest that the combination of most active dose of P. vulgaris seeds with glibenclamide may play an important role to reduce the blood glucose levels in chronic diabetic conditions. Moreover, further study is pharmacological and biochemical required. investigations are under way to elucidate the mechanism of the anti diabetic effect of *P.vulgaris* seeds. In the same way, it could be interesting to carry out isolation, purification and characterization of bioactive active components from seeds, which might be a good independent and/or complementary regiment for the treatment of diabetes mellitus, seem to be necessary.^[20]

Ace Inhibitory Activity: In vitro simulated digestion of lentil and whey by digestive proteases produced ACE-inhibitory activity that was dependent on the time of digestion and the source of protein.Pepsin digestion in the stomach phase produced increasing ACE-inhibitory activity with increased digestion time. The subsequent action of trypsin and chymotrypsin in the intestinal phase initially produced peptides with higher ACEinhibitory activity than the products of pepsin digestion, but decreasing inhibitory activity was observed with longer digestion time. Whey proteins possess more bioactive fractions than lentil proteins. It may be due to the presence of much more bioactive sequences within different whey proteins and also the specificity of digestive enzymes for releasing these sequences.^[21]

Anti-Cancer Activity: Beans (*Phaseolusvulgaris*) are an important food staple in many traditional diets. There is limited evidence to suggest an inverse relationship between bean consumption and colon cancer. The consumption of black beans and/or navy beans would reduce colon carcinogenesis in rats. Rats were feda modified AIN-93G diet (control) or diets containing 75% black beans or 75% navy beans for 4 week, and then colon cancer was initiated by administration of two injections of azoxymethane 1 week apart. At 31 week after the second injection, the incidence of colon adenocarcinomas was significantly lower (P<0.05) in rats fed the black bean (9%) and navy bean (14%) diets than in rats fed the control diet (36%). Total tumor multiplicity was also significantly lower in rats fed the black bean and navy bean diets than in rats fed the control diet. The 44-75% reduction in colon carcinogenesis in rats fed beans was attributed to 1) more controlled appetites, leading to significantly less body fat, and 2) much greater concentrations of butyrate in the distal colon. It was concluded that eating black beans and navy significantly lowered colon cancer beans incidence and multiplicity.^[22]

Antioxidant Activity: The antioxidant effect of an aqueous extract of Phaseolus vulgaris pods, an indigenous plant used in Ayurvedic medicine in India was studied in rats with streptozotocininduced diabetes. Oral administration of Phaseolus vulgaris pod extract (PPEt; 200mg/kg body weight) for 45days resulted in a significant reduction in thiobarbituric acid reactive substances and hydroperoxides. The extract also causes a significant increase in reduced superoxide glutathione, dismutase, catalase, glutathione-Sglutathione peroxidase and ransferase in the liver and kidneys of rats with streptozotocin-induced diabetes.^[23]

Enhance Weight Loss in Overweight Man and Women: *Phaseolus vulgaris* extract has been shown in vitro to inhibit the activity of alphaamylase and may help promote weight loss by interfering with the digestion of complex CHO to simple, absorbable sugars, potentially reducing carbohydrate-derived calories. Before crossing the intestinal wall, all complex CHO (ie, starches) must be hydrolyzed to their monosaccharide units, in most cases glucose 30. There are several enzymes involved in this process - alphaamylase present in saliva and pancreatic juice, which converts complex CHO into oligosaccharides, and various other enzymes (maltase, lactase, etc.) present in the brush border of the small intestine oligosaccharides that convert these to monosaccharides that can then be absorbed. ^[24]

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