RESEARCH ARTICLE

Extraction, Characterization and Therapeutic Evaluation of Seeds of *Phaseolus vulgaris* L. for Weight Management

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ABSTRACT

The frequency of overweight concerns and obesity is progressively growing in high-income countries in the west and low-income and middle-income countries. Those suffering from these conditions may find that taking measures to cut their carbohydrate consumption and their absorption rate is beneficial. To slow the pace at which glucose is taken into the bloodstream, blocking amylase, a key enzyme in the digestion of carbs, is highly recommended. AIs, or beta-amylase inhibitors, are chemicals found in plants that work to prevent this enzyme from doing its job. Interestingly, the common bean, or *Phaseolus vulgaris*, has protein-based α-Amylase inhibitors that regulate the activity of this enzyme through protein-protein interaction. In this article, we cover the numerous extracts of common bean seeds, the protein inhibitors that those extracts include, and what the most recent research has shown about the molecular structure of those extracts and the method by which they exert their inhibitory effects. Evidence for the possible benefits of *P. vulgaris* protein inhibitors, including clinical research conducted on both animals and humans, is also covered in this article. However, more research is necessary to validate the therapeutic efficacy of the drugs already on the market. This research has the ability to provide a succinct review of the current state of information on *P. vulgaris* extract and its prospective application as a nutraceutical alternative to control possible weight gain if observed to be successful.

Keywords: Characterization, Extraction, *Phaseolus vulgaris L.,* Therapeutic evaluation, Weight management.

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INTRODUCTION

In recent time there has been a serious concern in the general population's health and obesity is on the verge being termed as an epidemic. Obesity is responsible for 5% of all fatalities around the globe. According to estimates released by the world health organization (WHO) for the year 2016, more than 1.9 billion individuals were overweight and more than 650 million were obese. Obesity is a major public health concern at any age, but especially in children and teenagers. Since 1975, there has been a 14% rise in the number of children and adolescents in the age range of 5 to 19 years old that are overweight or obese. In 2016, this number was over 340 million. It is generally considered that there are more people who are overweight than people who are underweight throughout the whole world, except for specific locations in sub-Saharan Africa and Asia. This disorder of obesity may be regarded as a condition wherein the person develops inability to maintain a healthy calorie-to-energy-output ratio.[1-3] The primary reasons include a lack of physical activity and a diet with high sugar load but lacking in fibre and other essential minerals. Heart disease, high blood pressure, type 2 diabetes, high cholesterol, sleep apnea, knee osteoarthritis, and numerous forms of cancer are just some of the many serious health problems that are associated to being overweight or obese. During the current coronavirus disease (COVID-19) outbreak, obesity has emerged as the predominant risk factor for hospitalization and poor health outcomes among severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) patients. This is the case even though obesity was not previously considered a risk factor for any of these outcomes. Many different dietary strategies have been described as possible answers to these problems. These strategies try to change the way in which the body metabolizes carbs and lipids. It's possible that the easiest solution would be to consume less carbs or to consume more fibre, which would hinder the body from absorbing carbohydrates. The vast majority of people, on the other hand, are opposed to these strategies resulting in gastric disturbances. It is possible that consuming resistant starches, which are carbohydrates that do not ferment in the small intestine, would have the same results. Thus naturally occurring occurrences of these

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Figure 1: Plant of *P. vulgaris L.*

starches, which have a low glycemic index, may be obtained from a variety of seeds, legumes, and unprocessed whole grains (GI). Phytochemicals can be an alternate way to lower the amount of glucose absorbed by the body.[4,5] The intestines are responsible for the breakdown of these carbs; however, if the enzymes amylase and glucosidase were suppressed, it would be possible to prevent these carbohydrates from being absorbed. Specific glycoprotein enzyme beta-amylase is able to hydrolyze polysaccharides more rapidly if they contain three or more (14)-linked D-glucose units than other polysaccharides.

Phaseolus vulgaris L.

Common kidney bean, also known as *P. vulgaris L.,* is now considered a "new global crop." Around 7,000 years ago, it made its way across the Atlantic ocean from South and north america to europe. The *Leguminosae* family, the *Papilionoideae* subfamily, the Phaseoleae tribe, and the Phaseolinae subtribe are all home to the genus *Phaseolus*. Despite the fact that the genus *Phaseolus* has over 70 distinct species, this specific legume is the one that is farmed and used the most often out of all of them. Before Europeans came, the area was home to the development of five different cultivated kinds of the plant. There are many different kinds of beans, but the most well-known ones include the common bean, the lima bean, the red bean, the tepary bean, and the bean. There is no one strategy that all of them are used to overcome environmental obstacles and reproduce (Figure 1).[6]

More than 90% of the world's total agricultural output may be traced back to the *P. vulgaris L.* species. It is considered a non-centric crop because it was domesticated in at least two different places, and its wild forebears might be found anywhere in Central and South America. The evergreen vine *P. vulgaris L.,* which may be found in the intermediate latitudes of the neotropics and subtropics from Mexico to Argentina, is the crop's wild parent. This crop has been domesticated from it. The urban and rural inhabitants of East Africa and Latin America depend largely on this species as a food source. Both of these regions are home to this species. However, as people in wealthier countries make more of an attempt to enhance the quality of their diets, the consumption of this food is becoming more popular. In comparison to other food crops, *P. vulgaris L.* has a greater degree of diversity and versatility in terms of its development, seed appearance (including size, shape, and colour), duration to maturity, and adaptability. The most beneficial kind of legume, both for people and other animals, is the common bean. Most users have low wages.[7-9] In places like Mexico, Central and South America, and Africa, where annual per capita consumption can reach 40 kgs, they constitute a staple food. More than 90% of the world's annual output comes from countries south of the Sahara and in Latin America. Dry grain production was assessed at 26.8 million tonnes in 2016 by the Food and Agriculture Organization of the United Nations (FAO), while production of green beans was estimated at 23.5 million tonnes. Green beans, red kidney beans, black beans, Mexican, pinto beans, tirage beans, great northern beans, navy beans, and pink beans are among the most often consumed dry beans. Dried beans can come in a variety of colors and sizes, from pink to great northern to navy to large navy beans.[10-13]

MATERIALS AND METHODS

Collection of Phytochemical Material

The *P. vulgaris* utilised in the manufacture comes from seeds acquired legally from reputable firms. Same were confirmed in the appropriate amount of time.[14]

Pharmacognostic Study

P. vulgaris Linn seeds were studied from several different perspectives, including morphology, microscopy, chemistry, and physicochemical characters.

Seeds and Preparation of Crude Bean Extract

The ground seeds were subjected to a soaking for 5 hours, followed by boiling in fresh water for 15 minutes (for deactivation of Phytohaemogltinin). The aqueous extraction process will produce dried aqueous extract using either a cold press or hot water. The next processes are screening and then drying in a vacuum. After the extraction procedure has been completed and a concentrated extract has been obtained, the extractive value were determined.[15,16]

Pharmacological Screening

The estimation of the potency of crude drug or its preparation was studied by means of enzyme inhibition activity.

Weight Management Activity

Phytohemagglutinin has been shown to bind to the intestinal brush border membranes, as has been reported (the stomach, the small intestine, the cecum, and the colon). Therefore, both glucagon-like peptide and cholecystokinin secretion are stimulated. These two hormones play a crucial role in regulating fullness and digestion. In a laboratory investigation, lorglumide, an antagonist of the cholecystokinin receptor type A (CCKA), counteracted the effects of a *P. vulgaris* extract (Bean Block®) on food intake. According to these results, phytohemagglutinin in *P. vulgaris* extracts activates

CCKA receptors, leading to the release of cholecystokinin and a subsequent reduction in hunger. It has been shown that phytohemagglutinin stimulates CCKA receptors in the pancreatic tissue of rats, comparable to effects that can be correlated with human pancreatic tissue.[17,18] Since, there is an increase in the amount of amylase that is produced, the rats' metabolism is able to rapidly process the starch that is ingested. Theoretically, this should have caused the animals to feel hungrier and raise their blood sugar levels. However, it became evident that this effect does not have much influence on the degree of hunger or the amount of food consumed. This probably is owing to the fact that phytohemagglutinin and -amylase inhibitors have different effects on satiety and the metabolism of glucose.[19]

RESULT AND DISCUSSION

Pharmacognostic Study

Macroscopy of P. vulgaris L. Seed

The length, width, ratio of length to width, weight per 100 seeds, color, number of colours, primary/main color, secondary/accent color, distribution of accent color, veining, shape, primary/secondary color, and coat pattern were evaluated subjected to quantitative and qualitative analysis.

Microscopy of P. vulgaris L. Seed

This image shows a transverse section of a ripe kidney bean fruit. The pericarp forms the outer wall of an ovary that has reached maturity. The exocarp is the most exterior layer, followed by the mesocarp in the middle, and then the endocarp is the most inside layer. The exocarp is made up of the epidermis and a subepidermal layer that consists of very few cells. Together, they constitute the exocarp. Each of these layers is made up of chambers that have very thick walls around. Next to the mesocarp, which has many layers, is the exocarp. These layers are made up of parenchymatous cells, which may be identified by their thick and slightly lignified cell walls. They are observed in many different places, and there is a distinct length difference between the two ends. The mesocarp of the pod consists of connecting and transporting

Table 1: Percentage yield of the Folic acid fraction

Parameters	*P. vulgaris L. seed (Folic acid fraction)*
Color	Dark brown
Consistency	Crystalline powder

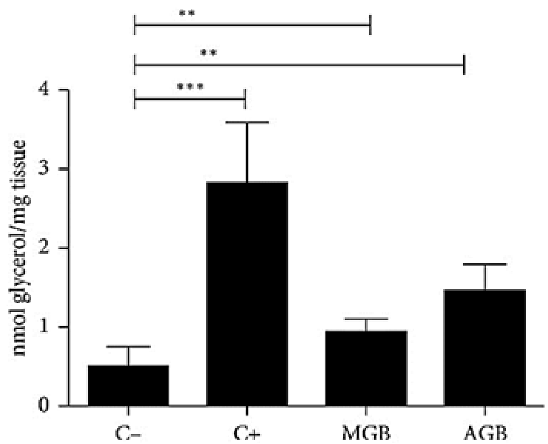

Figure 2: Effect of methanol and aqueous green bean extracts (MGB and AGB) on *ex-vivo* lipolysis.

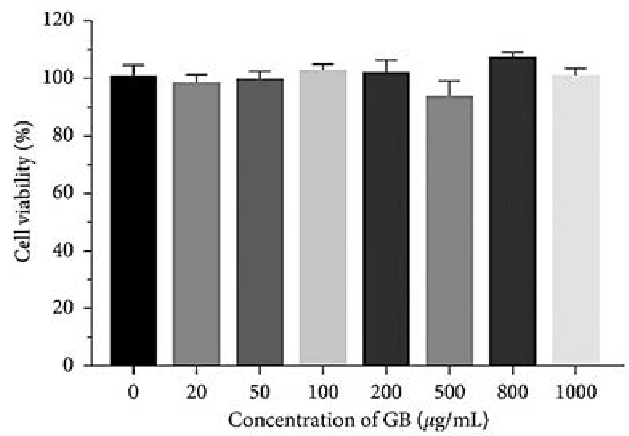

Figure 3: Green bean extract's impact on mitochondrial dehydrogenase.

Table 2: Concentrations of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides in the serum of rats fed a variety of different diets for four weeks

Glycerol/ mg tissue (nmol L−1)	*Normal diet*		*High fat diet*	
	C-	*C+*	*MGB*	*AGB*
Total cholesterol	1.99 ± 0.11	1.64± 0.08*	1.69 ± 0.09	1.46 ± 0.05
HDL cholesterol	0.70 ± 0.03	0.67 ± 0.02	0.63 ± 0.03	0.61 ± 0.02
Non-HDL	1.29 ± 0.08	0.97 ± 0.06*	1.06 ± 0.07	0.86 ± 0.03*
cholesterol Triglyceride	1.08 ± 0.13	0.43 ± 0.09*	0.77 ± 0.08	0.54 ± 0.08

Table 3: Test substances' cytotoxic effects (IC_{50} g/mL) and selectivity index.

Test compound	*IC_{50} (μg/mL)*		*Selectivity index*
	HeLa cells	*Fibroblast cells*	
P. vulgaris Seed extract 20 to 1000 μg/mL	182.4±5.5	493.9±2.1	2.7
Preadipocytes 20 to 1000 μg/mL	0.73±0.05	4.87±0.24	6.7

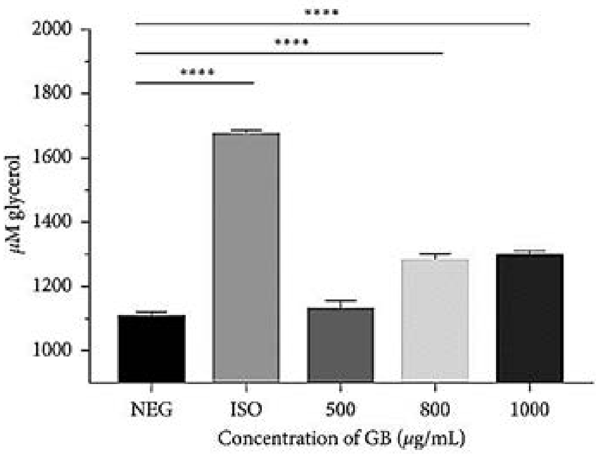

Figure 4: Green bean extract has lipolytic action on 3T3-L1 mature adipocytes.

Table 4: IC_{50} values

Test compound	*IC_{50} (μg/mL)*	
	Glycerol	*Fatty acid*
P. vulgaris Seed extract 800 to 1000 μg/mL	1082.4 ± 5.5	1393.9 ± 2.1
3T3-L1 adipocytes 1000 to 2000 μg/mL	1700 ± 0.05	14.87 ± 0.24

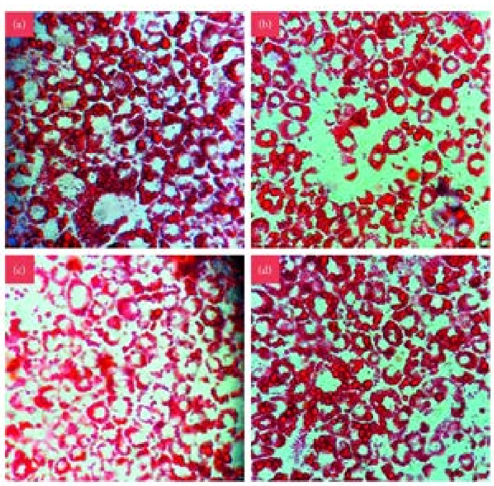

Figure 5: Oil Red O staining to show how green bean extract affects adipogenesis in 3T3-L1 cells.

tissues of the pod. The endocarp is formed when the pericarp and the inner epidermis are fused together. At this level, the fruit wall still contains a few parenchymatous cells that have not been removed. Due to the thinness of their cell walls and the fact that their sizes vary in different planes, parenchymatous cells do not stain very well. There is still an inner epidermis, but the cells that make up this layer are less differentiated. Following the completion of an ovule's developmental process, the integuments are responsible for producing the many layers that comprise the seed coat. This exterior layer, also referred to as the epidermis, is responsible for the development of the palisade wall that can be seen on the seed coats of most legumes.

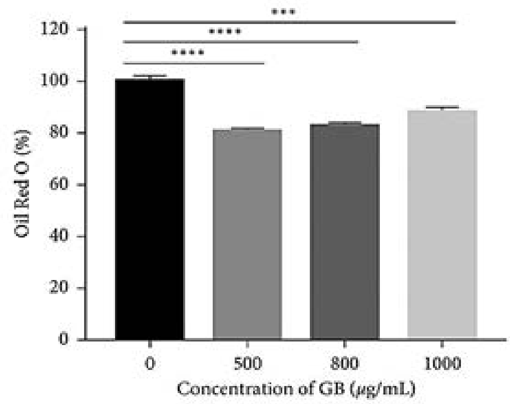

Figure 6: The accumulation of lipids within 3T3-L1 cells is suppressed by a green bean extract.

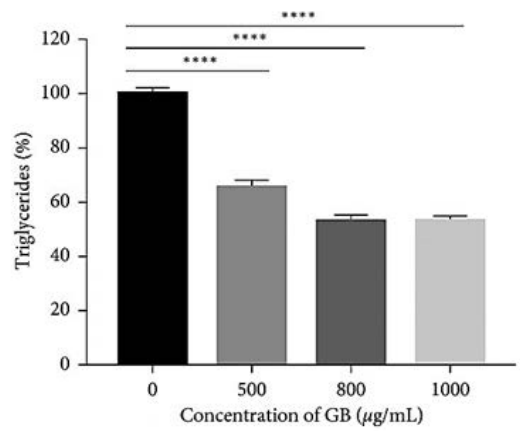

Figure 7: Green bean extract suppresses 3T3-L1 triglyceride buildup.

Processes of Fractionation and Extraction

The proportion of *P. vulgaris L.* that is extracted as a folic acid fraction (FAF). Data listed in Table 1 to 4.

Pharmacological Screening

The Impact of Bean Extracts on Lipolysis In-vitro

The capacity of bean extracts to activate lipolysis in a way is comparable to the process which takes place in a healthy organism. This was evaluated using rat adipose tissue in an *ex-vivo* environment and tested on bean extracts. After being extracted from the rats' dorsal body parts adipose tissue were rinsed with saline. Such isolated tissues were then put in an incubator containing 1-mg/mL of each extract. The measurement of lipolysis was made possible by determining the quantity of glycerol that was discharged into the medium as a result of the hydrolysis of triglycerides.

Figure 2 illustrates the outcomes of extracting green beans using water and methanol as the solvents. Both extracts, as can be shown, have a considerable impact on the pace at which lipolysis occurs ($p < 0.05$ compared to the negative control). It is important to note that both negative and positive controls have statistical significance.

Effects of Bean Extracts on Cytotoxicity

A cytotoxicity test was carried out on the cell line in advance of using the extracts to investigate their impacts on the cell line. The objective of the experiment was to determine the bare-bones minimum extract concentration that would be required to cause cell death. This step was referred to make certain that the extract do not potential risk to cells. According to the estimates of the researchers, more than 90% of the population survived. For the purpose of generating this cell viability curve, quantities of green bean extract ranging from 20 to 1000 µg/mL were utilized. In order to assess whether or not *P. vulgaris* is detrimental to cellular health, the activity of mitochondrial dehydrogenase was tested using the MTT assay.

When these experiments were conducted using the amounts of green bean extract indicated in Figure 3, it was observed that the activity of the mitochondrial dehydrogenase enzyme was not affected in any way.

Green bean extract was applied to preadipocytes derived from the mouse strain 3T3-L1 at doses ranging from 20 to 1000 µg/mL for a total of 48 hours. The results showed that the preadipocytes were able to differentiate into adipocytes (GB). The results of the MTT test were reported as a % of those obtained from cells that had not been treated in any way.

Effects of Bean Extracts on In-vitro Lipolysis

Despite the fact that green bean extract concentrations ranging from 800 to 1000 µg/mL stimulated lipolysis, with the greatest stimulation occurring at 800 µg/mL (p 0.001), the amount of glycerol released from 3T3-L1 adipocytes was increased after treatment with the extracts and isoproterenol, a nonspecific adrenergic agonist. Despite doses of green bean extract ranging from 800 to 1000 µg/mL, this was the case. Despite the fact that green bean extract concentrations ranged from 800 to 1,000 µg/mL, this was the case. (Figure 4).

Lipid Droplet Formation in 3T3-L1 Adipocytes and the Role of Bean Extract.

There is a correlation between the appearance of cells that have been stained with oil red O and the development of fat stores, which suggests that the two processes are related during the transition of preadipocytes to mature adipocytes. This is supported by the fact that there is a correlation between the two. According to the oil red O staining results, cells that had been treated with GB at one of three distinct doses formed fewer and smaller lipid droplets than control cells or cells that had not been treated during the terminal stage of differentiation (Figure 5).

On day 8 of the experiment, the mature adipocytes were stained. At this point in the experiment, the differentiation process had already been sped up. At the end of day 8^{th}, or 9^{th} days after differentiation first started, mature adipocytes were stained with GB at concentrations of 500 (b), 800 (c), and 1000 g/mL (d). After being treated with green bean extract, the cells were analysed using spectrophotometry to detect the amount of fat that had accumulated in them. As can be seen in Figure 6, the different amounts of green bean extract had no impact on the build-up of lipids. When compared to the control group, concentrations between 500 and 1000 µg/mL demonstrated a 20% reduction in the amount of fat intracellular fat deposited ($p < 0.001$). This is a significant reduction.

Extracts Effects on 3T3-L1 Adipocyte Intracellular TG

At all of the dosages that were evaluated, which varied from 500 to 1000 µg/mL, the extract of green bean beans was shown to lower the quantity of triglycerides that accumulated in the cells, as shown in Figure 7. When compared to the group without any therapy, these results were significantly better. Green bean extracts have been shown to reduce the accumulation of triglycerides by 34.4% at a concentration of 500 g/mL, 47.1% at 800 g/mL, and 50.1% at 1000 µg/mL. ($p< 0.0001$).[20]

DISCUSSION

With rise in the cardiovascular diseases current pandemic situation also has contributed as the stress induces cause of death in various countries. Because of this, it is essential to build a "new nutrition" that emphasizes the relevance of nutrient-dense food to the significance of nutrient-dense food to health and quality of life. It is of the utmost importance to encourage healthy lives and diets. It is also very important to spread awareness about the Mediterranean diet, which comprises of fresh vegetables and fruits. Fruits and vegetables, for instance, may be eaten not just for the nutrients they contain but also because they include functional foods and bioactive phytochemicals that are good for one's health. This is due to the fact that plants contain diverse assortment of important nutrients that have enormous health benefits. Being overweight or obese is another factor that might increase one's likelihood of developing cardiovascular disease or experiencing an ischemic episode. For a very long time, adipose tissue was thought of incorrectly as a straightforward energy storage method. On the other hand, recent research suggests that adipose cells may also perform autocrine, paracrine, and endocrine functions. Misconceptions such as this one lingered for a very considerable amount of time. When the body stores extra fat in a number of different sites, it releases a number of chemicals and hormones, some of which cause inflammation and others control the metabolism and the amount of food consumed. Endothelial dysfunction is the first link in the chain of events that leads to the development of cardiovascular disease. This dysfunction is a precursor to the sickness. The inflammatory cytokines tumour necrosis factor alpha (TNFα) and interleukin-2 (IL-2) play a crucial part in this process. Both of these cytokines are known as TNF-alpha. Endothelial dysfunction is the first step in the evolution of heart disease, which happens in stages cardiovascular disease (CVD). Bean extracts were used in this study to determine whether adipose tissue may be directly stimulated to initiate through the process of fat breakdown. Based on the data, it seems that bean extracts considerably increase the rate of lipolysis in isolated rat adipose tissue. According to the data obtained, the chemical or molecules responsible for the action change depending on the stage of growth the grain is in.

CONCLUSION

Diet has a vital part in lowering worldwide mortality and morbidity rates and preserving cardiovascular health. Diet also plays a role in preventing cardiovascular disease. According to the findings of this investigation, the common bean is observed to have the highest nutritional density of all of the edible legumes that have been taken into consideration here. Because of this, demand has grown not just in developing countries, where people are becoming more concerned about the quality of the food they eat, but also in nations where the economy is more secure. There have been a number of studies suggesting that a diet that is rich in beans may lower the chance of developing cardiovascular disease. The phenolic acids, flavonoids, and fatty acids contained in this legume provide preventive characteristics against various ailments. These disorders include those that impact the endothelium, inflammation, atherosclerosis, metabolic syndrome, and many more. The inhibition of lipid peroxidation and the reduction of cholesterol and blood sugar are among the most important impacts of this product on fundamental biological systems. Since it has been shown that eating beans on a daily basis may improve health and lower the chance of developing cardiovascular diseases.

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