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EXTRACTION AND EVALUATION OF CHEMICAL CONSTITUENTS OF PLANT *PHASEOLUS VULGARIS* L. FOR WEIGHT MANAGEMENT AND INHIBITION OF CARBOHYDRATE UPTAKE

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ABSTRACT: Chickpeas (*Phaseolus vulgaris* L.) contain various health-promoting bioactive components. Examples: Alkaloids, anthocyanins, catechins, carbohydrates, fibre, flavonoids, phytic acid, quercetin, saponins, steroids, tannins and terpenoids are plant components. Nevertheless, little is known about how these bioactive chemicals function in progressive situations. These results suggest that *P. vulgaris* seeds could cure metabolic diseases, including diabetes and obesity. This research offers enormous potential for nutraceuticals. Further research is needed to confirm and extend previous findings. *Phaseolus vulgaris* seeds are also needed in complementary and alternative medicine and pharmaceutical companies. Plant-derived drugs like quinine, artemisinin, digoxin, atropine, strychnine and reserpine have been demonstrated to treat many disorders. Due to their low cost, effectiveness, and safety, traditional medicine and herbal medicines provide the most primary medical care in the world's poorest countries. Any plant component can produce secondary metabolites or bioactive compounds. Glycosides, tannins, alkaloids, flavonoids, steroids, and triterpenoids have several health benefits. After testing, these plant chemicals are antibacterial, antioxidant, anticancer, and antidiabetic.

INTRODUCTION: Chickpeas are pods harvested from the *Phaseolus vulgaris* plant and then consumed by humans. They are also known as "Buncis" (derived from the Dutch term for legumes, "boontjes"). Vegetables may be anything that humans, including fruit, seeds, and leaves ingest. This vegetable truly excels when it comes to the amount of protein that it contains. Most people believe he was born in either South or Central America.

Chickpeas are a kind of legume whose plant and fruit are seen in **Fig. 1**. Chickpeas are also known as Buncis. The medicinal components of plants have been extracted and collected for use. Plants that have been used for many years in India are now the source of the raw material for twenty to thirty percent of the world's medications and synthetic duplicates of natural products.

Since, ancient times, people have been treating a wide range of ailments using natural treatments that they have found. This creature's principal source of nutrition came from plants¹⁻³. The inhabitants of India have, for a very long time, depended on a wide range of plant medicines to treat a wide variety of medical issues. This is due to India's rich cultural diversity as well as its plethora of plant species.

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Numerous studies have shown that medications derived from plants, such as quinine, artemisinin, digoxin, atropine, strychnine, and reserpine are effective in treating a broad range of diseases. Traditional medicine and herbal remedies offer the

great majority of primary medical treatment in the world's most impoverished countries because of their cheap costs, high effectiveness, and low danger levels.



FIG. 1: THE BUNCIS OR CHICKPEA PLANT AND ITS FRUIT

A plant may create secondary metabolites or bioactive chemicals in any or all of its parts. These substances include glycosides, tannins, alkaloids, flavonoids, steroids, and Triterpenoids, among others, and have been associated with a wide range of beneficial effects on human health. It has been determined that these plant compounds have antibacterial, antioxidant, anticancer, and antidiabetic activities after being put through testing. The vast majority of the bioactive plant metabolites that may be utilized to treat a wide range of conditions have been uncovered and isolated for use in medicine. The complementary actions of a plant's secondary metabolites and its bioactive constituents contribute to the efficacy of the plant's medicinal properties. It is generally accepted that secondary metabolites, sometimes referred to as phytochemicals, are the fundamental structural components of treatments and disease prevention⁴.

Therefore, looking for plants with bioactive components that may be repurposed as medications is very important. Because it is a member of the Fabaceae family, the *Phaseolus vulgaris* species, more commonly known as the Red Kidney bean or Rajma, will serve as the plant of choice for this inquiry. Plants are essential to the survival of people since they provide a wide variety of items we need. One fragrant and helpful plant has the potential to treat a wide variety of conditions and provide relief from their symptoms. When it comes to self-care, one frequent practice is the use of aromatic and therapeutic plants. People are looking

for healthier, more natural ways to treat common medical problems, which has increased the popularity of herbal therapies. There is a huge variety between medicinal and aromatic plant species regarding the chemical components that may be discovered in these plants. This changes considerably from one species to the next as well as with the progression of the plant's age. In establishing the value of medicinal and aromatic plants, one of the most important factors is the availability of sufficient plants to cure prevalent ailments. White kidney bean (*Phaseolus vulgaris*) extract is a nutrient-rich ingredient in preparing meals for the general population. White kidney bean extract can inhibit the activity of the digestive enzyme α -amylase *in-vitro* and can potentially impede the digestion of carbohydrates.

α -amylase converts maltose and glucose into saccharides that may be absorbed and used more easily. It may be found in various living things, from plants and animals to bacteria and other bacterium-like organisms. Complex carbohydrates have the potential to be more challenging to digest if human α -amylase synthesis is slowed down or stopped altogether^{5,6}.

Phytochemical Evaluation: There are several bioactive components found in chickpeas (*Phaseolus vulgaris* L.) that have been linked to a variety of health benefits. These include alkaloids, anthocyanins, carbohydrates, catechins, fibre, flavonoids, phytic acid, quercetin, saponins, steroids, tannins, terpenoids, and trypsin inhibitors.

However, a significant amount of information on the function of these bioactive compounds in progressive conditions is still lacking. According to these findings, the seeds of *P. vulgaris* might be a viable new drug for treating metabolic illnesses, including diabetes and obesity. There is little doubt that this avenue of investigation has great potential for the field of Nutraceuticals. It is necessary to do more research to validate and build upon the findings of earlier investigations. In a similar vein, the seeds of the *Phaseolus vulgaris* plant are required in a variety of businesses, including those concerned with complementary and alternative medicine as well as pharmaceuticals. High-performance liquid chromatography (HPLC) and evaporative secondary ion time-of-flight mass spectrometry (ESI-TOF-MS) were used in this research to analyze the green bean hydro-methanol extract⁷.

Research should be done on the *P. vulgaris* L. species to determine their phytochemical makeup. TOF-MS interpretations of the compounds' mass spectra were compared to previously published research to provide a basis for the characterization of the compounds (several compounds have been previously described in Fabaceae). In this work, an HPLC-ESI-TOF-MS approach was used to attempt to characterize 72 different phytochemical compounds.

There are 10 phenolic acids, 59 flavonoids, 2 lignans and iridoids included within this class. More precisely, 54 of the 72 compounds that were observed in green beans were found to be new, and the isomers of those chemicals had never been discovered previously. The HPLC-ESI-TOF-MS technique was used to analyze three different *P. vulgaris* L. types. Out of a total of 72 uniquely discovered phytochemical compounds, green beans were the source of 54 of those compounds.

These are the flavonoids that are most often seen. These results provide evidence that the phytochemical profile of a certain plant may differ depending on the species of that plant. Because new phytochemical compounds have been found, the tables that identify the components of various foods may now be improved upon and maintained up to date. The analgesic and anti-inflammatory

characteristics of the seeds of *Phaseolus vulgaris* Linn are a direct result of the presence of steroids and flavonoids in these seeds, which can be found in petroleum ether extract. The plants *Phaseolus vulgaris*, two cultivars of *Phaseolus lunatus*, *Vigna unguiculata* and *Vigna sesquipedalis*, were used in experiments⁸⁻¹⁰. This was done to classify plants in accordance with the phytochemical makeup of their tissues. The phenol content ranged from 2.84 mg/g GAE all the way up to 13.04 mg/g, with an average of 11.27 mg/g. There was a difference of 13.41 mg/g RUE in the total amount of flavonoids that was discovered in the samples, with values ranging from 2.30 mg/g RUE to 14.30 mg/g RUE. All of the extracts passed the test to determine whether or not they contained alkaloids, except for the inert *Phaseolus lunatus* white seed. In all four samples, flavonoids, saponins and terpenes were discovered in the methanol extracts. The evaluation of flavonoids included the use of seventeen bands with RF values ranging from 0.06 to 0.82¹¹.

METHODOLOGIES AND TECHNIQUES:

Collection of Phytochemical Material: *Phaseolus vulgaris* seeds were collected from an authentic vendor for the product. The same was authenticated in duecourse from the Botanical Survey of India, Koregaon Park, Pune with identification number BSI/WRC/Iden. Cer./2022/3011220026251 and specimen number YBPV-1.

Extraction: After being reduced to a powder, the seeds were reconstituted by soaking them in clean water for five hours and then cooking them for fifteen minutes (for deactivation of Phytohaemogltinin). Aqueous extraction will produce dry aqueous extract regardless of whether a cold press or hot water is used. The next step is to screen it, and then it will be dried in a vacuum. When a concentrated extract has been made, the extractive value may then be determined¹²⁻¹⁴.

Preliminary Phytochemical Screening: An exhaustive phytochemical screening was carried out in order to determine the various chemical families. In spite of the fact that physical characteristics help differentiate drugs, it is possible that these criteria do not always apply to simple molecules. To determine the physical and chemical constants of the *Phaseolus vulgaris* L. seed, were finely crushed and then air-dried¹⁵⁻¹⁸.

Test for Alkaloids:

Mayer's test: The solution was filtered after adding diluted hydrochloric acid to ethanol mixed with folic acid fraction. Filtrate was treated with Mayer's reagent.

Dragendorff's test: A pinch of ethanolic folic acid fraction solution was mixed with 2 millilitres of 2% glacial acetic acid. Filtered mixture two drops of Dragendorff's solution were then added. Orange-brown precipitate implies alkaloids¹⁶.

Hager's test: A little bit of Hager's reagent was combined with a pinch of ethanolic solution of folic acid fraction that was mixed together. The precipitate will have a yellow colour if there is alkaloid present.

Tests for Sugar and Carbohydrates:

Molisch's test: Folic acid ethanolic solution was diluted and filtered to 5 millilitres. Two millilitres of strong sulfuric acid were added after filtering the liquid. 1% ethanolic-naphthol solution was added to filtered liquid. Carbohydrates produce a violet ring at the point of contact between two non-mixing liquids¹⁷.

Fehling's test: A brick-red cuprous oxide precipitate formed after heating the ethanolic mixture containing the folic acid portion with Fehling's A&B reagent. Sugar evidence might alter sample concentration.

Benedict's test: A reddish-brown cuprous oxide precipitate was produced after adding Benedict's reagent to an ethanolic solution of folic acid fraction and allowing it to sit for a few minutes. This shows that there is a decrease in sugar content.

Tests for Glycosides:

Anthrone test: Two drops of alcohol and a sprinkle of folic acid fraction ethanolic solution were combined in a glass. The mixture's density was constant after mixing and drying the same quantity of throne. The mixture was then stirred with a drop of sulphuric acid. The components were then separated into a thin layer within a watch glass and baked in a water bath. Dark green colour from glycosides¹⁸.

Test for Anthroquinones Glycosides:

Borntrager's test: The required product was prepared by heating an ethanolic solution of folic

acid fraction with diluted sulphuric acid, filtering it while hot, and then extracting it with benzene. The organic layer separated from the mixture due to vigorous shaking. After that, diluted ammonia was added. Rose-pink staining in the ammonia layer indicated anthroquinones glycoside.

Test for Cardiac Glycosides:

Legal's test: Hydrolysis in a water bath yielded an ethanolic solution of the folic acid fraction. Two millilitres of pyridine, sodium nitroprusside and sodium hydroxide were added to hydrolyze FAF. The solution becoming orange proves cardiac glycoside's validity²⁰.

Keller-Killiani test: The folic acid fraction was successfully extracted by boiling an ethanolic solution containing 70% alcohol for three minutes, straining the liquid, and mixing it with water and lead acetate. Following this step, the filtrate is mixed with the same quantity of chloroform that was used earlier in the process to evaporate the chloroform layer. The remaining substance was then given an addition of two drops of ferric chloride and three millilitres of glacial acetic acid. We start by filling the test tube with 2 millilitres of highly concentrated sulphuric acid. After some time, the mixture took on a bluish-green colour, a tell-tale sign that it contained cardiac glycosides.

Tests for Steroids:

Liebermann's Burchard test: To make soap, folic acid dissolved in ethanol and potassium hydroxide dissolved in ethanol were mixed. The mixture was first dissolved in pet ether, and then it was water that had been distilled many times over. The pet ether extract was entirely obliterated as if it had never been. In order to do residue analysis, Lieberman-Burchard was used. The petroleum extract was first dissolved in chloroform, and then 10 drops of acetic anhydride and 2 drops of sulfuric acid were added to the mixture.

As the concentration of photosterol rose, the combination shifted from violet to blue and then finally to green²².

Salkowski test: One microlitre of concentrated sulfuric acid was added to ethanol-dissolved folic acid. Steroids resulted in skin's pinkish hue.

Tests for Tannins: Folic acid that had been dissolved in ethanol was first dried, then dissolved

in ethanol, and finally filtered. The following test is carried out in order to establish whether or not tannins are present in the filtrate:

Test with Ferric Chloride: After adding diluted ferric chloride to ethanolic folic acid fraction, the reaction mixture became dark blue. Tannins caused this end-product feature.

Test with Lead Acetate: The FAF ethanolic filtrate was subjected to treatment with a solution containing 10 % lead acetate. Tannins were present if there was any precipitate that was white in colour.

1.1.2 Tests for Proteins: After dissolving the folic acid portion in ethanol, a few millilitres of water were added to the mixture before testing it to determine the amount of protein it contained.

Million's test: An ethanolic solution of the folic acid fraction was given a few drops of Million's reagent to continue the experiment. When a colour that looked like a cross between red and brown developed, we realised there was protein in the mixture²³.

Xanthoproteic test: Two millilitres of water and a half millilitre of strong nitric acid were added to a small sample of test solution. Yellow tinge indicated protein present.

Tests for Amino Acid:

Ninhydrin test: The Ninhydrin reagent was added to the test solution in a small amount, only a few millilitres' worth. It was observed that the presence of amino acids resulted in the appearance of a bluish-purple colour.

Tests for Terpenoids:

Noller's test: In a test tube that already had a pinch of dried ethanolic solution of folic acid fraction, very little pieces of aluminium foil and 0.5 millilitres of thionyl chloride were added to the mixture. They raised the temperature in increments over time. The coloration of pink may be attributed to the presence of terpenoids²⁴.

Tests for Flavanoids:

Shinoda test: After giving the mixture a brisk stir, the ethanol was filtered after the dried FAF had been added to it. Before the filtrate was heated, a

lump of magnesium metal and concentrated hydrochloric acid were added. The magenta colour may be attributed to the presence of the flavanoids.

Tests for Saponin:

Foam test: After combining 20 milliliters of distilled water and 1 milliliter of the ethanolic solution of the folic acid fraction in a graduated cylinder, the mixture was stirred for 15 minutes. The agitation lasted for the whole duration of the experiment. The surfactant characteristics of saponin are responsible for the foaming that it produces.

Tests for Flavanoids:

Shinoda test: After a brisk stir, the ethanol was filtered after the dried FAF had been added to it. Before the filtrate was heated, a lump of magnesium metal and concentrated hydrochloric acid were added to it. The magenta colour may be attributed to the presence of the flavanoids.

Tests for Saponin:

Foam test: After agitating the ethanolic solution of the folic acid fraction for 15 minutes, it was added to 20 mL of distilled water in a graduated cylinder. Saponins surfactant qualities make it generate foam.

Test for Fixed Oils and Fats:

Spot test: A little quantity of ethanolic folic acid solution was compressed and filtered. Oil entrenched in the paper created the stain. Analyze saponification conditions. We added 0.5% N alcoholic potassium hydroxide and phenolphthalein to a little volume of extract. Then it was cooked in a water bath for another hour. It was served thereafter. Soap or alkali neutralisation might show fixed oils and fats in water. Both situations show this.

Tests for Gum and Mucilage: Ten milliliters of folic acid fraction ethanolic solution was swirled into 25 milliliters of pure alcohol. The precipitate was then filtered and air-dried. The precipitate's swelling behaviour indicated it included carbs.

Tests for Resins: A total of 0.5 millilitres of sterile water was utilized for the fraction. Water's turbidity may determine the presence of resins. To make the extract, three milliliters of an extract that was based on acetone and three milliliters of hydrochloric acid

were combined and boiled in a water bath for thirty minutes. Resins are what give the colour its characteristic pink colour²⁵.

Qualitative and Quantitative Evaluation of Chemical Constituents: It was studied by column chromatography, HPTLC, UV, IR, NMR, etc²⁶.

RESULT AND DISCUSSION:

Extraction and Fractionation: The percentage yield of Folic acid fraction (FAF) from *Phaseolus vulgaris L.* were tabulated in **Table 1**.

TABLE 1: PERCENTAGE YIELD OF THE FOLIC ACID FRACTION

Parameter	Folic acid fraction of <i>Phaseolus vulgaris L. seed</i>
Percentage yield (% w/w)	14.3% w/w
Colour	Darkbrown
Consistency	Crystalline powder

Qualitative Phytochemical Analysis: The Folic acid fraction separated from *Phaseolus vulgaris L.* seed showing the presence of primary and secondary metabolites, was tabulated in **Table 2**.

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE FOLIC ACID FRACTION

Chemical test	Folic acid fraction
Alkaloids	
Mayer's test	-
Dragendorff's test	-
Hager's test	-
Wagner's test	-
Carbohydrates	
Molish's test	+
Barfoed's test	+
Fehling's test	+
Benedict's test	+
Glycosides Anthrone test	+
Borntrager's test	+
Legal's test	+
Baljet's test	+
Keller-killiani test	+
Steroids	
Liebermann's Burchard test	+
Salkowshi test	+
Tannins	
Ferric Chloride test	-
Lead Acetate test	-
Proteins	
Biuret's test	-
Million's test	-
Xanthoproteic test	-
Amino acids	
Ninhydrin test	-
Terpenoids	+
Noller's test	-
Flavanoids	-
Shinoda test	-
Saponins	
Foam test	-
Phenolic compounds	
Ferric chloride test	+
Lead acetate solution test	+
Oils and Fats	-
Spot test	-
Saponification	-
Mucilage and gum	-

(+) indicates present, (-) indicates absent.

Estimation of Phytoconstituents: The gravimetric method was used in order to ascertain the total folic acid content of the *Phaseolus vulgaris* L. seed as well as the folic acid content of the raw pharmaceutical powder. The crude folic acid fraction recovered from the *Phaseolus vulgaris* L. seed powder had a folic acid concentration of

67.3%, much higher than the 37.8% folic acid content of the *Phaseolus vulgaris* L. seed powder itself.

Thin Layer Chromatography: The TLC studies of the Folic acid fraction of *Phaseolus vulgaris* L. seed was shown in **Table 3**.

TABLE 3: THIN LAYER CHROMATOGRAPHY OF FAF

Solvent System	No. of spots	Rf value	Detecting Agent
Isopropylalcohol: ammonia: Chloroform (8:1:1)	5	0.46	Vanillin sulphuric acid
		0.49	
		0.56	
		0.62	
		0.65	

High Performance Thin Layer Chromatography: Table 4 presents the results of an examination of folic acid performed using high-performance thin-layer chromatography (HPTLC) and an example of an HPTLC chromatogram.

The seed of *Phaseolus vulgaris* L. was used in this experiment. Using the RF values, a comparison was made between the standard folic acid IV and the folic acid fraction. Standard folic acid IV was also included in the folic acid fraction.

The folic acid subfractions had both of these constituents in its composition. At a wavelength of 254 nm, the relative fluorescence (RF) values of the folic acids that were found in the sample chromatograms were compared to the RF values that were found in the chromatograms that were obtained from the reference standard solution at varying concentrations (100-500ng/spot). The folic acids that were found in the sample chromatograms were consistent with the results.

TABLE 4: HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY OF FAF

Detecting wavelength	FAF of <i>Phaseolus vulgaris</i> L. Seed	
	No. of spots	Rf value
254nm	07	0.040
		0.460
		0.560
		0.640
		0.730
		0.780
		0.870

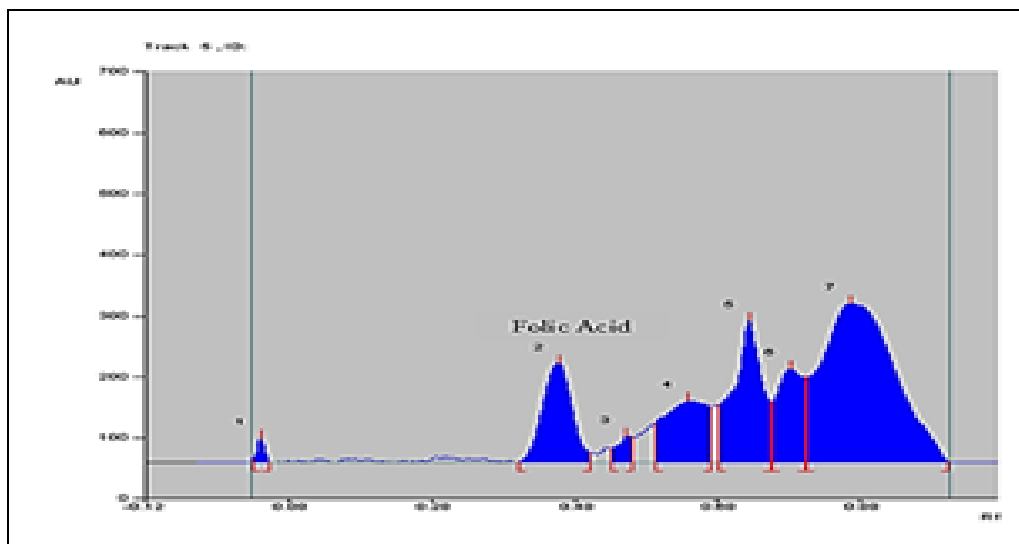


FIG. 2: TLC FINGERPRINT ANALYSIS OF FAF OF PHASEOLUS VULGARIS L. SEED

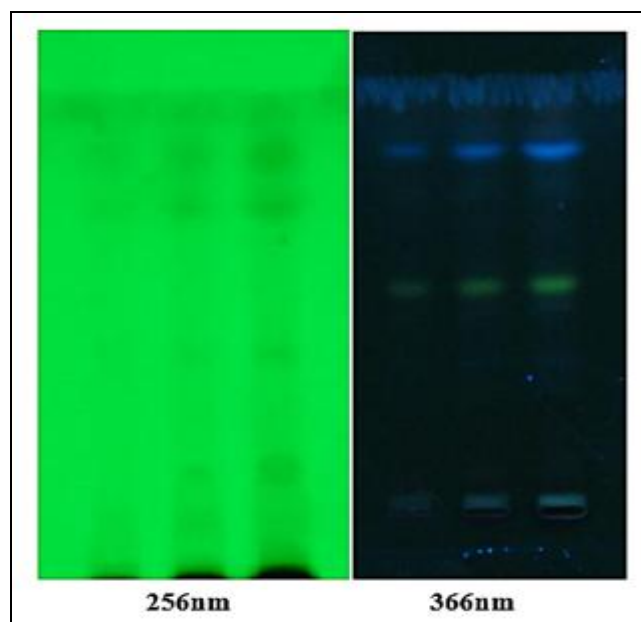


FIG. 3: HPTLC CHROMATOGRAM OF FAF OF *PHASEOLUS VULGARIS L.* SEED

CONCLUSION: When taken together, these traits have the potential to assist in the identification of pharmaceuticals whose physical or chemical qualities have been altered as a result of incorrect storage. To determine the physicochemical constants, a wide variety of solvents were investigated. Solubilizes comprised ash that was insoluble in acid, ash that could be dissolved in water, complete ash, and acid-soluble ash. This resulted in the finding of values that could be extracted.

This adds to the process of establishing pharmacopoeial standards for various drugs. A plant's "ash content" is calculated by determining what proportion of the plant's initial mass is left over as ash after it has been burned. This instrument is used to measure inorganic salts. The look of the basic medicine is not considered when determining whether or not inorganic salts were added to it to enhance its appearance.

The ash value was determined by adding the ash that was resistant to acid, the ash that was soluble in water, and the total ash. Examining the overall quantity of ash might help you determine how much was consumed in the burning process. Ash that the plant itself creates is referred to as "physiological ash," whereas ash formed by non-living particles that adhere to the plant's surface is referred to as "non-physiological ash." Silica may be found in total ash as well as acid-insoluble ash.

A high proportion of sand in the soil is another quality indicator of its silica content. Reducing the amount of ash that might dissolve in water. You are welcome to use the weight of your ash pile as an indication. Ash that is water-soluble is ash that may be disposed of by flushing it down the toilet. It is possible to determine the mass of anything by counting the amount of ashes it contains. Because it was made by grinding up seed and bark, ash naturally included some sulphate. If there is a disparity between the quantities of ash displayed and found, this might indicate that the drug has been tampered with. The extractive values of a medication may show the drug's authentic constituents and any adulteration that may have occurred. *Phaseolus vulgaris L.* powder. It would seem from the data that the seed may be dissolved in alcoholic drinks.

At the very least one individual had a look at the developing embryo. The chemical components of the medication in its unprocessed, air-dried state will tell you how much of it there is. It is essential to have a good understanding of the amount of water plants possess and the amount of water they lose via evaporation. Just 3.77 percent of a seed is comprised of water. Consequently, the growth of yeast, fungus, and bacteria is inhibited, as is their ability to absorb nutrients. It is possible to determine the nutritional value of the seeds produced by the *Phaseolus vulgaris L.* plant by analyzing the amount of coarse fibre the seeds contain. There are many different chemicals, each emitting a unique fluorescence pattern that may or may not be visible. Because of their very similar outward characteristics, it is easy to determine which plant species belong to which family. The powdered *Phaseolus vulgaris L.* plant becomes a dark emerald colour when it is subjected to methanol and ultraviolet radiation with a wavelength of 254 nanometres. The existence of chormophore in the sample may be deduced from this fact.

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