

## Total Chlorogenic acids standardized to more effective and absorptive form and branded as PureBeanFit®

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**ABSTRACT:** Coffee is a member of the genus *Coffea* and of the family Rubiaceae. Due to its antidiabetic activity, numerous investigations have been conducted. Green coffee is known to contain a wide range of active phytochemicals, including proteins, phenols, diterpenes, polysaccharides, melanoids, and lipids. Caffeine and Chlorogenic acid are significant chemical components. *Coffea robusta* is the primary species with the highest concentration of chlorogenic acid among all the species. Green coffee has several health benefits because of its diverse therapeutic and pharmacological action, drug interaction, and dosage. Initially, its good effects were linked to caffeine, but these have increased due to the drug's rising detrimental effects and interactions with other substances. PureBeanFit® is developed by Biomed ingredients. This product has been standardized to Total Chlorogenic acids 45%, enriched with higher antioxidant ingredients, which would serve as a major nutraceutical for health benefits such as antidiabetic, weight loss, and antioxidants. This product has wide applications which includes beverage, bakery, confectionary, vitamin pre-mixes, nutraceuticals and dietary supplements.

**KEYWORDS:** Green Coffee Extract, Coffee arabica, Chlorogenic acids, antidiabetic, weight loss and antioxidants.

### I. INTRODUCTION:

The most widely consumed beverage in the world, after water, is coffee. Eight million tonnes of coffee are consumed annually, or about 255 kg per second, worldwide. (35). Coffee is a member of the genus *Coffea* and of the family Rubiaceae. Over 80 different species of coffee have been identified worldwide. (17). The quality of the finished coffee beverage can be significantly impacted by the quantity and make-up of taste

precursors in green coffee beans. Just two of the 80 species of coffee are significant both commercially and medicinally. Arabica coffee is also known as *Coffea arabica*, and robusta coffee is also known as *Coffea canephora* var. *robusta*. But in the world coffee market, *Coffea arabica* is the most well-known variety. (36). The primary crop is grown in India, Brazil, Vietnam, Colombia, and Indonesia. Brazil is the world's largest producer of coffee. (20). Coffee contains around 700 components that give it its distinct and fragrant flavors. (34). Coffee growth is also influenced by temperature and height. Arabica coffee requires an altitude of 1000 to 2100 meters and a temperature of 18 to 22 degrees Celsius for growth, while robusta coffee requires an altitude of 100 to 1000 meters and a temperature of 22 to 26 degrees Celsius. (59,6). Green coffee extract is traditionally extracted from green coffee beans using alcohol as a solvent.

The evergreen plant has a maximum height of 10 meters. It is composed of a main stem from which secondary branches develop, and its dark green leaves are arranged in an opposing decussate arrangement. The fruits, called cherries or berries, contain the plant's seeds. The shape of the seeds is elliptical. [25, 29, 64]. Green coffee beans, along with several fruits and vegetables, are among the richest dietary sources of chlorogenic acid, an ester of caffeic acid with quinic acid, one of the most prevalent polyphenols. (18). There are reports on the antioxidant and antitumor effects of coffee extracts. (39, 53, 63). Robusta samples had much more reducing chemicals than Arabica samples, according to research of Robusta and Arabica coffees from six different nations. Additionally, roasted coffee had significantly higher *ex vivo* protective effect. (19). Numerous biological activities of green coffee beans have been reported, including the prevention of type II diabetes, the reduction of obesity, the improvement

of cardiovascular parameters, the reduction of the risk of chronic hepatic disorders, the reduction of oxidative stress, and the presence of neuroprotective, antitumor, antioxidant, anti-inflammatory, and antimicrobial effects. [49,44, 11, 62 ]14 .

Hormone secretions and glucose tolerance in humans can also be modified by the green coffee extract (37). A lower risk of diabetes, liver disease, and cancer is associated with the antioxidant activity of the green coffee extract. A different therapy for Parkinson's disease includes green coffee extract. (8,9). Additionally, there is a blood pressure-lowering effect of green coffee extract. (58).

**PureBeanFit<sup>®</sup>** is a branded product by Bio-Med Ingredients Pvt.Ltd, for Green Coffee Extract. Green coffee is unroasted coffee beans from *Coffea* fruits (*Coffea arabica*, *Coffea canephora*). It contains higher levels of chlorogenic acid than regular roasted coffee.

The chlorogenic acid in green coffee is thought to have health benefits. It might affect blood vessels so that blood pressure is reduced. It might also affect how the body handles blood sugar and metabolism. Green coffee also contains caffeine, but in lower amounts than regular coffee. **PureBeanFit<sup>®</sup>** is derived from *Coffea arabica*, belongs to Rubiaceae, the plant part used is beans, with trademark number 6131987, the recommended daily dose is 500 mg twice a day, shelf life of 3 years, it is soluble in water, the application includes, beverage, bakery, confectionary, vitamin premixes and dietary supplements. This product is enriched with antioxidants and vitamin C, which enhances the efficacy of the active ingredients. Hence it has more health benefits than the regular coffee bean extracts which are available in the market.

**PureBeanFit<sup>®</sup>** is standardized with green coffee bean extract, it aids in dealing with Weight loss, diabetes, high blood pressure, due to the presence of chlorogenic acid content. Total Chlorogenic acids, which is a more bioavailable form than the normal extracts, which enhances the efficacy of the ingredient. This product is used in weight loss, antioxidants, immunity booster and antidiabetic.



**Figure 1:Green Coffee Plant**

## PHARMACOLOGICAL EFFECTS OF GREEN COFFEE BEAN

### Decrease in blood glucose level (Type 2 diabetes)

Sarriá et al. (55) anticipate that 366 million people worldwide would have Type 2 diabetes by 2030, indicating an increasing global prevalence of the condition. Green coffee contains caffeine, which is an activator of mitochondria and a glucose stimulant. When trigonellindentrit is coupled with caffeine, oxidative stress is decreased, and axon regeneration is encouraged. (42). On the other hand, glucose homeostasis and the reduction of oxidative stress depend on chlorogenic acid.(66) In rats given a high-fat diet, green coffee extract has been shown to have anti-diabetic properties, lower triglyceride, glucose, and oxidized glutathione levels, shield cells from oxidative stress, and even help prevent and treat type 2 diabetes.(45)

### Hepatic Disorders

Based on epidemiological data, coffee use may help prevent liver cirrhosis and other chronic illnesses.(42) It has been demonstrated that coffee consumption is negatively connected with serum gamma glutamyl transferase and alanine aminotransferase (ALT) activity. (38).

### Figure 2:Effects of Green Coffee Extract

### Reduction in obesity and weight

Obesity is defined as an abnormal accumulation of body fat, or an excess of body fat based on internationally established criteria. (1). Obesity is considered as a severe public health issue worldwide (16). Obesity and overweight individuals with chronic illnesses such as diabetes, non-alcoholic fatty liver disease (NAFLD), coronary artery disease, hypertension, malignancies, and mental health issues have been

associated. Potential beneficial biological effects of consuming GCE and CGA include inhibiting fat accumulation, perhaps lowering body weight and BMI (31), lowering blood pressure (48), and modulating postprandial glucose metabolism through decreased intestinal absorption. The liver is stimulated by the green coffee beans, which results in increased bile production, accelerated metabolism, and systemic release of glucose (47). By freeing fatty acids from body fat that has been accumulated, caffeine also decreases the absorption of fat. Weight loss results from CGA's improved hepatic triglyceride levels and improved fatty acid processing in the liver (54). Extract from green coffee beans lowers hepatic triglyceride levels, and chlorogenic acid inhibits the absorption of fat (22). Hepatic carnitine palmitoyl transferase (CPT) activity is elevated by other phenolic components found in green coffee beans, including feruloylquinic acid and neochlorogenic acid (57). The anti-obesity effects, which also restrict the formation of triglycerides in the liver, are mediated by modifications in plasma adipokine levels, body fat distribution, and the regulation of fatty acid oxidation and cholesterol biosynthesis as well as the upregulation of PPAR expression and fatty acid biosynthesis in the liver (13).

#### Antioxidant effect

Chlorogenic acid is a phenolic compound found in green coffee that has the ability to trap hydroxyl or superoxide anions and has antioxidant qualities.(28) Among its many health advantages are its capacity to scavenge free radicals in vitro and prevent the progression of oxidative processes (12). Reactive oxygen species can be effectively scavenged by chlorogenic acids.(4)ROS are naturally produced by a variety of biological processes, including aerobic metabolism, and they ought to be dangerous when present in sufficient quantities.(3) Despite their known negative effects, ROS are necessary for redox cell signaling, which keeps cells in a state of homeostasis (50).

#### Anti-cancer effects

The International Agency for Research on Cancer determined in 2016 that coffee does not cause cancer in humans.(32) Numerous investigations have discovered a connection between coffee consumption and a decreased risk of some cancers(14)(56). Many studies on the anti-cancer properties of coffee have shown that kahweol is one of the main compounds involved in cancer chemoprevention.(15) By eliminating

reactive oxygen species and activating hemoxygenase-1 to control intracellular reactive oxygen species (ROS), the antioxidant kahweol shields DNA from oxidative damage brought on by hydrogen peroxides (10).(40, 51, and 60).-20

#### STRUCTURE & PROPERTIES:

##### Chlorogenic Acids

CGAs are phenolic compounds that are produced by esterifying cinnamic acids with (-)-quinic acid. Examples of these compounds are caffeic, ferulic, and p-coumaric acids. The primary hydroxycinnamic acids in green coffee bean extract are 3-, 4-, and 5-caffeoylquinic acids (3-, 4-, and 5-CQA), 3-, 4-, and 5-feruloylquinic acids (3-, 4-, and 5-FQA), and 3,4-, 3,5-, and 4,5-dicaffeoylquinic acids (3,4-, 3,5-, and 4,5-DCQA) (30). Theobromine, theophylline, and methylxanthines-caffeine (CAF) are additional bioactive ingredients in green coffee (33). Whereas *C. canephora* (robusta) has 5.17% -- 14.4% chlorogenic acid, *C. arabica* has 3.40% -- 7.24% (66).

{Green coffee beans, along with some fruits and vegetables, are among the foods that are highest in chlorogenic acids, one of the most prevalent polyphenols. Chlorogenic acids, like other polyphenols, have been demonstrated to support green coffee bean extracts' anti-inflammatory, antipyretic, antioxidant, and antitumor effects. (64)

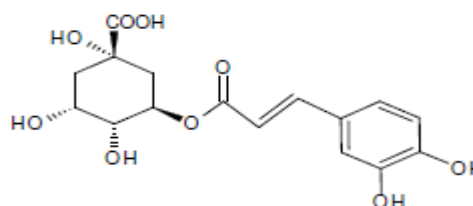


Figure 3:Structure of Chlorogenic Acids

##### Caffeine content

Among the purine alkaloidal components found in green coffee beans, caffeine (1, 3, 7-trimethylxanthine) makes up 99.78 percent of the total methylxanthines. As green coffee beans mature, their caffeine content remains constant. Due to its thermostability, excessive roasting of coffee beans cannot eliminate or lower its content (46). After roasting, proteins, carbohydrates, trigonelline, CGA, and fat can either be conserved or transformed into reactive compounds. Additionally present in trace levels are theophylline, theobromine, paraxanthine, libertine,

and methylxanthines. The caffeine content of Robusta coffee ranges from 1.7% to 4.0% (w/w), whereas Arabica coffee has a concentration of 0.8% to 1.4% (w/w) (5). Coffee's primary psychoactive ingredient is caffeine, an adenosine antagonist with stimulant properties. Its psychedelic qualities may be attributed to the presence of monoamine oxidase inhibitors like harman and carboline (52). Among the diterpenes present in green coffee are cafestol, kahweol, 16-O-methylcafestol, cafestal, and kahweal. About 20% of the lipid component is made up of them. Saturated long-chain fatty acids (LCFAs) in coffee oil esterify diterpenes (27).

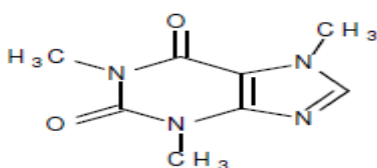


Figure 4: Caffeine structure

### PHARMACOKINETICS OF CHLOROGENIC ACIDS

Isoferulic, ferulic, and caffeic acid comprise the majority of CGA metabolites, which are detectable in blood circulation. One-third of the consumed or ingested CGAs in foods and beverages are physiologically absorbed by the small intestine and may be quantified by HPLC as 5-CQA, 4-CQA, and 3-CQA in plasma. The remaining two thirds are moved into the large intestine, where gastrointestinal bacteria significantly metabolizes and absorbs the phenolic acid. Biochemical reactions within the small intestine break down quinic acid into feruloyl quinic acid (FQA) and caffeine (CQA), with the subsequent release of ferulic acid and caffeic acid. Both the conversion of ferulic and caffeic acids into dihydroferulic acid and its absorption are significantly influenced by the colon. [23, 24]-24

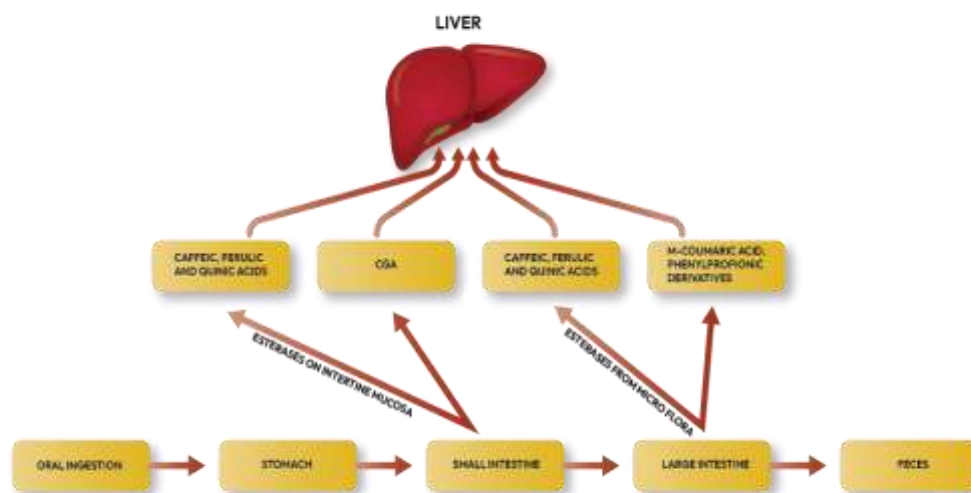


Figure 5: Pharmacokinetics of Green Coffee Extract

### Extraction

After sterilizing green coffee seeds for 20 minutes at 120 degrees Celsius, the seeds were flaked to produce flakes with a thickness of 0.5 to 1.0 mm. Using the Soxhlet device, coffee flakes were extracted using hexane. To reduce the moisture content to around 10%, these flakes were dried for two to three hours at  $55 \pm 5$  C using a

crossflow drier. The arid flakes were run through a hammer mill with a 30 mesh (500  $\mu$ m) screen to create a powder that served as the starting point for making the conserve. Each of these 100 g samples was placed into a different glass column and extracted at a material to solvent ratio of 1:10 using certain solvents. For extraction, solvent solutions including isopropanol and water in the ratios of



80:20, 70:30, and 60:40 were utilized. Over the course of five hours, ten 100 ml additions of solvent were applied, allowing for a 30-minute contact time. After draining, the extracts were combined. Pooled extracts were subjected to a

reduced pressure (40 millibar) distillation process at 50 C on a rotavapor, with the final product being kept at 4 C. Coffee constituent recovery was calculated using the yield of conserves, polyphenol content, and percentage of CGA. (41)



**Figure 6: Green Coffee Beans**



**Figure 7: Green Coffee Extract 45% CGA**



**Figure 8: PureBeanFit®**

#### Analysis by HPLC

The HPLC system utilized a Shimadzu LC-20AD with photodiode array (PDA) detection (Shimadzu SPD-M20A) at 325 nm for CGAs and caffeine at 275 nm. Chromatographic separation was achieved by C-18 column (4.6mm· 250 mm) with a pore size of 5  $\mu$ m. The 5-CQA standard was analyzed with a 12.5- 13 min gradient using the following conditions: (A) 0.1% formic acid in water; (B) 0.1% formic acid in acetonitrile; gradient elution, time 0 at 5% B to 12.5 min at 10% B then hold for 10 min at a flow rate of 1 mL/min. The retention time of the 5-CQA standard was 18 min. CGA and related compounds were first identified by the order of elution 13 and later by liquid chromatography (LC)/mass spectrometry (MS). All CGA had a similar ultraviolet (UV)-

visible spectrum by PDA detection. For the HPLC, the sample were analyzed using a batch analysis program at a flow rate of 1mL/min using 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with gradient elution as follows: 0min (5% B), 12.5min (10% B), 22.5min (10% B), and 60min (30% B).

**Reference Standard preparation:** Weigh 0.2mg/ml of USP chlorogenic acid RS in solvent.

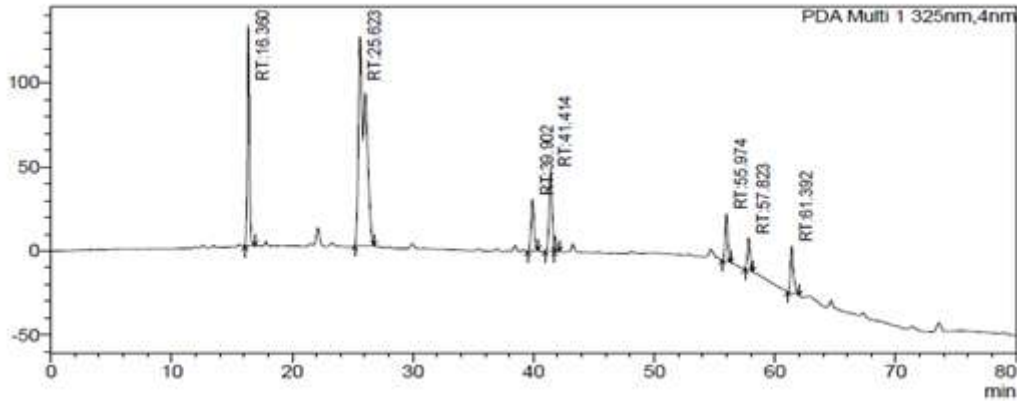
**Working Standard preparation:** Accurately weigh 10mg of working standard into a 25ml volumetric flask and add 15ml of solvent. Sonicate the sample for 10mins, cool to room temperature. Dilute with solvent and mix.

Before injection pass through filter 0.45-  $\mu$ m.

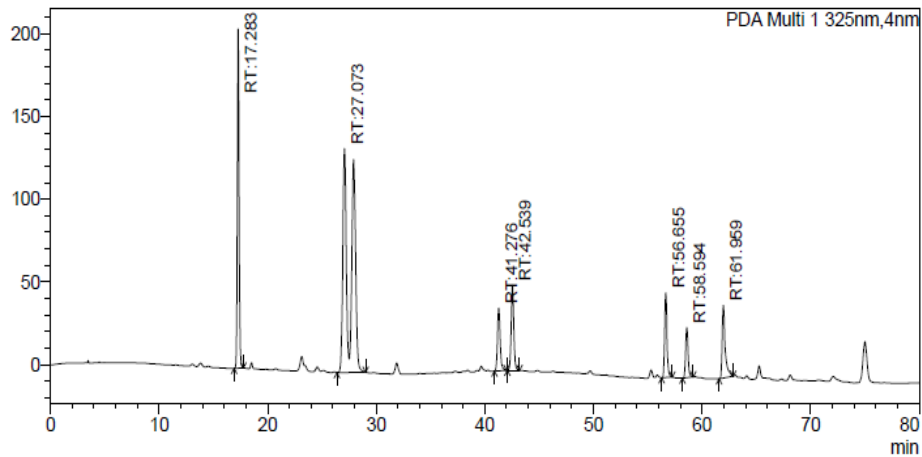
**Sample preparation:** Repeat the procedure given for working standard preparation for sample preparation. Before injection, pass through 0.45-

µm, discarding the first few milliliters of the filtrate.

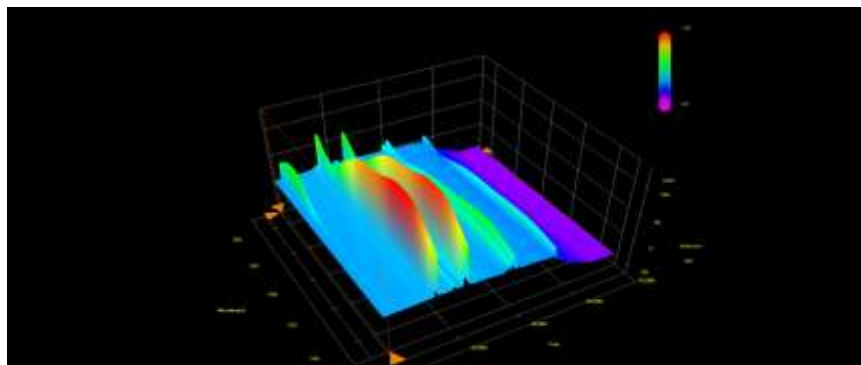
The sample was analyzed at 325 nm with a flow rate of 1.0ml/min.



**Figure 9:Chromatogram of Green coffee bean extract 45% CGA content.**



**Figure 10:Chromatogram of PureBeanFit® CGA content.**



**Figure 11:3D chromatogram of Green Coffee Bean extract 45% CGA**

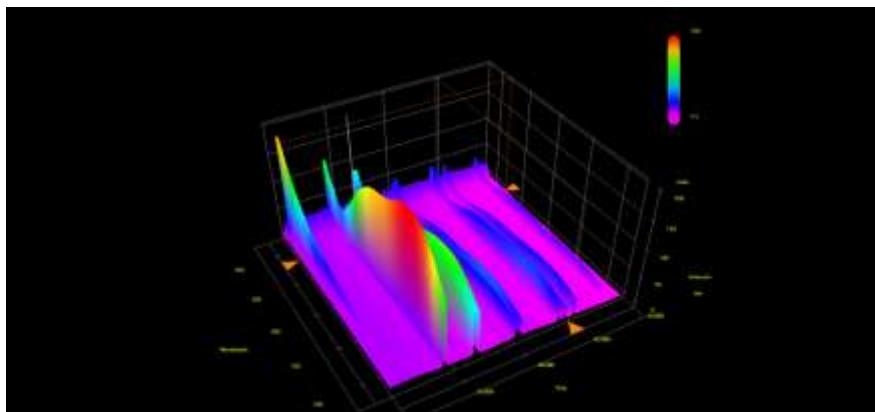


Figure 12:3D chromatogram of PureBeanFit®

## II. CONCLUSION:

**PureBeanFit®** has been developed by Bio-Med Ingredients and it contains active constituents which are known to biologically act as antioxidant and a potential nutraceutical for antidiabetic anti-hypertension, weight loss and other potential health benefits. The formulation is likely known to have higher Bio-absorptivity than regular marketed green coffee extract .Therefore this makes it more Bio available .

This product is used in weight loss, antioxidant, immunity booster and antidiabetic. The total chlorogenic acids in green coffee are thought to have health benefits. It might affect blood vessels so that blood pressure is reduced. It might also affect how the body handles blood sugar and metabolism. Green coffee also contains caffeine, but in lower amounts than regular coffee. It aids in dealing with Weight loss, diabetes, high blood pressure, due to the presence of chlorogenic acid content. Usually chlorogenic acid acts as scavengers , that liberate oxygen species that are generated by consumption of high fat diet .Hence, it suppresses ,the expression of inflammation and consequently reduces fat accumulation & weight gain .This is achieved as chlorogenic acids act on the LDL cholesterol in the body. Vitamin C obtained from Acerola cherry extract, which is a natural source, helps in maintaining the overall immunity .It is an antioxidant as it helps to absorb minerals in the body. Thereby ,the mechanism of action of vitamin C, that keeps the chlorogenic acid in active mode.

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