

Bioactive α -Amylase Inhibitors from *Phaseolus vulgaris*: Advances in Extraction, Structural Characterization, and Clinical Applications in Obesity Management

Nayab Aslam¹, Iman Aslam^{2,3}, Umm-E-Habiba⁴, Rida Arshad⁴, Faiza Zafar⁴, Sana Saleem⁴, Urooj Fatima⁴, Duaa Shakeel⁴, Momina Tariq⁴, María Rosa Bernal-López^{2,3,5}, Imran Mukhtar^{4*}

¹School of Food and Biological Engineering, Engineering Research Centre of Bio-process of Ministry of Education, Key Laboratory for Agricultural Products Processing of Anhui Province, Hefei University of Technology, Hefei-230009, China

²Unidad de Gestión Clínica de Medicina Interna, Hospital Regional Universitario de Málaga, Universidad de Málaga (UMA), 29010 Málaga, Spain

³Instituto de Investigación Biomédica de Málaga (IBIMA-Plataforma Bionand), 29590 Málaga, Spain

⁴Gut Microbiome Biostructural Research group (GMBR group), Gut Microbiome Metabolic Physiology Research Lab (GMPRL), Department of Physiology, Government College University, Faisalabad-38000, Pakistan

⁵Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III, 28029 Madrid, Spain

*Correspondence: drimranmukhtar@gcuf.edu.pk

Article Info

Keywords:

α -Amylase inhibitor;
Phaseolus vulgaris, Glycemic control, Weight management, Protein purification.

Date:

Received: 15 February 2026

Accepted: 01 June 2026

Published: 15 June 2026

Volume: 06, 2026



Copyright: © 2026 by the authors. This article is distributed under the Creative Commons Attribution (CC BY) license, allowing for open access and sharing under specified terms and conditions. (<https://creativecommons.org/licenses/by/4.0/>).

Abstract

The global prevalence of obesity has nearly tripled since 1975, now affecting an estimated 650 million adults and necessitating the urgent development of sustainable, evidence-based metabolic interventions. Functional dietary agents, specifically the α -amylase inhibitors sequestered from *Phaseolus vulgaris* (white kidney bean), occupy a critical niche at the intersection of molecular biochemistry and clinical therapeutics. By translating fundamental enzymatic kinetics into viable nutritional applications, these extracts offer a validated, non-pharmacological pathway to modulate body mass index (BMI) and mitigate chronic metabolic risks. This review synthesizes current data regarding the heterotetrameric $\alpha_2\beta_2$ configuration of the α -AII isoform while evaluating four primary purification methodologies: pH-modulation, ammonium sulfate fractionation, ethanol precipitation, and ion-exchange chromatography. Mechanistically, the inhibitory potency of α -AII is dictated by the non-covalent occlusion of the enzyme's catalytic triad (Asp197, Glu233, and Asp300), a process mediated by the strategic orientation of the L1 and L2 hairpin loops. High-resolution biochemical profiling identifies the inhibitor as a 36–43 kDa glycoprotein composed of approximately 88.2% protein. Furthermore, clinical meta-analyses demonstrate that standardized supplementation (1,500–3,000 mg/day) effectively attenuates postprandial glycemic excursions and facilitates measurable adipose reduction, averaging a 2.93 kg weight loss over 30 days. Consequently, *P. vulgaris* extracts serve as a high-safety-index therapeutic for metabolic regulation, though future optimizations must prioritize green extraction technologies to preserve the bioactivity of specific isoforms. This review is to provide a comprehensive structural and clinical evaluation of *Phaseolus vulgaris* α -amylase inhibitors as a standardized intervention for obesity and glycemic management.

How to cite: Aslam N, Aslam I, Habiba U, Arshad R, Zafar F, Saleem S, Fatima U, Shakeel D, Tariq M, Bernal-López MR, Mukhtar I, 2026. Bioactive α -Amylase Inhibitors from *Phaseolus vulgaris*: Advances in Extraction, Structural Characterization, and Clinical Applications in Obesity Management, *Frontiers in Microbiology and Biotechnology* 6, 1-13. DOI: <https://doi.org/10.54219/fmb.06.01.2026.485>

Introduction

Current epidemiological data identify obesity and overweight as two of the most critical global health challenges, ranking as the fifth primary risk factor for mortality worldwide (EASO). According to the World Health Organization (WHO) estimates, the global burden reached over 1.9 billion overweight adults in 2016, with more than 650 million individuals meeting the clinical criteria for obesity (Salam et al., 2023). The pediatric and adolescent populations are also significantly impacted by this crisis; in 2016, more than 340 million individuals aged 5–19 were classified as either overweight or obese. This reflects a staggering 14% increase in prevalence since 1975. Currently, global epidemiological trends indicate that the number of individuals living with obesity exceeds those who are underweight in nearly every geographical region, except for specific areas within sub-Saharan Africa and Asia (Peddio et al., 2022).

The pathophysiology of obesity is intricately linked to a spectrum of comorbid conditions, most notably type 2 diabetes mellitus (T2DM), chronic cardiovascular disorders, and a diverse range of malignancies, including colorectal and breast carcinomas. Type 2 diabetes can be brought on by an unhealthy lifestyle and weight, regardless of genetic risk (Misra et al., 2025). Various dietary interventions and nutritional frameworks, characterized by fluctuating macronutrient ratios of proteins, lipids, and carbohydrates, have been advocated for the management of body weight (Iqbal et al., 2015). Recently, the restriction of dietary carbohydrates, specifically refined and processed variants, has gained significant traction as a primary weight-loss intervention (Iqbal et al., 2016; Iqbal et al., 2021; Chandrakanth et al., 2025). Since carbohydrates represent the foundational caloric intake in the majority of global diets, the strategic inhibition or attenuation of starch hydrolysis is considered a potent pharmacological and nutritional target for weight control (Bhujle et al., 2025). In this context, *Phaseolus vulgaris* (white kidney bean) has emerged as a focal point of scientific inquiry due to its high concentration of bioactive alpha amylase inhibitors, which facilitate weight management by modulating carbohydrate digestion (Jiang et al., 2024).

White kidney beans, scientifically known as *Phaseolus vulgaris* L., are a subspecies of the common bean. The white kidney bean has a high concentration of bioactive compounds such as peptides, polyphenols, oligosaccharides, and lectins, and contains between 22 and 27 % protein by seed weight. The high concentration of lectins caused some concern because these peptides might act as toxins and allergens in addition to their purported anti-cancer and anti-obesity effects. Consumption of white kidney beans has

been linked to some cases of clinical illness (Petrovičova et al., 2024).

Phaseolus vulgaris extracts (PVE) have been created to isolate the action of phaseolin, a -amylase inhibitor (-AI) capable of non-covalently interfering with the breakdown of complex carbohydrates, resulting in decreased absorption of these nutrients through the gut wall. PVE has antioxidant, anticarcinogenic, anti-inflammatory, glycemic-reducing, and cardioprotective characteristics, as well as the ability to cause weight loss (Ganesan & Xu, 2017). The extracted protein is rich in cysteine, serine, leucine, threonine, glutamic acid, and aspartic acid, with a molecular weight of about 36,000 kDa. The α -amylase inhibitor contains a polypeptide part of about 88.2%, and 11.8% is carbohydrate in nature. The enzyme commonly contains 3 different isoforms named α -AIL, α -A1, and α -A12. All three forms of α -amylase inhibitors have 50-90% similarity in their structure. In contrast, the nucleotide sequence indicates that the similarity index ranges from 40% to 53% (Kasar et al., 2022). The enzyme is observed to be in cotyledons and embryonic axes inside the seedlings, while absent in other plant body organs. The inhibitor contains two primary components (α and β), which are linked by the β -elimination reaction, creating glycopeptides.

The second isoform α -AI2 of α -amylase inhibitor is observed to have anti-amylase activity among humans. The α -amylase inhibitor is commonly inactive against various plant α -amylase enzymes and is classified as an anti-feeding or seed defense protein (Singh et al., 2025). An evolutionary relationship between α -amylase and lectins proved influentially that some amino acid sequences were (Ćorković et al., 2022) deleted in this inhibitor used the docking techniques were used to determine the additional loops and occurrence of an extra glycan chain, and the non-existence of proteolytic activity in PHA is basically accountable for the absence of inhibitory action in subtype α -AI1 of α -amylase inhibitor. The presence of an additional loop also creates steric hindrance, which prohibits the inhibitor enzyme from further functioning at the active site (Martinez-Gonzalez et al., 2019; Zhang, 2016).

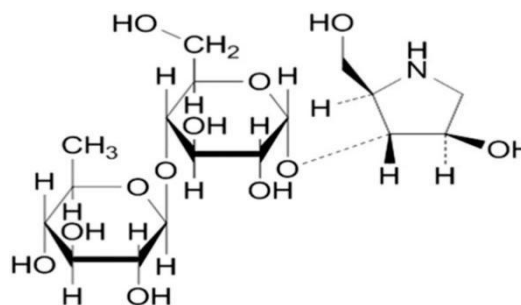


Figure 1. Structure of the α -amylase inhibitor

As illustrated in Figure 1, the α -amylase inhibitor is a **heterodimeric glycoprotein** (molecular weight of ~ 36 kDa). The structure is characterized by a complex of α and β subunits that form a specific globular fold. This protein structure allows for non-covalent, high-affinity binding to the active site of human pancreatic α -amylase, creating a steric hindrance that prevents the enzyme from accessing starch molecules.

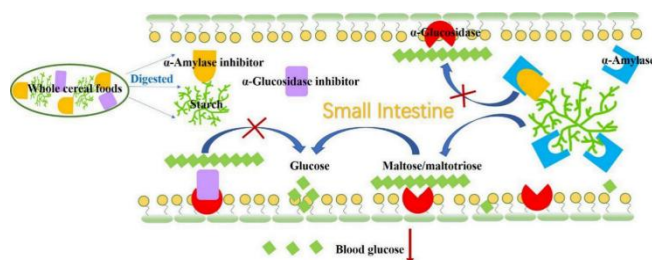


Figure 2. Action mechanism of α -amylase in the human body (Gong et al., 2020)

Figure 2 illustrates the mechanism by which α -amylase inhibitors modulate carbohydrate metabolism within the small intestine. Specifically, the inhibitor binds to the enzyme, preventing the hydrolysis of starch into smaller oligosaccharides like maltose and maltotriose. Consequently, complex carbohydrates remain undigested and pass through the gastrointestinal tract, reducing the net glucose absorption into the bloodstream (Gong et al., 2020).

Various nutritional strategies have been developed to address the global obesity epidemic and its role in the pathogenesis of insulin resistance and type 2 diabetes mellitus (T2DM). While calorie restriction and increased fiber intake are conventional approaches, they often face challenges such as low patient compliance and gastrointestinal discomfort, including flatulence and diarrhea (de Toro-Martin et al., 2017). Therefore, the use of bioactive compounds to inhibit key digestive enzymes, specifically α -amylase and α -glucosidase, presents a more targeted therapeutic pathway. By delaying or preventing the conversion of poly- and oligosaccharides into absorbable monosaccharides, these inhibitors effectively manage postprandial glycemic levels and support weight management (Udani et al., 2018).

This review provides a comprehensive synthesis of the biochemical properties and therapeutic potential of *Phaseolus vulgaris* α -amylase inhibitors. By critically evaluating recent advancements in extraction protocols alongside modern clinical trial outcomes, this work identifies the most effective strategies for utilizing bean-derived bioactive compounds in obesity management. Furthermore, it addresses current research gaps and provides

a strategic framework for the future development of high-potency, plant-based metabolic interventions

Application of α -amylase Inhibitor

The primary clinical application of α -amylase inhibitors from *Phaseolus vulgaris* is as a specialized dietary supplement for weight management. By facilitating the "starch-blocker" effect, these inhibitors prevent the enzymatic hydrolysis of complex carbohydrates in the small intestine, allowing undigested starch to transit into the large intestine. This process mimics the metabolic benefits of a low-glycemic-index diet (Peddio et al., 2022).

Metabolic Impact and Lipid Mobilization

The physiological impact of α -amylase inhibitors extends beyond simple caloric restriction. When undigested starch reaches the distal ileum and colon, it undergoes bacterial fermentation, which can be measured via breath hydrogen analysis, an indicator of carbohydrate malabsorption rather than a direct "fat-melting" mechanism. This process is associated with a reduction in net energy absorption of approximately 20% to 50% from starch sources. The resulting energy deficit necessitates the mobilization of endogenous lipid stores to meet metabolic demands, leading to a reduction in adipose tissue and circulating triglycerides (Dhital et al., 2017; Mohiuddin et al., 2025).

Evaluation of Clinical Trial Data

In a randomized clinical study involving 60 subjects with obesity (excess body weight between 5–15 kg), the efficacy of a 445 mg daily dose of α -amylase was evaluated over 30 days. Participants consumed a carbohydrate-rich diet (2000–2200 kcal/day). The treatment group demonstrated significant reductions in total body weight, adipose tissue mass, and anthropometric measurements (waist, hip, and thigh circumference). On average, the test group lost 2.93 kg compared to 0.35 kg in the placebo group. Furthermore, the test group showed a decrease in Body Mass Index (BMI) from 25.9 ± 2.0 to 24.9 ± 1.9 and a 10.45% reduction in body fat, while the placebo group remained relatively stagnant. Notably, these clinical outcomes were achieved without significant adverse effects (Celleno et al., 2007).

Synergistic Effects and Long-term Clinical Outcomes

In a double-blind study involving 40 participants with obesity, a combination of *Phaseolus vulgaris* extract (200 mg), and *Garcinia cambogia* (50 mg) was administered three times daily alongside a 1200 kcal/day diet. After a three-month interval, the treatment group showed a significant average weight reduction of 3.5 kg, compared to 1.3 kg in the control group. Body composition analysis indicated that the weight loss was primarily derived from a reduction in adipose tissue mass (Topping et al., 2003).

Further open-label analysis of healthy subjects (BMI 23–30) utilizing a standardized dose of Phase 2® (750 mg) supplemented with amino acids (lysine, arginine, alanine) demonstrated significant metabolic improvements over eight weeks. Dietary caloric intake decreased from 1,742 to 1,525 kcal/day ($P = 0.01$), resulting in a 2.4% reduction in total body weight (from 74.5 kg to 72.7 kg; $P = 0.002$). Significant decreases were also observed in BMI and body fat percentage ($P < 0.001$), alongside improved lipid profiles, including reductions in triglycerides ($P = 0.019$) and total cholesterol (Shi et al., 2020).

Glycemic Regulation and Safety

Preceding research has confirmed that α -amylase inhibitors attenuate postprandial spikes in glucose, C-peptide, insulin, and gastric inhibitory polypeptide (GIP) by slowing the rate of starch digestion. Interestingly, the efficacy of α -amylase may depend on the baseline metabolic state of the subject. In animal models, no significant differences were found in weight loss among healthy rats, suggesting that the inhibitor's impact is more pronounced in obese models where baseline amylase activity may differ. This suggests that α -amylase inhibitors are specifically effective as a therapeutic intervention for metabolic dysfunction rather than a general weight-loss agent for healthy-weight individuals (Balasubramaniam et al., 2024).

Synthesis of Clinical Trial Outcomes

Table 1 summarizes pivotal clinical investigations into the efficacy of α -amylase inhibitors, demonstrating a consistent trend toward weight reduction and metabolic improvement across various delivery formats (powders, capsules, and functional foods). Trial durations typically range from 30 days to six months. Notably, the administration of α -AI before carbohydrate-rich meals significantly attenuates postprandial glycemic spikes. While earlier studies utilizing low-potency extracts

showed negligible effects on plasma glucose, modern standardized extracts like Phase 2® have demonstrated significant clinical weight loss in systematic reviews (Barrett & Udani, 2011).

However, a balanced scientific perspective requires acknowledging contradictory findings. For instance, a meta-analysis by (Onakpoya et al., 2011) suggested that some commercial PVE products may lack sufficient potency for universal efficacy. This discrepancy is often attributed to variations in extract quality, dosage, and trial duration. The largest and longest trial ($n = 123$; 24 weeks) reported a mean weight maintenance at $99.34 \pm 2.96\%$ of baseline ($P < 0.001$). Recent Randomized Controlled Trials (RCTs) in Chinese cohorts (Wang et al., 2020) further corroborate these findings, reporting a placebo-adjusted weight loss of 1.95 kg after only 35 days of 2,400 mg daily supplementation. These cumulative data suggest that standardized PVE is an effective intervention for reducing BMI, adipose tissue thickness, and waist circumference.

Molecular Mechanism of Enzymatic Inhibition

The inhibitory profile of *Phaseolus vulgaris* is defined by three distinct isoforms: α -AI1, α -AI2, and α -AIL. These specialized glycoproteins are synthesized during seed development and are primarily sequestered within the cotyledons (Peddio, 2023). The fundamental mechanism involves the formation of a stable, high-affinity complex between the inhibitor and the target enzyme. This interaction is strictly non-covalent, relying on a combination of Van der Waals forces, hydrogen bonding, and hydrophobic interactions to occlude the catalytic site of α -amylase.

The efficacy of this "starch-blocking" activity is highly sensitive to the microenvironment, including fluctuations in pH, thermal stability, and the presence of specific stabilizing cations. Structurally, the α -AI1 isoform utilizes two prominent loops, designated L1 and L2, to penetrate and block the active site cleft of human pancreatic α -amylase (HPA). Molecular docking analyses suggest that specific amino acid residues, most notably histidine, tryptophan, arginine, and tyrosine, are critical for maintaining the structural integrity of this inhibitory complex (Peddio et al., 2022). Unlike many plant lectins, α -AI1 functions as a potent non-competitive inhibitor against both mammalian and specific coleopteran amylases (Kasar et al., 2022). The steric hindrance

Table 1. A review of research on α -amylase inhibitor from white bean extract and its specific dose used for a group of volunteers for weight-loss clinical trials

Reference (Author, date)	Study Duration	Subjects	Preparation/Dose	Results
(Thom, 2000)	RPCT, 12 weeks	n = 40 (BMI 28-39)	Phaseolamin: 1200 mg/day (400 mg 3x/day) (other ingredients: inulin & <i>Garcinia</i> extract)	Reduction of 2.5 ± 0.7 kg
(Rothacker, 2003)	RPCT, 12 weeks	n = 88 (BMI 24-32)	StarchAway: 3000 mg/day (1000 mg 3x/day)	Mean weight loss of 2.8 kg
(Erner & Meiss, 2003)	RPCT, 12 weeks, Then open 12 weeks	n = 54 (BMI 24-36)	Thera-Slim: 2000 mg/day (1000 mg 2x/day)	Mean reduction of 2.2 kg
(Koike et al., 2005)	Open, 8 weeks	n = 10 (BMI 23-30)	3 capsules Phaseolamin 1600 diet 2X daily; 750 mg Phase 2 daily	Weight reduction of 2.4 ± 0.2 kg over 8 weeks.
(Celleno et al., 2007)	RPCT, 30 days	n = 60 (BMI avg 26)	Phase 2 + Chromium: 445 mg/day	Mean loss of 2.93 ± 1.16 kg vs 0.35 kg in placebo
(Vinson et al., 2009)	PCT, X-over, single dose	Part 1: n = 11, Part 2: n = 7	Phase 2 mixed with margarine or gravy. 750 mg or 1500 mg	Reduction in body fat of 1.5 ± 0.5 %
(Udani et al., 2004)	RPCT, 8 weeks	n = 39 (BMI 30-43)	Phase 2: 3000 mg/day (1500 mg 2x/day)	AUC reduced by 40.5%
(Osorio & Gamboa, 2005)	Open, 30 days	n = 39 (overweight)	PreCarb: 3000 mg/day (1000 mg 3x/day)	Triglycerides decreased by 15.3%
(Wu et al., 2010)	RPCT, 60 days	n = 101 (BMI 25-40)	Phase 2: 3000 mg/day (1000 mg 3x/day)	Average loss of 1.9 kg
(Udani et al., 2009)	RPC, X-over, single dose	n = 13 (BMI 18-25)	Phase 2: 1500, 2000, or 3000 mg (Capsules or mixed with butter)	WHR reduction of 1.3 cm

provided by the L1 and L2 loops prevents the enzyme from engaging with complex starch polymers.

By effectively arresting the hydrolysis of polysaccharides into absorbable monosaccharides, this mechanism facilitates the significant reductions in postprandial glycemia and adipose accumulation observed in clinical cohorts (León-Galván & Loarca-Piña, 2015). This structural framework not only explains the weight-loss efficacy of white kidney bean extracts but also provides a

template for the design of future enzyme-targeted metabolic therapies.

Physicochemical Properties of Purified α -Amylase Inhibitors

The primary isoforms, α -AI1 and α -AI2, exhibit a structural framework analogous to the lectin tetrameric configuration ($\alpha_2 \beta_2$). The maturation of the α and β polypeptide chains involves a complex, two-step proteolytic processing within the protein storage vacuoles.

This activation sequence requires the removal of a C-terminal short-chain fragment and subsequent proteolytic cleavage.

Specifically, the endopeptidase-mediated cleavage occurs at the carboxyl side of Asparagine-77 (Asn-77), often assisted by asparagyl-specific endopeptidases, resulting in the formation of two distinct subunits (Li et al., 2021).

Structural analyses indicate a localized conformational restriction near the Asn-77 residue, which is critical for the inhibitor's functional geometry (Ahmad & Choudhary, 2024). The stability of the inhibitor protomers is maintained through non-covalent interactions, primarily hydrophobic bonding. While N-glycosylation is present, studies have demonstrated that glycan chains do not directly modulate inhibitory performance, as their positioning does not interfere with the mammalian amylase binding interface (Shen et al., 2023). Even after the enzymatic removal of glycan chains, the purified α -AI from *Phaseolus vulgaris* retains its inhibitory potency. However, the glycan chain at Asn-12 remains solvent-exposed, contributing to the overall solubility and conformation of the enzyme-inhibitor complex through peripheral hydrogen bonding and Van der Waals interactions (Brain-Isasi et al., 2017).

Comparative Analysis: α -AI1 vs. Acarbose

A comparative analysis reveals that the α -AI1 inhibitor from *Phaseolus vulgaris* exhibits higher peak inhibitory potency than the pharmaceutical agent Acarbose, specifically achieving 74% inhibition of amylose digestion compared to 71% for the latter. While Acarbose possesses a lower molecular weight and faster reaction kinetics, α -AI1 provides a superior, plant-derived alternative for modulating starch catabolism through its distinct non-competitive binding mechanism and higher sustained inhibitory rates for complex carbohydrate hydrolysis (Santimone et al., 2004).

Structural Determinants of Inhibitory Activity

The inhibitory efficacy of α -AI1 is fundamentally dependent on its primary amino acid sequence and the strategic orientation of two specific hairpin loops, designated L1 (residues 29–46) and L2 (residues 171–190). As illustrated in Figure 3, these loops facilitate high-affinity binding through a network of direct and water-mediated hydrogen bonds. The structural interference is centered on the projection of key residues—Tyr37 (L1), Asp38, Tyr186, and Tyr190

(L2)—into the catalytic site of the enzyme (Nguyen et al., 2014).

Molecular docking reveals that Tyr37 forms critical hydrogen bonds with Glu233 and Asp300 within the enzyme's active site, while Asp38 interacts with His201. Furthermore, the L2 loop orientation allows Tyr186 to bond with the catalytic nucleophile, Asp197, effectively arresting the enzymatic displacement process. This complex is further stabilized by interactions between Ser189 and Tyr190 of the inhibitor and the catalytic triad of the amylase (Asp197, Glu233, and Asp300) (Rane et al., 2020).

While the common bean contains several isoforms, α -AI1 is characterized as a glycoprotein complex (~36–43 kDa) stabilized by three disulfide bridges (Bompard-Gilles et al., 1996). The inhibitory action is categorized as non-competitive and lectin-like, effective against both mammalian pancreatic amylase and specific insect amylases (Li et al., 2021). The presence of the L1 and L2 loops on the "front face" of the protein is the definitive structural requirement for its ability to occlude the substrate-binding cleft, thereby preventing the hydrolysis of starch into absorbable saccharides.

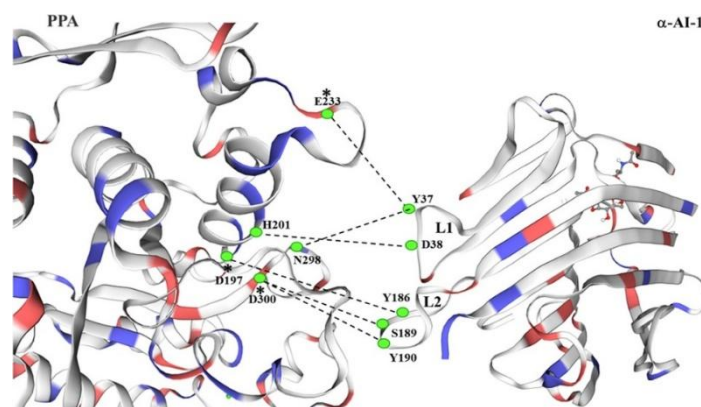


Figure 3. Three-dimensional model structure of the α -amylase inhibitor 1 (α -AI1) complex

Figure 3 depicts the structural basis for enzymatic inhibition, showing the α -AI1 hairpin loops (L1 and L2) deeply inserted into the catalytic cleft of porcine pancreatic α -amylase (PPA). High-affinity binding is stabilized by a non-covalent network between inhibitor residues (Y37, F38, D186, S189) and the enzyme's catalytic triad (E233, D197, D300). This direct occlusion of the active site effectively arrests starch hydrolysis,

providing the biochemical foundation for the extract’s metabolic benefits.

Identification of α -amylase inhibitor isomer

Isoforms α -AI1 and α -AI1’ are characterized as 43 kDa globular heterotetramers ($\alpha_2\beta_2$) with a 29.0 Å radius. Despite sharing similar molecular masses, they exhibit distinct isoelectric points and significant glycosylation (14–16% w/w), primarily consisting of mannose and trace xylose, which contribute to the structural stability of the inhibitor (Nguyen et al., 2014).

Extraction and Purification Methodologies

The isolation of α -amylase inhibitors (α -AI) from *Phaseolus vulgaris* typically employs four primary methodologies: (i) pH-dependent extraction, (ii) ammonium sulfate precipitation followed by dialysis, (iii) fractional ethanol precipitation, and (iv) advanced column chromatography.

pH-Dependent Extraction

This method leverages the solubility of proteins at specific acidic or basic ranges. A-AI isoforms exhibit distinct pH optima for stability and activity; specifically, extracts from KR-9 cultivars show optimal performance within a pH range of 5.0 to 6.9, and also identified a peak inhibitory pH of 5.0 for standardized white bean extracts (Kasar et al., 2022).

Ammonium Sulfate Precipitation and Dialysis

A common protocol involves dissolving white kidney bean powder in a specialized extraction buffer (10 mM Tris-HCl, pH 7.5) containing stabilizing agents such as 500 mM NaCl, 1% 2-mercaptoethanol, 0.1% Triton X-100, and 2 mM phenylmethylsulphonyl fluoride (PMSF). The crude protein is precipitated using ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$). Following precipitation, the supernatant is dialyzed against a 10 mM sodium phosphate buffer (pH 6.0) containing 15% ammonium sulfate to remove low-molecular-weight impurities. Purified samples are typically stored at -20°C for long-term stability (Mundi, 2012).

Fractional Ethanol Precipitation and Chromatography

Fractional precipitation using ethanol serves as an effective initial concentration step, followed by ion-exchange chromatography to achieve homogeneity. The purity of the resulting glycoprotein is traditionally

confirmed via Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and gel filtration. As shown in Figure 4, the purified α -AI manifests as a distinct band corresponding to a molecular weight of approximately 36 kDa.

Biochemical analysis reveals that the glycoprotein consists of approximately 88.2% protein, characterized by a high concentration of acidic and neutral amino acids (Glutamic acid, Aspartic acid, Leucine, Serine, and Threonine). The glycan component comprising mannose, glucose, galactose, and xylose in a molar ratio of 2.42:1.50:1.52:1.00—is predominantly linked to the protein core via O-glycosidic linkages (Mahajan, 2016).

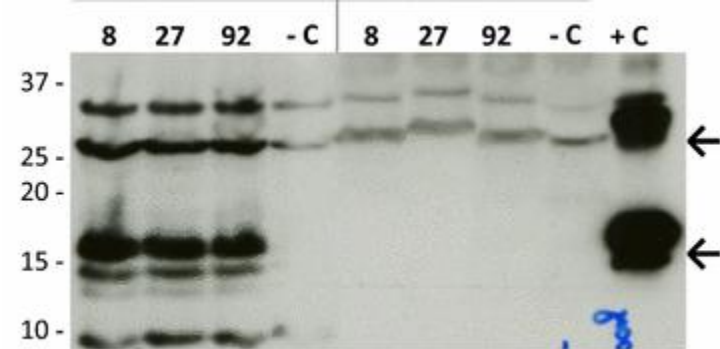


Figure 4. SDS-PAGE analysis of purified α -amylase inhibitor from white kidney bean extracts, indicating a molecular weight of approximately 36 kDa.

The efficiency of α -amylase inhibitor isolation is highly dependent on the choice of extraction and purification protocols. While traditional saline extraction offers high raw yields, advanced chromatographic techniques are essential for achieving the high-purity isoforms required for clinical applications. The table 2 provides a critical comparison of these methodologies, highlighting their respective yields, advantages, and technical limitations.

Table 2. Comparative Analysis of Extraction and Purification Methods for α -Amylase Inhibitors

Method	Optimal Conditions	Yield / Purity	Advantages	Disadvantages
Aqueous/Saline Extraction	pH 4.0–7.0; Temp: 25°C	High Yield / Low Purity	Simple, cost-effective, maintains protein stability.	High volume of waste requires further purification.
Ethanol Precipitation	60–80% Ethanol concentration	Moderate Yield / Moderate Purity	Rapid; effective for the initial concentration of proteins.	Potential for protein denaturation; uses flammable solvents.
Ammonium Sulfate Precipitation	30–60% Saturation	High Yield / Moderate Purity	Scalable; protects enzyme activity during storage.	Requires subsequent dialysis to remove residual salts.
Dialysis	Buffer pH 6.9; 4°C	High Recovery	Effectively removes low-molecular-weight impurities/salts.	Time-consuming; requires large buffer volumes.
Ion-Exchange Chromatography	Buffer-dependent (DEAE-cellulose)	Low Yield / High Purity	Excellent separation of specific α -AI isoforms.	Expensive equipment; complex protocol; low throughput.
Affinity Chromatography	Bound α -amylase ligand	Very High Purity	Single-step purification with high specificity.	High cost of ligands; difficult to scale for industry.

Column Chromatography and Purification Refinements

Standardized extraction via column chromatography, as pioneered by Lee and Whitaker (2000), has undergone significant optimization. Modern protocols utilize a DEAE-32 cellulose matrix with a 0.025 mol/L sodium acetate/acetic acid buffer (pH 4.5) and a linear NaCl gradient (0–1.0 mol/L) to enhance isoform separation (Parvin et al., 2025). A typical laboratory workflow involves suspending white kidney bean flour in deionized water at 37 °C, followed by centrifugation at 10,000 rpm. The resulting supernatant is heat-treated at 70 °C and fractionated with 70% ethanol at 4 °C. The precipitate is then resolved using a CM-cellulose column at a flow rate

of 0.6 mL/min to isolate the high-purity inhibitor (Szewczuk et al., 1993).

Further purification can be achieved through Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) using a C18 column and an acetonitrile-water mobile phase (70:30) with 0.1% trifluoroacetic acid (TFA). Additionally, the use of zinc hydroxide Zn(OH)₂ as an inorganic adsorbent has emerged as a rapid alternative for separating α -AI from other soluble bean proteins, achieving high specificity before final DEAE chromatography (Szamos et al., 2012).

Industrial Production: Phase 2® vs. Laboratory Extraction

Phase 2® is a standardized, water-extracted supernatant derived from non-GMO white kidney beans. Unlike laboratory-scale purification, the industrial process involves large-scale aqueous mixing, vacuum concentration, pasteurization, and spray-drying. This results in a stable, odorless, and tasteless powder standardized to at least 3,000 α -amylase inhibiting units (AAIU) per gram (Jeganathan, 2023). Commercially available in over 200 brands, Phase 2® is typically administered in 500 mg capsules (1,500–3,000 mg/day). Safety panels have established a maximum daily intake of 10,000 mg, confirming its high therapeutic index (Peter, 2018).

Safety and Acute Toxicity Analysis

The safety profile of α -amylase inhibitors is quantified via LD (Ahmad & Choudhary) (lethal dose for 50% of the test population) values. In murine models, acute oral administration of 1,668 mg/kg of Phase 2® (equivalent to a 3 g/kg dietary supplement) showed no significant deleterious effects or clinical signs of toxicity. Crucially, these extracts do not exhibit the toxicity associated with phytohemagglutinin (PHA), a common bean lectin, confirming that properly processed α -AI is safe for human consumption (Barrett & Udani, 2011).

Future Directions

While the efficacy of α -amylase inhibitors (α -AI) from *Phaseolus vulgaris* is well-established, several avenues for future research remain. Current challenges include the high variability in the inhibitory potency of commercial extracts. Future studies should focus on optimizing extraction through green chemistry, such as supercritical carbon dioxide extraction, to improve yield and maintain the structural integrity of the L1 and L2 loops. Furthermore, investigating microencapsulation techniques could protect the inhibitor from premature gastric degradation, ensuring higher activity in the small intestine. Additionally, long-term longitudinal studies are required to evaluate the impact of chronic starch blockade on the gut microbiome and the potential synergistic effects of combining α -AI with other plant-based metabolic modulators.

Conclusion

This review confirms that α -amylase inhibitors derived from white kidney beans are potent tools for metabolic

regulation. These glycoproteins can be successfully isolated using pH-dependent extraction, ammonium sulfate precipitation, and column chromatography. Mechanistically, α -AI1 functions by occluding the catalytic triad (Asp197, Glu233, and Asp300) of human pancreatic amylase via its specific hairpin loops. Clinical evidence consistently demonstrates that standardized doses of these inhibitors significantly reduce postprandial glucose and insulin spikes, leading to measurable reductions in body mass index (BMI) and adipose tissue. By lowering the rate of carbohydrate absorption, α -AI not only assists in weight management but also mitigates the risk factors associated with insulin resistance and cardiovascular disorders.

Credit Authorship Contribution Statement

Nayab Aslam spearheaded the conceptual design of the review, which was conducted under the primary supervision and project administration of Imran Mukhtar. The initial manuscript was drafted through the collaborative efforts of Nayab Aslam, Iman Aslam, Rida Arshad and Faiza Zafar with Sana Saleem providing essential data validation and resource support. Umm-E-Habiba, Urooj Fatima, Duaa Shakeel and Momina Tariq helped in write up and refined the structural figures and comparative tables. Maria-Rosa Bernal-Lopez contributed significantly to the critical intellectual content and ensured the manuscript's alignment. The final technical editing and critical revisions were managed by Imran Mukhtar. All listed authors have reviewed, refined, and formally approved the final version of the manuscript for publication.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Acknowledgment

The writers appreciate School of Food and Biological Engineering, Engineering Research Centre of Bio-process of Ministry of Education, Key Laboratory for Agricultural Products Processing of Anhui Province, Hefei University of Technology, Hefei 230009, China and Government College

University Faisalabad, Pakistan for giving resources for their literature collection.

Data Availability

Even though adequate data has been given in the form of tables and figures, all authors declare that if more data is required, then the data will be provided on a request basis.

Conflict of Interest

The authors declare no conflict of interest.

References

- Ahmad, R., & Choudhary, M. (2024). Design and synthesis of copper (II) and nickel (II) complexes as potential inhibitors against SARS-CoV-2 viral RNA binding protein and Omicron RBD protein. *New Journal of Chemistry*, 48(34), 15053-15076.
- Balasubramaniam, A. K., Elangovan, A., Rahman, M. A., Nayak, S., Richards, A., Swain, D., & Rahman, M. A. (2024). Purple Tea (*Camellia sinensis* var. assamica) Leaves and Obesity Management: A Review of 1, 2-Di-Galloyl-4, 6-Hexahydroxydiphenoyl- β -D-Glucose's (GHG) Potential Health Benefits, and Future Prospects. *Cureus*, 16(12).
- Barrett, M. L., & Udani, J. K. (2011). A proprietary alpha-amylase inhibitor from white bean (*Phaseolus vulgaris*): a review of clinical studies on weight loss and glycemic control. *Nutrition journal*, 10(1), 24.
- Bhujle, R. R., Nayak, N., Gowda, N. N., Pandiselvam, R., & Sunil, C. K. (2025). A comprehensive review on influence of millet processing on carbohydrate-digesting enzyme inhibitors and implications for diabetes management. *Critical Reviews in Biotechnology*, 45(4), 743-765.
- Bompard-Gilles, C., Rousseau, P., Rougé, P., & Payan, F. (1996). Substrate mimicry in the active center of a mammalian α amylase: Structural analysis of an enzyme-inhibitor complex. *Structure*, 4(12), 1441-1452.
- Brain-Isasi, S., Álvarez-Lueje, A., & Higgins, T. J. V. (2017). Heterologous expression of an α -amylase inhibitor from common bean (*Phaseolus vulgaris*) in *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. *Microbial Cell Factories*, 16(1), 110.
- Celleno, L., Tolaini, M. V., D'Amore, A., Perricone, N. V., & Preuss, H. G. (2007). A dietary supplement containing standardized *Phaseolus vulgaris* extract influences body composition of overweight men and women. *International journal of medical sciences*, 4(1), 45.
- Chandrakanth, M., Kumar, N., Sura, C., & Tung, S. (2025). Context-dependent effects of developmental and adult diet on life-history traits in *Drosophila melanogaster*. *Journal of evolutionary biology*, 38(10), 1396-1409.
- Ćorković, I., Gašo-Sokač, D., Pichler, A., Šimunović, J., & Kopjar, M. (2022). Dietary polyphenols as natural inhibitors of α -amylase and α -glucosidase. *Life*, 12(11), 1692.
- de Toro-Martín, J., Arsenault, B. J., Després, J.-P., & Vohl, M.-C. (2017). Precision nutrition: a review of personalized nutritional approaches for the prevention and management of metabolic syndrome. *Nutrients*, 9(8), 913.
- Dhital, S., Warren, F. J., Butterworth, P. J., Ellis, P. R., & Gidley, M. J. (2017). Mechanisms of starch digestion by α -amylase—Structural basis for kinetic properties. *Critical reviews in food science and nutrition*, 57(5), 875-892.
- Erner, S., & Meiss, D. (2003). Thera-Slim for weight loss: A randomized double-blind placebo controlled study. Unpublished internal report, Pharmachem Laboratories, Inc, Kearny, NJ.
- Ganesan, K., & Xu, B. (2017). Polyphenol-rich dry common beans (*Phaseolus vulgaris* L.) and their health benefits. *International journal of molecular sciences*, 18(11), 2331.
- Gong, L., Feng, D., Wang, T., Ren, Y., Liu, Y., & Wang, J. (2020). Inhibitors of α -amylase and α -glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia. *Food Science & Nutrition*, 8(12), 6320-6337.
- Iqbal, Z., Aftab, A., Swar, S.O., Hussain, R., Mohiuddin, M. (2021). Determination of Lipid Lowering Effect of Corn Flour in Hyperlipidemic Albino Mice. *Pakistan Veterinary Journal*, 42(1): 112-116.
- Iqbal, Z., Ashraf, T., Khan, A. A., Hussain, R., Mohiuddin, M. (2016). Antihyperlipidemic efficacy of cinnamon in albino rats. *Asian Journal of Agriculture and Biology*, 4(1): 8-16
- Iqbal, Z., Iqbal, K., Mohiuddin, M. (2015). Hepatoprotective effect of cinnamon on cholesterol induced fatty changes in albino rats. *Isra Medical Journal*, 7(4): 225-7
- Jeganathan, B. (2023). Novel mild fractionation of faba bean (*Vicia faba* L.) and physicochemical and functional characterization of protein as a food ingredient.
- Jiang, C., Li, S., Su, H., Zhou, N., & Yao, Y. (2024). Kidney bean protein prevents high-fat and high-fructose diet-induced obesity, cognitive impairment, and disruption of gut microbiota composition. *Foods*, 13(11), 1718.

- Kasar, S. S., Maheshwari, V. L., & Pawar, P. K. (2022). Bioactive α -amylase inhibitors: Sources, mechanism of action, biochemical characterization, and applications. *Natural Products as Enzyme Inhibitors: An Industrial Perspective*, 59-104.
- Koike, T., Koizumi, Y., Tang, L., Takahara, K., & Saitou, Y. (2005). The antiobesity effect and the safety of taking "Phaseolamin (TM) 1600 diet". *J New Rem & Clin (Japanese)*, 54, 1-16.
- León-Galván, F., & Loarca-Piña, G. (2015). Potential role of bioactive compounds of *Phaseolus vulgaris* L. on lipid-lowering mechanisms.
- Li, H., Zhou, H., Zhang, J., Fu, X., Ying, Z., & Liu, X. (2021). Proteinaceous α -amylase inhibitors: purification, detection methods, types and mechanisms. *International journal of food properties*, 24(1), 277-290.
- Mahajan, R., (2016). Molecular analysis for micronutrients in common bean (*Phaseolus vulgaris* L.) Kashmir University].
- Martinez-Gonzalez, A. I., Díaz-Sánchez, Á., De La Rosa, L., Bustos-Jaimes, I., & Alvarez-Parrilla, E. (2019). Inhibition of α -amylase by flavonoids: Structure activity relationship (SAR). *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 206, 437-447.
- Misra, S., Khunti, K., Goyal, A., Gable, D., Armocida, B., Tandon, N., Sachdev, P., Wild, S. H., Hivert, M.-F., & Beran, D. (2025). Managing early-onset type 2 diabetes in the individual and at the population level. *The Lancet*, 405(10497), 2341-2354.
- Mohiuddin, M., Asghar, T., Hameed, H., Din, A.M., Siddique, A., Younas, S., Mohi ud din, M. and Ara, R. (2025). The psychobiotic revolution: comprehending the optimistic role of gut microbiota on gut-brain axis during neurological and Gastrointestinal (GI) disorders. *World Journal of Microbiology and Biotechnology*, 41(10), p.401.
- Mundi, S. (2012). Structural and functional characterization of red kidney bean (*Phaseolus vulgaris*) proteins and enzymatic protein hydrolysates. University of Manitoba (Canada).
- Nguyen, P. Q., Wang, S., Kumar, A., Yap, L. J., Luu, T. T., Lescar, J., & Tam, J. P. (2014). Discovery and characterization of pseudocyclic cystine-knot α -amylase inhibitors with high resistance to heat and proteolytic degradation. *The FEBS journal*, 281(19), 4351-4366.
- Onakpoya, I., Aldaas, S., Terry, R., & Ernst, E. (2011). The efficacy of *Phaseolus vulgaris* as a weight-loss supplement: a systematic review and meta-analysis of randomised clinical trials. *British Journal of Nutrition*, 106(2), 196-202.
- Osorio, L., & Gamboa, J. (2005). Random multicenter evaluation to test the efficacy of *Phaseolus vulgaris* (Precarb) in obese and overweight individuals.
- Parvin, N., Aslam, M., Joo, S. W., & Mandal, T. K. (2025). Nano-phytomedicine: harnessing plant-derived phytochemicals in nanocarriers for targeted human health applications. *Molecules*, 30(15), 3177.
- Peddio, S. (2023). Proteinaceous inhibitors of α -amylase and α -glucosidase from common bean (*Phaseolus vulgaris* L.): biochemical characterization and phylogenetic analysis of Sardinian cultivars.
- Peddio, S., Padiglia, A., Cannea, F. B., Crnjar, R., Zam, W., Sharifi-Rad, J., Rescigno, A., & Zucca, P. (2022). Common bean (*Phaseolus vulgaris* L.) α -amylase inhibitors as safe nutraceutical strategy against diabetes and obesity: An update review. *Phytotherapy Research*, 36(7), 2803-2823.
- Peter, R. (2018). Properties of aqueous-alcohol-washed protein concentrates prepared from air-classified pea protein and other air-classified pulse protein fractions
- Petrovičova, O. D., Djuričić, I., Ivanović, N., Dabetić, N., Dodevska, M., & Ilić, T. (2024). Dietary interventions in obesity: A narrative review. *Archives of Pharmacy*, 74(Notebook 3), 281-297.
- Rane, A. S., Joshi, R. S., & Giri, A. P. (2020). Molecular determinant for specificity: Differential interaction of α -amylases with their proteinaceous inhibitors. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1864(12), 129703.
- Rothacker, D. (2003). Reduction in body weight with a starch blocking diet aid: StarchAway comparison with placebo. Leiner Health Products.
- Salam, M. M., Yousuf, R., Salam, M. W., & Haque, M. (2023). Obesity and overweight: A global public health issue. *Advances in Human Biology*, 13(1), 154-156.
- Santimone, M., Koukiekolo, R., Moreau, Y., Le Berre, V., Rougé, P., Marchis-Mouren, G., & Desseaux, V. (2004). Porcine pancreatic α -amylase inhibition by the kidney bean (*Phaseolus vulgaris*) inhibitor (α -AI1) and structural changes in the α -amylase inhibitor complex. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, 1696(2), 181-190.
- Shen, J.-X., Du, W.-W., Xia, Y.-L., Zhang, Z.-B., Yu, Z.-F., Fu, Y.-X., & Liu, S.-Q. (2023). Identification of and mechanistic insights into SARS-CoV-2 main protease non-covalent inhibitors: an in-silico study. *International journal of molecular sciences*, 24(4), 4237.
- Shi, Z., Zhang, X., Zhu, Y., Yao, Y., & Ren, G. (2020). Natural extracts from white common bean

- (*Phaseolus vulgaris* L.) inhibit 3T3-L1 adipocytes differentiation. *Applied Sciences*, 11(1), 167.
- Singh, S., Patni, S., Singh, A., Bhatt, S., & Mehta, S. L. (2025). α -Amylase Inhibitors: A Safe Nutraceutical Approach for Obesity Management. In *Nutraceuticals in Obesity Management and Control* (pp. 353-378). Apple Academic Press.
- Szamos, J., Macz , A., & Gelencs r,  . (2012). Application of zinc hydroxide in the purification of bean alpha-amylase inhibitor. *Acta alimentaria*, 41(2), 272-276.
- Szewczuk, Z., Gibbs, B. F., Yue, S. Y., Purisima, E., Zdanov, A., Cygler, M., & Konishi, Y. (1993). Design of a linker for trivalent thrombin inhibitors: Interaction of the main chain of the linker with thrombin. *Biochemistry*, 32(13), 3396-3404.
- Thom, E. (2000). A randomized, double-blind, placebo-controlled trial of a new weight-reducing agent of natural origin. *Journal of International Medical Research*, 28(5), 229-233.
- Topping, D. L., Fukushima, M., & Bird, A. R. (2003). Resistant starch as a prebiotic and synbiotic: state of the art. *Proceedings of the Nutrition Society*, 62(1), 171-176.
- Udani, J., Hardy, M., & Madsen, D. C. (2004). Blocking carbohydrate absorption and weight loss: a clinical trial using Phase 2TM brand proprietary fractionated white bean extract. *Alternative medicine review*, 9(1), 63-69.
- Udani, J., Tan, O., & Molina, J. (2018). Systematic review and meta-analysis of a proprietary alpha-amylase inhibitor from white bean (*Phaseolus vulgaris* L.) on weight and fat loss in humans. *Foods*, 7(4), 63.
- Udani, J. K., Singh, B. B., Barrett, M. L., & Preuss, H. G. (2009). Lowering the glycemic index of white bread using a white bean extract. *Nutrition journal*, 8(1), 52.
- Vinson, J. A., Al Kharrat, H., & Shuta, D. (2009). Investigation of an amylase inhibitor on human glucose absorption after starch consumption. *Open Nutraceuticals J*, 2(1), 88-91.
- Wang, S., Chen, L., Yang, H., Gu, J., Wang, J., & Ren, F. (2020). Regular intake of white kidney beans extract (*Phaseolus vulgaris* L.) induces weight loss compared to placebo in obese human subjects. *Food Science & Nutrition*, 8(3), 1315-1324.
- Wu, X., Xu, X., Shen, J., Perricone, N. V., & Preuss, H. G. (2010). Enhanced weight loss from a dietary supplement containing standardized *Phaseolus vulgaris* extract in overweight men and women. *Journal of Applied Research*, 10(2), 73-80.
- Zhang, X. (2016). Binding of substrates and inhibitors to human pancreatic alpha amylase [University of British Columbia].

Disclaimer/Publisher's Note: The views, opinions, and data presented in all publications are exclusively those of the respective author(s) and contributor(s) and do not reflect the stance of Pagepal or its editorial team. Pagepal and the editor(s) disclaim any responsibility for harm to individuals or damage to property resulting from the use of any ideas, techniques, instructions, or products mentioned in the content.