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Nutritional Profiling and Antihyperglycemic Potential of Traditional Barley Preparations: A Comparative Biochemical Study of Manda, Peya, and Vilepi

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ABSTRACT: Diabetes mellitus is a global health crisis, prompting interest in dietary strategies from traditional medicine. Barley (Hordeum vulgare L.) is used in Ayurveda for its antidiabetic properties through three preparations: Manda, Peya, and Vilepi, differing in water-to-grain ratio and processing. This study compared their nutritional profiles and antihyperglycemic effects. Preparations followed Ayurvedic protocols: Manda (filtered supernatant, 14:1), Peya (homogenate, 14:1), and Vilepi (whole slurry, 4:1). Protein was measured by BCA assay, fat by solvent extraction, carbohydrates by phenol-sulfuric acid method, antioxidant activity by DPPH assay, and antihyperglycemic potential by α-amylase inhibition using DNS method. Protein content increased from Manda (0.20 ± 0.04 mg/mL) to Peya (0.60 ± 0.02 mg/mL) to Vilepi (1.12 ± 0.06 mg/mL). Fat was highest in Manda (0.52%), then Vilepi (0.21%) and Peya (0.20%). Carbohydrates were highest in Vilepi and Peya at 1000 µg/mL. Antioxidant activity inversely correlated with density: Manda had strongest DPPH scavenging (IC50 16 ± 0.2 mg/mL), followed by Peya (17 ± 0.8 mg/mL) and Vilepi (20 ± 0.6 mg/mL). α-Amylase inhibition was greatest in Vilepi (20.19 ± 0.23 µg/mL), then Peya (14.02 ± 1.03 µg/mL), and Manda (10.16 ± 2.3 µg/mL). These differences reflect distinct physicochemical, nutritional, and antihyperglycemic properties, supporting formulation-specific therapeutic diets and positioning these barley preparations as functional foods for diabetes management.

1. INTRODUCTION

Diabetes mellitus (DM) is a complex, multifactorial metabolic disorder characterized by chronic hyperglycemia resulting from absolute or relative deficiency of insulin secretion, impaired insulin function, or both [ElSayed et al., 2023]. According to the International Diabetes Federation (IDF), DM is a major global health burden, affecting an estimated 537 million adults as of 2021, and the number is predicted to reach 783 million by 2045 [Hossain et al., 2024]. Persistent hyperglycemia drives the pathogenesis of micro- and macrovascular complications, including nephropathy, retinopathy, peripheral neuropathy, and cardiovascular disease, substantially reducing quality of life and imposing enormous economic costs on healthcare systems globally [Hussain, 2025; Islam et al., 2025]. Current pharmacological management of type 2 DM involves a diverse therapeutic armamentarium, including biguanides (metformin), sulfonylureas, thiazolidinediones, GLP-1 receptor agonists and SGLT-2 inhibitors [Kuo & Li, 2026]. Although effective, these agents are associated with significant adverse effects, including lactic acidosis and gastrointestinal intolerance with metformin, hypoglycemia with sulfonylureas, fluid retention and increased fracture risk with thiazolidinediones, and diabetic ketoacidosis with SGLT-2 inhibitors [Dugbartey et al., 2026]. The complexity of long-term pharmacotherapy, concerns about adverse event profiles and the economic burden of treatment, particularly in resource-limited settings, have renewed interest in evidence-based complementary strategies derived from traditional medicine systems [Gieroba et al., 2025].

Barley (Hordeum vulgare L., family Poaceae) is one of the oldest cultivated cereal grains and has been extensively studied for its metabolic benefits [Kaur et al., 2024]. It is uniquely rich in bioactive compounds, including β-glucan, phenolic acids, tocols, phytosterols and resistant starch, which collectively modulate postprandial glycemia, attenuate oxidative stress and favorably alter colonic fermentation profiles [Idehen et al., 2017; Zeng et al., 2020; Ahmed et al., 2025]. Within the classical Ayurvedic pharmacopeia, barley (Yava) is classified as a major dietary and medicinal grain, specifically indicated for prameha (a clinical entity encompassing disorders with polyuria and glycosuria, similar to DM) owing to its Kapha- and meda-pacifying properties [Sharma, 2009].

The graduated barley preparations belong to a formal framework of Ayurvedic clinical dietetics rather than to folk or culinary practice. Within the classical literature they are recognized as components of Pathya Kalpana, the system of therapeutic food preparations, and of Samsarjana Krama, the strictly sequenced post-purification dietary protocol described in the Charaka Samhita and Sushruta Samhita. Samsarjana Krama prescribes a graded reintroduction of nourishment after Shodhana (bio-purification) procedures such as Vamana and Virechana, when the digestive and metabolic capacity (Agni) is temporarily weakened. The sequence begins with simple carbohydrate-dense liquid preparations and progresses stepwise through Peya, Vilepi, unseasoned and seasoned Yusha and meat soups, with the number of meals per day determined by the degree of purification achieved [Bhatt et al., 2020; Lahari & Shivakrishna, 2020; Panigrahi & Snehamayee, 2024; Lata et al., 2024]. The classical texts therefore treat these barley gruels not as ordinary foods but as a titratable therapeutic continuum: Manda (thin barley water), Peya (semi-viscous gruel incorporating both supernatant and solubilized solids) and Vilepi (a thick, dense paste prepared with a low water-to-grain ratio) represent a graded series of increasing nutrient density and therapeutic potency, selected according to disease severity, digestive capacity (Agni) and therapeutic intent [Panigrahi & Snehamayee, 2024]. This graduated design anticipates the modern concept of therapeutic dietetics, in which the physicochemical form of a food determines its rate of digestion, glycemic impact and clinical application. In this sense the series can be read as an ancient form of therapeutic gastronomy: a deliberately engineered dietary instrument that encodes an empirical understanding of metabolic recovery and stepwise nutritional repletion, rather than a set of traditional recipes.

Despite their longstanding empirical use, no study has systematically compared the three barley formulations using modern biochemical and pharmacological assays. While individual preparations have been investigated in isolation, the absence of head-to-head comparative data precludes evidence-based selection of formulations for specific therapeutic contexts, such as acute glycemic control versus long-term metabolic support. Bridging this gap requires both classical textual authority and mechanism-anchored scientific validation, an approach consistent with the ethnopharmacological program of validating traditional claims through contemporary analytical methods [Acharya, 2026].

Postprandial hyperglycemia is primarily governed by the rate of carbohydrate digestion, in which pancreatic α-amylase plays a pivotal rate-limiting enzymatic role by hydrolyzing α-1,4-glycosidic bonds in starch to produce maltose and shorter oligosaccharides [Gong et al., 2020]. Therefore, inhibition of α-amylase activity is an established mechanistic target for reducing postprandial glucose excursions [Tundis et al., 2010]. Oxidative stress is both a cause and consequence of hyperglycemia, and the antioxidant capacity of dietary preparations represents an important complementary biomarker of their therapeutic potential [Adom & Liu, 2002]. Based on a systematic literature search, no prior study has systematically compared all three formulations using standardized biochemical and pharmacological assays. We hypothesized that formulations with higher solid retention (Vilepi and Peya) would demonstrate greater α-amylase inhibitory activity due to increased β-glucan and protein content, whereas the aqueous supernatant (Manda) would exhibit superior antioxidant capacity due to the enrichment of water-soluble phenolic compounds. Therefore, the present study aimed to: (i) characterize the physical and organoleptic properties of Manda, Peya and Vilepi; (ii) determine and compare their macronutrient (protein, fat and carbohydrate) profiles; (iii) evaluate their in vitro antioxidant activity using the DPPH radical scavenging assay; and (iv) assess their in vitro antihyperglycemic potential through α-amylase inhibitory activity, with the goal of establishing formulation-specific bioactivity profiles that can guide evidence-based selection in Ayurvedic diabetic management and inform the design of clinical glycemic index studies.

2. MATERIALS AND METHODS

2.1 Authentication and Source of Raw Material

Barley grains (Hordeum vulgare L., cultivar RD 2035) were obtained from a certified supplier. Botanical authentication was performed at the Department of Dravyaguna, Yenepoya Ayurveda Medical College and Hospital (YAMCH), Mangalore, Karnataka, India. Morphological evaluation confirmed the grain to be ovoid to narrowly oblong, pale whitish-yellow, dorsally

compressed and laterally flattened with a distinct shallow longitudinal furrow, characteristics consistent with Hordeum vulgare L. (family Poaceae).

2.2 Preparation of Barley Formulations

All three preparations were prepared independently in triplicate using standardized procedures under aseptic conditions following the formulation methods mentioned in the Sushruta Samhita, Sutrasthana, chapter 46/344, on the major preparations of Pathya [Sharma, 2004] . For the preparations, the glassware and apparatus were autoclaved at 121°C for 15 min prior to use. For each formulation, 100 g of barley grains were accurately weighed using a precision analytical balance and coarsely crushed in a traditional stone mortar (Khalva Yantra) to rupture the husk while preserving endosperm integrity. All three formulations were prepared according to the traditional procedure described in Dravyaguna Vijnana [NCISM, 2024]. The differences in preparation procedures were intended to generate formulations with distinct proportions of liquid and grain-derived constituents.

For Manda preparation, crushed barley (100 g) was suspended in 1400 mL of double-distilled water (water-to-grain ratio of 14:1 w/v) in a borosilicate glass vessel. The mixture was heated over a controlled, mild flame with continuous stirring until the grains completely gelatinized. The cooked mass was filtered through a double-layered, sterile muslin cloth. The clear aqueous supernatant collected constituted Manda. This preparation corresponds to the classical Manda, described as the liquid fraction obtained after thoroughly boiling the grain [Medindia, 2019; Krishnendu, 2021].

For Peya preparation, the same 14:1 water-to-grain ratio was used. After complete cooking, both the supernatant and softened solid grain fractions were retained and combined in equal proportions. This mixture was homogenized using a high-speed laboratory mixer for 3 min and subsequently filtered through a sterile muslin cloth to remove coarse fibrous particles. The resulting semi-viscous, homogeneous gruel was designated Peya, consistent with the classical formulation that includes both liquid and solubilized grain constituents [Singh, 2024].

For Vilepi preparation, crushed barley (100 g) was cooked with a substantially reduced volume of 400 mL of distilled water (water-to-grain ratio of 4:1 w/v) over a mild flame until complete gelatinization. The entire cooked mass (including both solid and liquid fractions) was mechanically blended until a uniform slurry was formed and then filtered through sterile muslin cloth to yield a semi-solid, dense paste. Vilepi represents the most concentrated formulation, with the highest solid content [Kshirsagar & Santosh, 2022]. All preparations were stored at 4°C in sterile, airtight glass containers and processed within 24 h for biochemical and pharmacological analyses.

2.3 Physical and Organoleptic Characterization

The physical consistency, color, texture, viscosity and flowability of each preparation were assessed by three trained evaluators using standardized, organoleptic descriptors. Viscosity was qualitatively graded as thin/watery, semi-viscous or thick/paste-like.

2.4 Biochemical Analyses

Total protein content: Total protein content was determined using a bicinchoninic acid (BCA) protein assay kit (Millipore-Merck), following the manufacturer's instructions and method described previously [Smith, 1985]. Briefly, samples were centrifuged at 10,000 rpm for 10 min at 4°C, and the clear supernatant was used. Bovine serum albumin (BSA) standards (25-2000 µg/mL) were prepared in parallel. Samples and standards (25 µL each) were loaded in triplicate onto a 96-well microplate, and 200 µL of working reagent (reagent A:B = 50:1) was added. The plates were incubated at 37°C for 30 min, and the absorbance was measured at 562 nm (Systronics microplate reader, India). Protein concentrations were interpolated from the BSA standard curve and expressed as mg protein/mL.

Crude fat content: Total lipid content was determined gravimetrically using solvent extraction with diethyl ether [DuBois et al, 1956]. Samples were oven-dried at 100-105°C to a constant weight. Dried samples (1-5 g) were extracted with 25-50 mL analytical-grade diethyl ether for 2 h under continuous stirring in a fume hood. Following filtration through Whatman No. 1 filter paper, ether was evaporated at 50-60°C on a water bath. The lipid residue was then cooled in a desiccator and weighed. The fat content was expressed as a percentage of the total sample weight (% w/w).

Total carbohydrate content: Carbohydrate content was determined using the phenol-sulfuric acid method described by Dubois et al, (1956). A 200 µL aliquot of freshly prepared 5% phenol solution was added to each sample, followed immediately by the rapid addition of 1 mL concentrated sulfuric acid. After vortexing and 10-minute incubation at room temperature, the

absorbance was measured at 490 nm (UV-Vis spectrophotometer, Shimadzu, Japan) against a reagent blank. D-Glucose serial dilutions (10-100 µg/mL) were used as calibration standards. The results are expressed as mg equivalent glucose per mL at varying extract concentrations (125, 250, 500 and 1000 µg/mL).

2.5 DPPH Radical Scavenging Assay

Antioxidant capacity was evaluated using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as described previously [Brand-Williams, 1995]. A 0.1 mM DPPH solution in ethanol was freshly prepared and protected from light. Sample extracts (Manda, Peya and Vilepi) were serially diluted in methanol (1-1000 µg/mL). Equal volumes (1 mL each) of DPPH solution and the sample extract were mixed in microplate wells, and the reaction was allowed to proceed in the dark at 25 ± 2°C for 30 min. Ascorbic acid was used as a positive control. The absorbance was measured at 517 nm. The percentage DPPH scavenging activity was calculated as: % inhibition = $[(A_0 - A_s) / A_0] \times 100$, where A_0 is the absorbance of the control and A_s is the absorbance of the test sample. The IC_{50} (concentration required for 50% radical scavenging) was derived from the nonlinear regression of the dose-response curves. All assays were performed in triplicates.

2.6 α-Amylase Inhibition Assay

The inhibition of α-amylase activity was assessed using the dinitrosalicylic acid (DNS) method [Hossain et al., 2025; Iqbal et al., 2023]. α-Amylase (2 U/mL in 20 mM sodium phosphate buffer, pH 6.9, with 6.7 mM NaCl) was preincubated with test extracts (0.1 mL each) for 10 min at 25°C prior to substrate addition. Soluble starch (1% w/v in 20 mM sodium phosphate buffer) was solubilized by boiling for 15 min and cooled before use. The enzyme reaction was initiated by adding 0.1 mL α-amylase solution to the extract-substrate mixture and was conducted for exactly 3 min at 20°C. The reaction was terminated by adding 0.5 mL of DNS reagent and incubating in a boiling water bath for 5 min. Samples were centrifuged at 3000 rpm for 5 min, and the absorbance of the supernatant was measured at 540 nm. A maltose standard curve was used to quantify the reducing sugars. The percent inhibition was calculated as follows:

$$\% \text{ inhibition} = [(A_{c+} - A_{c-}) - (A_s - A_b) / (A_{c+} - A_{c-})] \times 100$$

where A_{c+} is the absorbance of the positive control (α-amylase + substrate, without extract, representing 100% enzyme activity), A_{c-} is the absorbance of the negative control blank (substrate only, without enzyme or extract), A_s is the absorbance of the test sample (extract + α-amylase + substrate) and A_b is the absorbance of the sample blank (extract + substrate, without enzyme, used to correct for any inherent color or reducing sugar content of the extract).

2.7 Statistical Analysis

All data are expressed as the mean ± standard deviation (SD) of three independent experimental replicates. One-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to compare the three formulations. IC50 values were calculated using nonlinear regression analysis with GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Physical and Organoleptic Characteristics

The three barley preparations exhibited markedly distinct physical properties, governed by their respective water-to-grain ratios and processing techniques (Table 1). Manda, prepared as a filtered aqueous supernatant at a 14:1 ratio, was presented as a clear, thin, watery fluid with minimal viscosity and no perceptible sediment. Peya, incorporating both the supernatant and blended solid grain fractions at the same 14:1 dilution, yielded a semi-viscous, opaque, homogeneous gruel of moderate flowability and notably higher apparent viscosity than that of Manda. Vilepi, prepared from a concentrated 4:1 ratio and retaining all cooked solids, was a thick, dense, paste-like formulation with markedly elevated viscosity, minimal flowability and substantially higher solid density.

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Figure1. Representative images of different barley-based preparations: Manda, Peya, and Vilepi. The images illustrate the distinct physical consistencies and textures of each formulation tested.

Table 1. Physical and organoleptic characteristics of Manda, Peya, and Vilepi barley preparations.

Parameter	Manda	Peya	Vilepi
Water-to-grain ratio	14:1	14:1	4:1
Physical form	Clear supernatant	Homogeneous gruel	Dense paste/slurry
Viscosity (qualitative)	Low (thin/watery)	Moderate (semi-viscous)	High (thick/paste-like)
Color	Pale straw-yellow	Cream/opaque white	Cream-white, opaque
Solid content	Absent (filtered)	Moderate (blended)	High (all solids retained)
Processing	Filtered supernatant	Blend of supernatant + solids	Whole cooked blend

3.2 Nutritional Content Analysis

Protein: Vilepi demonstrated the highest protein concentration at 1.12 ± 0.06 mg/mL, which is approximately 5.6 times greater than that of Manda (0.20 ± 0.04 mg/mL) and 1.9 times greater than that of Peya (0.60 ± 0.02 mg/mL), reflecting the enhanced retention of soluble proteins in more concentrated formulations (Table 2). One-way ANOVA indicated significant differences in total protein content among the three formulations ($p < 0.001$). Post-hoc analysis using Tukey's test confirmed that Vilepi differed significantly from both Peya ($p < 0.01$) and Manda ($p < 0.001$), whereas Peya also differed significantly from Manda ($p < 0.01$).

Fat: The total fat content exhibited an unexpected distribution pattern among the formulations. Manda demonstrated the highest fat concentration at 0.52 ± 0.08%, significantly exceeding the levels observed in Peya (0.20 ± 0.06%) and Vilepi (0.21 ± 0.05%), which showed comparable and markedly lower fat contents, respectively. This pattern suggests preferential partitioning of lipid-soluble compounds into the aqueous supernatant fraction during the high-dilution preparation process specific to Manda (Table 2). These findings indicate that the formulation methodology critically influences lipid retention and distribution, potentially affecting the physicochemical properties and biological performance of the final product.

Carbohydrates: At the highest extract concentration tested (1000 µg/mL), Vilepi demonstrated the highest total carbohydrate content at 0.259 mg/mL, slightly surpassing Peya (0.256 mg/mL) and Manda (0.252 mg/mL) (Table 2). Both Peya and Vilepi

exhibited a consistent, concentration-dependent decline in carbohydrate yield with serial dilution, indicating predictable solubility and extract stability profiles. In contrast, Manda showed irregular and non-linear carbohydrate values across dilutions, ranging from 0.154 to 0.252 mg/mL, which may be attributed to assay matrix interference or variable gelatinized starch solubility at lower concentrations. These observations suggest formulation-specific differences in carbohydrate retention and solubility dynamics, which could influence the bioavailability and functional properties of the extracts.

Table 2. Summary of macronutrient content of Manda, Peya, and Vilepi preparations.

Parameter	Manda	Peya	Vilepi	p-value
Protein (mg/mL)	0.20 ± 0.04	0.60 ± 0.02	1.12 ± 0.06	< 0.001
Total Fat (%w/w)	0.52 ± 0.08	0.20 ± 0.06	0.21 ± 0.05	< 0.05
Carbohydrate at 1000 µg/mL (mg/mL)	0.252	0.256	0.259	NS*
Carbohydrate at 500 µg/mL (mg/mL)	0.154	0.247	0.242	< 0.05
Carbohydrate at 250 µg/mL (mg/mL)	0.170	0.242	0.235	< 0.05
Carbohydrate at 125 µg/mL (mg/mL)	0.238	0.185	0.210	NS*

*NS = not significant at $p < 0.05$. Values for protein and fat content are expressed as mean ± SD (n = 3). The carbohydrate values were single representative measurements at each dilution.

3.3 Antioxidant Activity (DPPH Radical Scavenging)

Antioxidant activity showed a clear inverse relationship with formulation density (Table 3, Figure 2). Manda exhibited the most potent DPPH radical scavenging effect, as evidenced by the lowest IC50 value of 16 ± 0.2 mg/mL, indicating superior efficacy in neutralizing 50% of DPPH radicals at a lower extract concentration than the other formulations. Peya displayed moderate antioxidant capacity with an IC50 of 17 ± 0.8 mg/mL, whereas Vilepi showed the weakest activity, with an IC50 of 20 ± 0.6 mg/mL. All formulations demonstrated a dose-dependent increase in radical scavenging activity. The substantially lower IC50 values recorded for the ascorbic acid positive control validate the relative antioxidant potential of these barley extract preparations, underscoring formulation density as a critical factor influencing bioactivity.

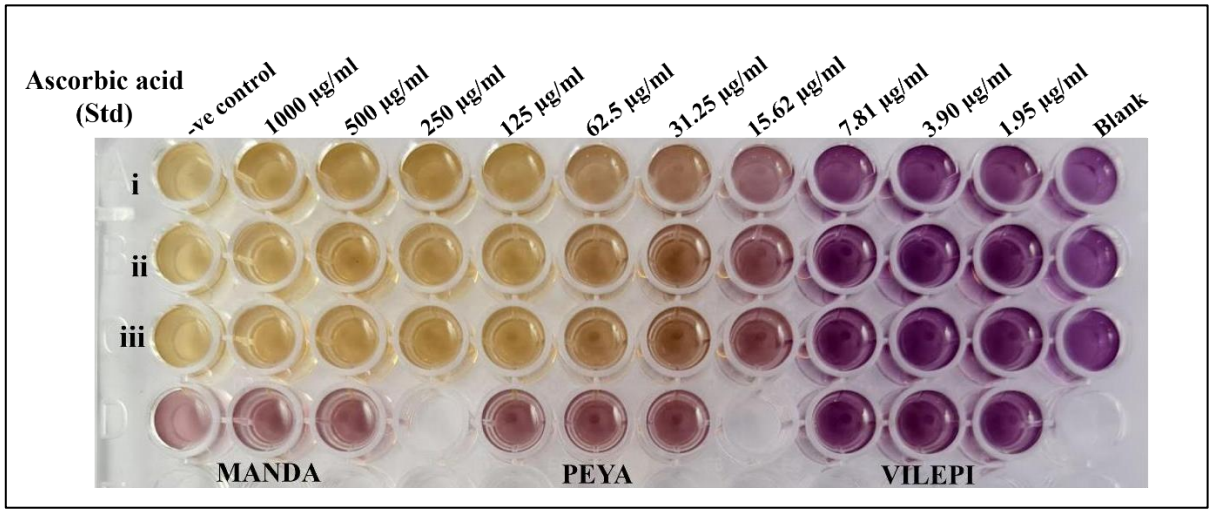

Figure 2. Microtiter plate representing the DPPH radical-scavenging activity of different barley preparations.

3.4 α-Amylase Inhibitory Activity

α-Amylase inhibitory activity was positively correlated with formulation concentration and protein content (Table 3). Vilepi showed the most potent inhibitory effect with an IC50 value of 20.19 ± 0.23 µg/mL, followed by Peya at 14.02 ± 1.03 µg/mL, while Manda demonstrated the lowest inhibitory potency at 10.16 ± 2.30 µg/mL. These results indicate that the macromolecular components retained in more concentrated formulations, such as proteins, β-glucan and insoluble dietary fiber, play a significant role in enhancing enzyme inhibition. This suggests that formulation density and associated biochemical composition are positively correlated with the α-amylase inhibitory potential of these preparations.

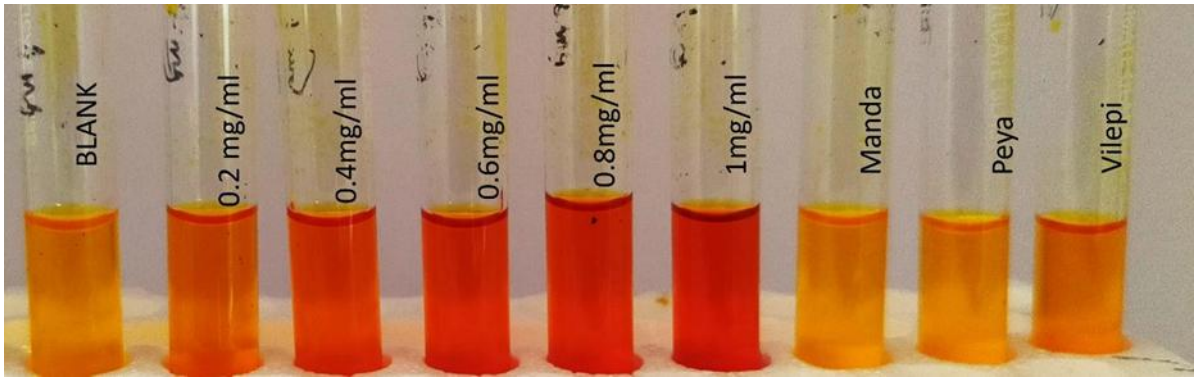

Figure 3: Alpha-amylase activity in different barley preparations.

Table 3. Antioxidant and antihyperglycemic activity of Manda, Peya, and Vilepi.

Parameter	Manda	Peya	Vilepi	p-value
DPPH IC_{50} (mg/mL)	16.0 ± 0.2	17.0 ± 0.8	20.0 ± 0.6	< 0.05
α-Amylase Inhibition (µg/mL)	10.16 ± 2.30	14.02 ± 1.03	20.19 ± 0.23	< 0.001

Values are expressed as mean ± SD (n = 3). A lower IC_{50} value for DPPH indicates higher antioxidant activity. Higher α-amylase inhibition (µg/mL of reducing sugar released) indicates lower inhibitory efficacy. p-values from one-way ANOVA with Tukey's post-hoc test.

3.5 Discussion

The progressive increase in protein content from Manda to Peya to Vilepi directly reflects the increasing retention of grain solids across these preparations. The comparative analysis of Manda, Peya and Vilepi demonstrates that nutritional density and antioxidant activity do not necessarily follow the same distribution pattern. Vilepi exhibited the highest protein concentration, consistent with its lower water-to-grain ratio and greater retention of cooked grain material. Protein solubilization during cooking releases albumins, globulins, prolamins (hordeins) and glutelins into the aqueous phase; however, the higher dilution and filtration steps in Manda limit the total protein yield per unit volume [Shewry & Halford, 2002; Jaeger et al., 2021]. Conversely, the mechanical blending and reduced dilution approach in Vilepi maximize protein recovery by incorporating structurally integrated endosperm proteins. These findings align with established reports that food-processing techniques significantly modulate protein bioaccessibility in cereal-based foods [Oboh et al., 2010; Fu et al., 2023].

The unexpectedly higher fat content observed in Manda compared with Peya and Vilepi is particularly intriguing. We hypothesized that during prolonged aqueous boiling at a high water-to-grain ratio, polar and amphiphilic lipid fractions, such as glycolipids or phospholipids, may preferentially partition into the aqueous phase and be retained in the Manda supernatant. This hypothesis requires validation through lipid class profiling using analytical methods to identify the specific lipid species that contribute to the elevated fat content. This phenomenon parallels the emulsification of cereal lipids during wet processing [Cozzolino et al., 2014; Banas & Harasym, 2021]. In concentrated Vilepi preparations, starch gelatinization may encapsulate lipids within a gel matrix, limiting their extractability using standard solvent methods. This requires further investigation using more sensitive lipid profiling techniques, such as GC-MS [Hammann et al., 2019; Zhou 2025].

The total carbohydrate content was highest in Vilepi and Peya at saturating extract concentrations, consistent with their higher gelatinized starch and solubilized β-glucan contents. β-Glucan, the primary bioactive polysaccharide in barley, exhibits high water solubility and forms viscous solutions that are retained in the gel fraction of concentrated preparations [Marconi et al., 2014; Izydorczyk et al., 2000; Lazaridou & Biliaderis, 2007]. The erratic carbohydrate signal from Manda at lower concentrations likely reflects the sensitivity limits of the phenol-sulfuric acid assay at low polysaccharide concentrations, compounded by potential matrix interference from residual aromatic compounds in the aqueous extract [Masukoet al., 2005; Nielsen, 2010; Mecozzi, 2005].

The divergent antioxidant and α-amylase inhibitory profiles of the three preparations can be traced directly to how the specific preparation variables, the water-to-grain ratio and the duration of heat treatment, govern the bioavailability and activity of barley phytochemicals. Most cereal phenolics exist predominantly in insoluble-bound forms esterified to cell-wall arabinoxylans; ferulic acid, the dominant phenolic acid of barley, is concentrated in the aleurone and pericarp and occurs mainly in the bound fraction [Zeng et al., 2020; Amic et al., 2020; Calinoiu & Vodnar, 2018; Shahidi et al., 2021]. Prolonged boiling in water, the central step of all three preparations, disrupts cell walls and cleaves ester and glycosidic bonds, converting bound phenolics into free and soluble-conjugated forms and thereby raising both total phenolic content and antioxidant activity; thermal processing of cereal matrices has been shown to increase free ferulic acid several-fold and to elevate total antioxidant capacity markedly [Dewanto et al., 2002; Malunga et al., 2021]. Heat treatment also deactivates endogenous oxidative and hydrolytic enzymes, protecting released phenolics from degradation [Calinoiu & Vodnar, 2018]. These considerations explain why Manda, which is prepared by prolonged boiling at 14-fold dilution followed by filtration, emerges as the most potent antioxidant formulation: its extended thermal treatment maximizes release of low-molecular-weight, water-soluble phenolics such as ferulic, caffeic and p-coumaric acids, which partition into the aqueous phase and pass through the muslin cloth into the final supernatant [Adom & Liu, 2002; Zhao et al., 2006; Abdel-Aal E-SM et al., 2012]. The inverse relationship between antioxidant activity and formulation density observed here replicates patterns reported for grain-based beverages versus porridges in the broader functional food literature [Zagol-Ikapitte et al., 2010]. From a mechanistic perspective, barley phenolics exert their antioxidant effects primarily through hydrogen atom transfer (HAT) and single-electron transfer (SET) mechanisms, with flavonoids additionally chelating redox-active metal ions [Amic et al., 2020].

The same preparation variables act differently on macromolecular constituents. The α-amylase inhibition observed in the present study provides preliminary biochemical evidence regarding the potential of barley preparations to modulate enzymatic starch digestion. However, in vitro inhibition of α-amylase should not be considered equivalent to demonstrated antihyperglycemic efficacy in living organisms. The experimental assay represents a simplified biochemical system and does not account for the complex physiological processes involved in carbohydrate digestion, gastrointestinal absorption, gastric emptying, intestinal transport and systemic glucose regulation. β-Glucan is a high-molecular-weight, water-soluble polysaccharide: at high dilution it remains dispersed, while in Vilepi, where solids are retained and the mass is concentrated, it accumulates and contributes to a viscous gel matrix together with gelatinized starch and endosperm proteins [Marconi et al., 2014; Izydorczyk et al., 2000; Lazaridou & Biliaderis, 2007, Zhang et al., 2024]. The markedly superior α-amylase inhibitory activity of Vilepi relative to Peya and Manda underscores the critical role of this macromolecular solid fraction in mediating enzyme inhibition. Multiple mechanisms are plausible and require future investigation. β-Glucan, likely retained at the highest concentration in Vilepi (though not directly quantified here), may form a viscous gel that physically impedes enzyme-substrate interaction by increasing diffusional resistance; viscosity measurements and β-glucan quantification are required to confirm this mechanism [Zhang et al., 2024; Yang et al., 2024; Yu et al., 2018]. This viscosity-mediated mechanism has been extensively documented in clinical studies of β-glucan-enriched oat and barley products [Tapola et al., 2005]. Proteinaceous fractions, including hydrolysate-derived peptides, may directly inhibit α-amylase through competitive or non-competitive mechanisms at substrate-binding or allosteric sites, as demonstrated for barley-derived trypsin-chymotrypsin inhibitors [Idehen et al., 2017; Gong et al., 2020; Pekkarinen et al., 2007]. Resistant starch and insoluble dietary fiber in Vilepi may competitively sequester amylase, thereby reducing net hydrolytic activity [He et al., 2023; Ou et al., 2001; Asp et al., 1987]. The convergence of these complementary mechanisms accounts for the substantially higher α-amylase inhibition observed in the most solid-rich preparation. While α-amylase inhibition in vitro suggests a potential mechanism for slowing starch hydrolysis, in vivo efficacy depends on factors not captured in this assay, including gastric pH, enzyme secretion dynamics and gastrointestinal transit. It should also be noted that clinical and mechanistic studies attribute part of the glycemic effect of cereal β-glucan to delayed gastric emptying and modulation of intestinal glucose transporters rather than to direct α-amylase inhibition alone [Thondre et al., 2013; Acharya , 2026]. Clinical validation through postprandial glucose or glycemic index studies in human volunteers is

essential before any therapeutic claims can be made. This is conceptually related to, although mechanistically distinct from, the action of α-glucosidase inhibitors, such as acarbose, a clinically approved α-glucosidase inhibitor [Cecilia et al., 2012]. Dietary strategies employing high-β-glucan cereal preparations have demonstrated reductions in postprandial blood glucose area under the curve (AUC) in randomized controlled trials in both healthy subjects and individuals with type 2 DM [Tapola et al., 2005; Zurbau et al., 2021].

The divergent bioactivity profiles of Manda, Peya and Vilepi provide biochemical support consistent with the differential Ayurvedic prescriptions described in classical texts. Classical texts indicate Manda for acute illness, febrile states and severely impaired digestive capacity, where its superior antioxidant properties and light digestibility are the primary therapeutic attributes. Vilepi, with its superior α-amylase inhibitory activity and the highest protein and carbohydrate density, is prescribed for convalescent and nutritionally depleted states requiring energy repletion alongside glycemic modulation. Peya occupies an intermediate therapeutic niche, balancing both attributes. The graduated prescription of Samsarjana Krama, from Manda through Peya to Vilepi, can thus be understood as an empirically derived titration of nutrient density and bioactivity against the recovering metabolic capacity of the patient: each stepwise escalation in solid retention corresponds to the stepwise escalation in protein, carbohydrate and enzyme-inhibitory potential documented here, while the aqueous Manda stage preferentially delivers heat-released polyphenolic antioxidants at minimal digestive load [Lata et al., 2024; Panigrahi & Snehamayee, 2024; Gebre et al., 2024; Kadam et al., 2020]. This convergence between classical graduated dietetics and modern formulation science represents an empirically derived optimization of bioactivity that is supported by our biochemical data. Previous studies have documented the hypoglycemic effects of barley in whole grain, flour and extract forms [Ahmed et al.,2025; Gebre et al., 2024]. Barley β-glucan at concentrations ≥3 g per serving significantly reduced postprandial blood glucose levels in human trials [Tosh, 2013]. Yao et al. reported significant α-glucosidase inhibitory activity of grain polyphenols in colored barley cultivars, with IC50 values comparable to the α-amylase inhibitory potencies observed in our Vilepi preparation [Yao et al., 2010]. Idehen et al., (2017) comprehensively reviewed the antioxidant phytochemicals of barley, consistent with the phenolic-mediated antioxidant activity we report. To our knowledge, this is the first study to comparatively characterize all three traditional Ayurvedic barley formulations using standardized biochemical and pharmacological methods.

The higher solid content of Vilepi implies substantially greater insoluble and soluble dietary fiber densities, including β-glucan and arabinoxylan [Feng et al., 2024; Henrion et al., 2019]. Dietary fiber exerts antihyperglycemic effects through multiple synergistic mechanisms, including delayed gastric emptying, reduced glucose absorption rate, modulation of incretin hormone release and selective prebiotic stimulation of SCFA-producing gut microbiota that improve insulin sensitivity [Jenkins & Jenkins, 1985]. Future glycemic index (GI) studies in human volunteers would be valuable for directly quantifying the in vivo glycemic impact of each preparation and determining optimal preparation-specific serving sizes for therapeutic use [Vlachos et al., 2020].

3.6 Limitations

The present study had several limitations that need consideration. The in vitro α-amylase inhibition assay does not replicate the complex physiological conditions of the human gastrointestinal tract, such as variations in gastric pH, the presence of other macronutrients, compensatory pancreatic enzyme secretion and gastrointestinal transit time, all of which influence starch digestion and glucose absorption. Therefore, extrapolation of these findings to clinical antidiabetic efficacy requires further validation. Future research should include in vivo postprandial glucose response and glycemic index studies in human participants to validate these preliminary results. Additionally, variability in barley cultivars, harvest seasons and storage conditions may affect the concentrations and profiles of bioactive compounds. These factors were not controlled in the present study and should be standardized in future investigations. Antioxidant capacity was assessed solely using the DPPH assay, which measures hydrogen-donating activity but does not capture other relevant antioxidant mechanisms, such as metal ion chelation or inhibition of lipid peroxidation. Employing a broader panel of antioxidant assays would provide a more comprehensive evaluation of antioxidant activity. Furthermore, this study did not quantify specific carbohydrate subtypes, particularly β-glucan, which is hypothesized to be a key contributor to α-amylase inhibition through viscosity-mediated mechanisms. The absence of direct β-glucan measurements limits mechanistic interpretation. Future studies should incorporate enzymatic or chromatographic methods to quantify β-glucan, resistant starch and soluble versus insoluble dietary fiber fractions in each formulation to better elucidate their contributions to observed bioactivities. Finally, the preparation-specific bioactivity differences reported here were characterized for a single barley cultivar prepared under laboratory conditions; confirmation across cultivars and traditional preparation settings, and profiling of individual phenolic species by HPLC or LC-MS, would strengthen the translation of these findings.

4. CONCLUSION

This study provides a systematic and comparative characterization of Manda, Peya and Vilepi, revealing formulation-specific bioactivity profiles. Manda, the high-dilution aqueous supernatant, excels in antioxidant capacity due to its enrichment in water-soluble polyphenols, while paradoxically exhibiting elevated fat content. Vilepi, the most concentrated and solid-retaining preparation, delivers superior protein and carbohydrate density alongside the most potent α-amylase inhibitory activity, which is mechanistically attributable to the synergistic actions of β-glucan, insoluble fiber and proteinaceous macromolecules. Peya occupied a balanced intermediate position for all measured parameters. Processing methodology and water-to-grain ratio are the primary determinants of the physicochemical, nutritional and bioactive properties of these preparations.

Beyond these biochemical findings, the present results reframe the classical preparations in a broader scientific context. Manda, Peya and Vilepi are not merely heritage foods; they constitute a graduated, formulation-specific therapeutic diet whose classical prescriptions align closely with the bioactivity gradients documented here. The ancient practice of adjusting the water-to-grain ratio and processing intensity to modulate nutrient density, phenolic release and enzyme-inhibitory capacity represents an empirical, culturally embedded form of therapeutic dietetics whose rationale is now measurable in biochemical terms. By grounding a Samsarjana Krama-derived dietary sequence in comparative macronutrient, antioxidant and enzymatic data, this work illustrates how traditional knowledge systems and evidence-based functional food science can be integrated in a single analytical framework, an approach of direct relevance to ethnopharmacology and metabolic science. These data provide a scientific rationale for the differential therapeutic prescriptions of these preparations in Ayurvedic diabetes management and lay the groundwork for translational research. Priorities for future investigations include in vivo glycemic index studies in human volunteers, clinical trials evaluating adjunctive use in type 2 DM management, comprehensive metabolomic profiling of bioactive constituents and standardization of preparation protocols for potential functional food product development.

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AUTHOR CONTRIBUTIONS

Netra Bansal: Methodology, Investigation, Formal Analysis, Writing - review & editing.

Saptami Kanekar: Methodology, Investigation, Formal Analysis, Writing - original draft

Chrysilla Espy Vaz: Formal Analysis, Writing - review & editing

Sreeraj P: Conceptualization, Methodology, Validation, Writing - original draft

All the authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Ethics Statement

This study involved only in-vitro experiments; no human subjects or animal experiments were conducted. Therefore, ethical approval was not required.

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Data Availability Statement

No datasets were generated or analyzed during the current study. All the data are within the manuscript.

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