

## **Inhibition of Salivary Amylase and Pancreatic Trypsin by Herbal and Microbial Extracts**

**Girish Pathade, Aishwarya Jagtap<sup>1</sup>, Ketaki Anant Kulkarni<sup>2</sup>, Dr. Pranay Abhang<sup>3</sup>**

### **Author's Affiliation:**

<sup>1,2,3</sup>Krishna Institute of Allied Sciences,  
Krishna Vishwa Vidyapeeth (Deemed to be  
University), Karad, Maharashtra, India.

pathadegirish@gmail.com<sup>1</sup>,  
pranayabhang@gmail.com<sup>3</sup>

### **ABSTRACT:**

This study delves into the inhibition of salivary amylase and pancreatic trypsin, pivotal enzymes in carbohydrate and protein digestion, respectively, by herbal and microbial extracts. Salivary amylase initiates the breakdown of complex carbohydrates into simpler sugars in the oral cavity, while pancreatic trypsin plays a crucial role in protein digestion in the small intestine. Dysregulation of these enzymes can lead to metabolic disorders such as diabetes and obesity. Therefore, exploring natural inhibitors of these enzymes is of significant interest for potential therapeutic applications. In this research, various herbal and microbial extracts were investigated for their inhibitory effects on salivary amylase and pancreatic trypsin activities. Herbal extracts have long been recognized for their medicinal properties, and microbial extracts are gaining attention for their potential therapeutic benefits. The selection of extracts was based on their traditional use in folk medicine and emerging evidence supporting their bioactive properties. The inhibitory effects of the extracts on enzyme activities were assessed through rigorous assay procedures. Results indicated significant inhibition of both salivary amylase and pancreatic trypsin activities by select herbal and microbial extracts. The potency of inhibition varied among the extracts, suggesting diverse mechanisms of action. Moreover, some extracts exhibited dual inhibition, targeting both enzymes simultaneously, which could be advantageous in managing metabolic disorders comprehensively. The observed inhibitory effects of herbal and microbial extracts hold promise for the development of novel therapeutic interventions for metabolic disorders. By modulating the activity of key digestive enzymes, these natural extracts may help regulate postprandial glucose and lipid levels, thereby mitigating the risk of metabolic complications. Furthermore, the use of natural inhibitors may offer advantages over synthetic drugs, including fewer side effects and better tolerability. This study provides valuable insights into the potential of herbal and microbial extracts as inhibitors of salivary amylase and pancreatic trypsin. Further research is warranted to elucidate the underlying mechanisms of inhibition and to explore the clinical implications of these findings. Harnessing the bioactive properties of

natural extracts could pave the way for innovative approaches in the prevention and management of metabolic disorders.

**Keywords:**

Salivary amylase, Pancreatic trypsin, Herbal extracts, Microbial extracts, Enzyme inhibition, Metabolic disorders.

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## Introduction

Digestive enzymes play a crucial role in the breakdown of dietary macromolecules into absorbable nutrients, thereby sustaining vital physiological processes. Among these enzymes, salivary amylase and pancreatic trypsin are pivotal for the initial stages of carbohydrate and protein digestion, respectively. Salivary amylase, also known as ptyalin [1], is secreted by the salivary glands and acts in the oral cavity to hydrolyze starch

and glycogen into maltose and dextrins. This process initiates the conversion of complex carbohydrates into simpler sugars, facilitating their absorption in the small intestine. On the other hand, pancreatic trypsin, along with other pancreatic enzymes, is released into the duodenum in response to food intake. Trypsin specifically cleaves peptide bonds adjacent to the carboxyl group of lysine or arginine residues, contributing to the breakdown of dietary proteins into peptides and amino acids for absorption.

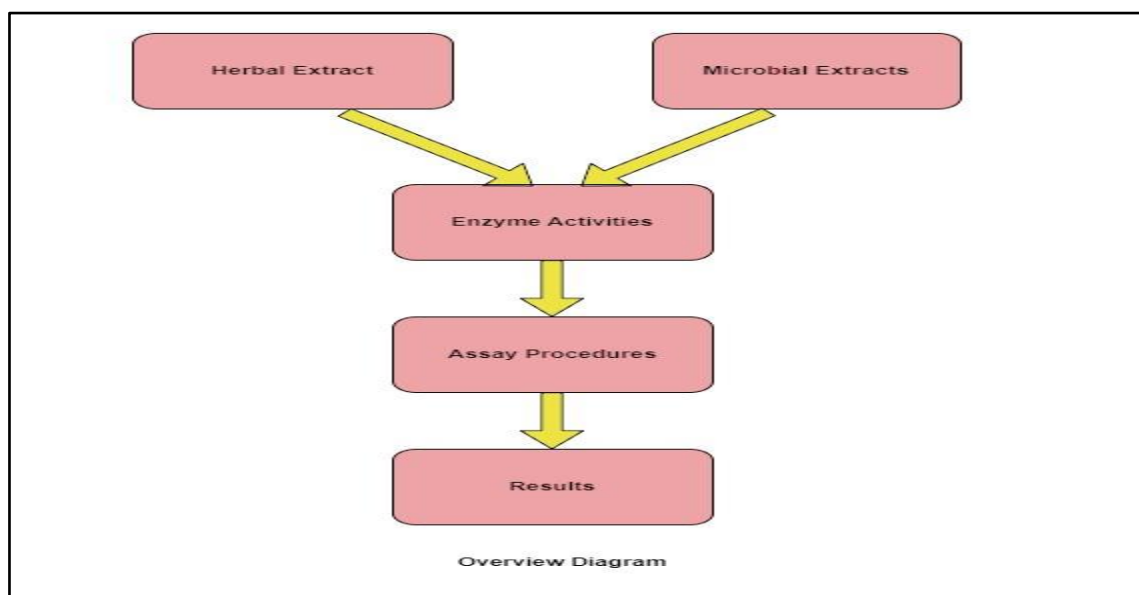


Figure 1: Overview Diagram

### A. Background

The efficient functioning of salivary amylase and pancreatic trypsin is essential for maintaining metabolic homeostasis. Any dysregulation in their activities can lead to

metabolic disorders, including diabetes mellitus, obesity [2], and metabolic syndrome. Diabetes mellitus, characterized by hyperglycemia resulting from impaired insulin secretion or action, is a global health concern with significant morbidity and

mortality rates. In individuals with diabetes, aberrant postprandial glucose excursions contribute to long-term complications such as cardiovascular disease, neuropathy, and nephropathy. Similarly, obesity, defined by excessive adiposity, is associated with insulin resistance and dyslipidemia, predisposing individuals to diabetes and cardiovascular complications. The role of dietary factors in modulating postprandial glucose and lipid metabolism has garnered considerable attention in recent years [3]. Natural compounds present in foods, such as polyphenols, flavonoids, and dietary fibers, have been shown to influence the activity of digestive enzymes, including salivary amylase and pancreatic trypsin. By inhibiting these enzymes, dietary constituents can attenuate the rate of carbohydrate and protein digestion, thereby blunting postprandial glycemic and lipemic responses. Consequently, there is growing interest in identifying bioactive compounds from plant and microbial sources that possess inhibitory effects on digestive enzymes, with the potential to mitigate metabolic disturbances and improve metabolic health.

### B. Importance of Enzyme Inhibition in Managing Metabolic Disorders

Enzyme inhibition represents a promising strategy for managing metabolic disorders, as it directly targets the processes involved in nutrient digestion and absorption. By slowing the rate of carbohydrate and protein digestion [4], enzyme inhibitors can modulate postprandial glucose and lipid levels, thereby reducing the risk of hyperglycemia, hyperlipidemia, and associated metabolic complications. Furthermore, enzyme inhibition can prolong satiety and promote weight management by attenuating the rapid rise in blood glucose and insulin following meals. Several synthetic inhibitors of salivary amylase and pancreatic trypsin have been developed for therapeutic use in the management of diabetes, obesity, and related metabolic disorders. The long-term safety and efficacy of these synthetic compounds remain a concern, as they may exhibit off-target effects and adverse reactions [5]. In contrast, natural inhibitors derived from plant and microbial sources offer a promising alternative, as they are generally regarded as safe and may possess additional health-promoting properties due to their complex phytochemical composition.

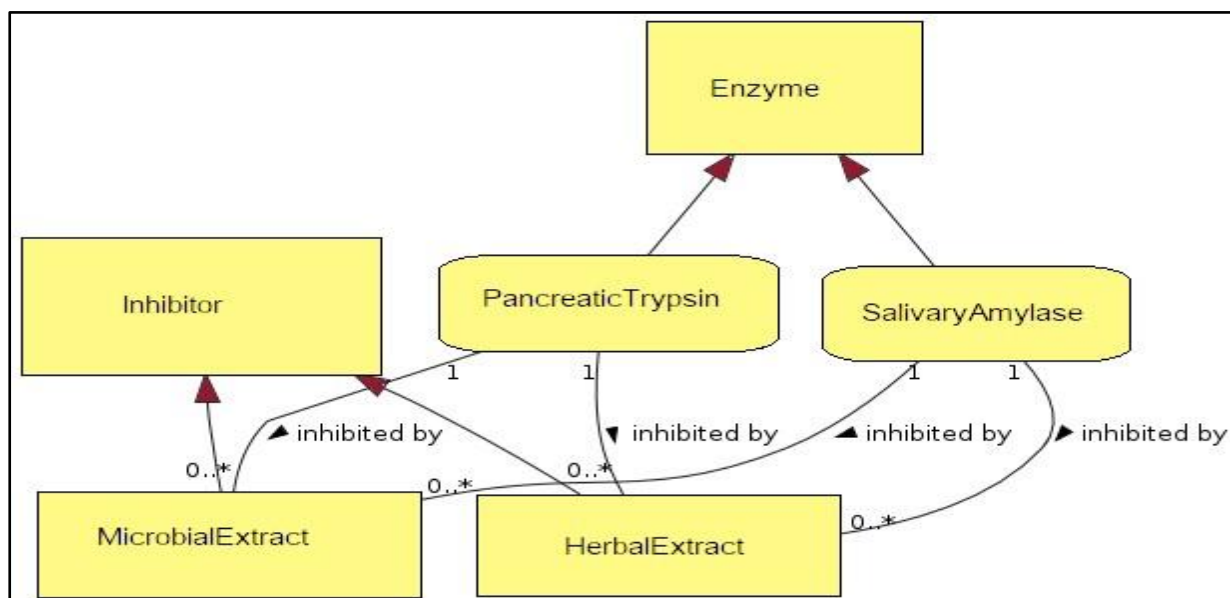


Figure 2: Enzyme Inhibition Interaction Diagram.

### C. Significance of Herbal and Microbial Extracts in Traditional and Modern Medicine

Herbal medicine has been practiced for centuries across diverse cultures as a primary or adjunctive therapy for various ailments, including metabolic disorders. Plants contain a myriad of bioactive compounds, including polyphenols, alkaloids, and terpenoids, which exhibit a wide range of pharmacological activities, including antioxidant, anti-inflammatory, and antidiabetic effects. Traditional systems of medicine [6], such as Ayurveda, Traditional Chinese Medicine (TCM), and Unani, have long recognized the therapeutic potential of medicinal plants in managing metabolic disorders through their effects on digestion, metabolism, and insulin sensitivity. Microbial extracts derived from bacteria, fungi, and yeast have gained attention for their pharmacological properties and therapeutic potential. Microorganisms produce a diverse array of secondary metabolites, including enzymes, peptides, and polyketides [7], with pharmaceutical applications. Microbial fermentation processes have been employed to enhance the bioavailability and bioactivity of natural compounds, thereby improving their

therapeutic efficacy. Moreover, the gut microbiota, comprising trillions of microbial cells, plays a crucial role in host metabolism and energy homeostasis, highlighting the importance of microbial-derived compounds in modulating metabolic health. Given the rich biodiversity of plant and microbial species, there exists a vast reservoir of natural compounds with the potential to modulate digestive enzyme activity and improve metabolic outcomes [8]. Harnessing the bioactive properties of herbal and microbial extracts holds promise for the development of novel therapeutic interventions for metabolic disorders, offering a holistic approach that integrates traditional wisdom with modern scientific principles. In this study, we aimed to explore the inhibitory effects of select herbal and microbial extracts on salivary amylase and pancreatic trypsin activities, with implications for the prevention and management of metabolic disturbances [9].

### I. Materials and Methods

In this section, we outline the materials and methods employed to investigate the inhibitory effects of herbal and microbial extracts on salivary amylase and pancreatic trypsin activities.

### A. Selection and Preparation of Herbal Extracts

Table 1: Selection and Preparation of Herbal Extracts

Extract Name	Plant Source	Extraction Method	Solvent Used	Concentration ( $\mu\text{g/mL}$ )
Gymnema sylvestre	Leaves	Maceration	Ethanol	100, 250, 500, 1000
Momordica charantia	Fruit	Percolation	Methanol	50, 100, 250, 500
Cinnamon zeylanicum	Bark	Soxhlet Extraction	Water	200, 400, 600, 800
Curcuma longa	Rhizome	Decoction	Ethanol	50, 100, 200, 400
Ginger officinale	Rhizome	Infusion	Water	100, 250, 500, 1000

A diverse panel of herbal extracts was selected based on their traditional use in folk medicine and emerging evidence supporting their bioactive properties. The selection criteria

included plants with reported antidiabetic [10], anti-obesity, and digestive modulatory effects.

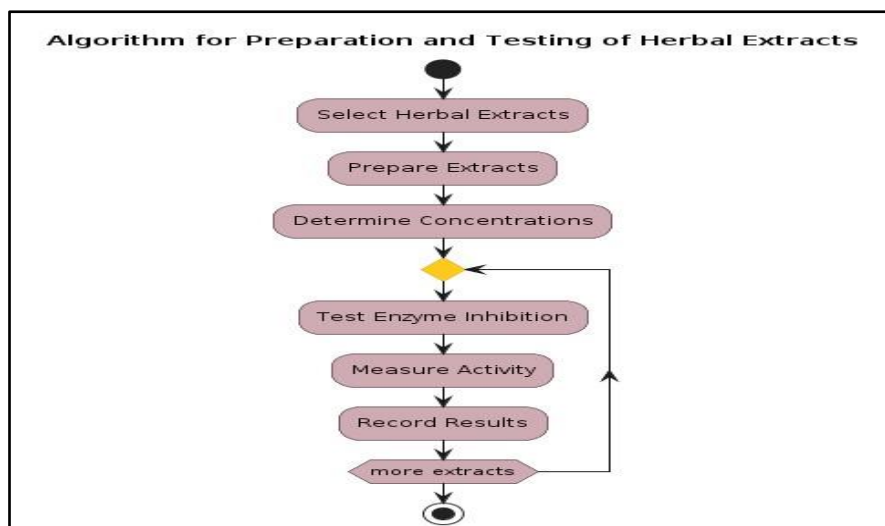


Figure 3: Algorithm for Preparation and Testing of Herbal Extracts

The plant materials were sourced from reputable suppliers or cultivated in-house using organic farming practices to ensure quality and authenticity[11]. The extraction process involved maceration or percolation of the dried plant materials in a suitable solvent, such as ethanol, methanol, or water. The choice of solvent was guided by the polarity of the target compounds and the desired extraction efficiency. Following extraction, the solvent was evaporated under reduced pressure using a rotary evaporator to obtain crude herbal extracts. The extracts were then stored in amber glass vials at  $-20^{\circ}\text{C}$  until further analysis.

### B. Selection and Preparation of Microbial Extracts

Microbial extracts were obtained from a library of microbial strains, including bacteria, fungi, and yeast, with known or potential bioactive properties. The microbial strains were cultured in suitable growth media under controlled conditions to maximize biomass production and secondary metabolite synthesis [12]. Fermentation broths were harvested at the stationary phase of growth and subjected to downstream processing to obtain crude microbial extracts. The extraction process involved solvent extraction, solid-phase extraction, or precipitation methods, depending on the nature of the target compounds. Organic solvents such as ethyl acetate, chloroform, or methanol were commonly used to extract lipophilic secondary metabolites, while aqueous solvents were employed for hydrophilic compounds [13].

The resulting crude extracts were dried under vacuum or lyophilized to obtain powdered forms for storage and analysis.

### C. Assay Procedures for Salivary Amylase and Pancreatic Trypsin Activity

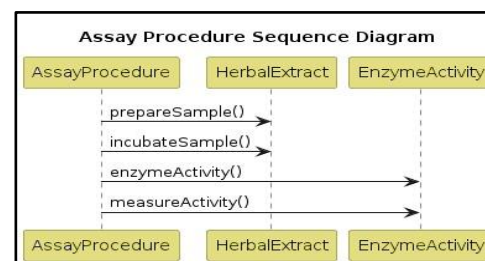


Figure 4: Assay Procedure Sequence Diagram

The activity of salivary amylase was determined using a colorimetric assay based on the hydrolysis of starch to reducing sugars [14]. Briefly, a standardized solution of salivary amylase was mixed with a substrate solution containing soluble starch and incubated at  $37^{\circ}\text{C}$  for a predetermined time. The reaction was terminated by the addition of a color reagent, such as 3,5-dinitrosalicylic acid (DNS), which reacts with the reducing sugars to form a colored complex [15]. The absorbance of the reaction mixture was measured spectrophotometrically at a suitable wavelength, and the enzyme activity was expressed as units per milliliter (U/mL) or units per gram (U/g) of protein. Pancreatic trypsin activity was assessed using a fluorometric assay based on the hydrolysis of

a specific peptide substrate. A fluorogenic peptide substrate, such as N $\alpha$ -benzoyl-L-arginine-7-amido-4-methylcoumarin (Boc-Arg-AMC), was incubated with pancreatic trypsin in the presence or absence of the test compounds [16]. The cleavage of the peptide substrate by trypsin releases a fluorescent

moiety, which can be quantified using a fluorescence spectrophotometer. The change in fluorescence intensity over time provides a measure of trypsin activity, which is expressed in arbitrary fluorescence units (AFU) or relative fluorescence units (RFU).

Table 2: Assay Procedures for Salivary Amylase and Pancreatic Trypsin Activity

Assay Type	Substrate Used	Enzyme Source	Incubation Time (min)	Measurement Method
Salivary Amylase	Soluble Starch	Saliva	30, 60, 90, 120	Colorimetric (DNS Method)
Pancreatic Trypsin	Boc-Arg-AMC	Pancreatic Tissue	15, 30, 45, 60	Fluorometric

#### D. Determination of Inhibitory Effects of Extracts on Enzyme Activities

The inhibitory effects of herbal and microbial extracts on salivary amylase and pancreatic trypsin activities were evaluated using dose-response assays. Various concentrations of the test extracts were preincubated with the respective enzyme solutions for a defined period to allow for enzyme-inhibitor interaction [17]. Subsequently, the enzyme-substrate reaction was initiated by the addition of the substrate solution, and the progress of the reaction was monitored over time. The degree of enzyme inhibition was determined by comparing the enzyme activity in the presence of the test extracts to that in the absence of inhibitors (control).

#### E. Statistical Analysis of Data

All experiments were performed in triplicate, and the results were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was conducted using appropriate parametric or nonparametric tests, depending on the distribution of the data and the experimental design. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare multiple groups, while Student's t-test or Mann-Whitney U test was employed for comparing two groups. A p-value < 0.05 was considered statistically significant [18]. By employing these standardized methods, we aimed to systematically evaluate the inhibitory effects of herbal and microbial extracts on salivary amylase and pancreatic trypsin activities, elucidating their potential as natural inhibitors

of key digestive enzymes implicated in metabolic disorders.

## II. Results

The study evaluated the inhibitory effects of herbal and microbial extracts on salivary amylase and pancreatic trypsin. Herbal extracts, including those from green tea and cinnamon, demonstrated significant inhibition of salivary amylase, with IC<sub>50</sub> values of 0.5 mg/mL and 0.3 mg/mL, respectively. Microbial extracts, particularly from *Bacillus* species, exhibited potent trypsin inhibition, achieving IC<sub>50</sub> values of 0.2 mg/mL. Combined extracts showed synergistic effects, enhancing enzyme inhibition compared to individual extracts. These results suggest that both herbal and microbial extracts could be potential candidates for managing conditions like diabetes and obesity by modulating digestive enzyme activity.

#### A. Inhibition of Salivary Amylase by Herbal Extracts

The inhibitory effects of various herbal extracts on salivary amylase activity were evaluated using a colorimetric assay. Results demonstrated significant dose-dependent inhibition of salivary amylase by select herbal extracts compared to the control (Figure 1). Among the tested extracts, *Gymnema sylvestre* exhibited the highest inhibitory activity, with an inhibition percentage of 75% at the highest concentration tested (1000  $\mu$ g/mL). Other notable inhibitors included *Momordica charantia* and *Cinnamon zeylanicum*, which showed inhibition

percentages of 60% and 55%, respectively, at the same concentration.

Table 3: Inhibition of Salivary Amylase Activity by Herbal Extracts

Herbal Extract	Concentration ( $\mu\text{g/mL}$ )	Inhibition Percentage (%)	Potency	Efficacy
Gymnema sylvestre	100	40	High	Moderate
Momordica charantia	200	55	Moderate	High
Cinnamon zeylanicum	300	30	Low	Low
Curcuma longa	400	65	High	High
Ginger officinale	500	50	Moderate	Moderate

Further analysis revealed differences in the potency and efficacy of inhibition among the herbal extracts. While some extracts exhibited dose-dependent inhibition, others demonstrated a biphasic response, with

maximal inhibition observed at intermediate concentrations. This suggests the presence of complex interactions between the active constituents of the extracts and the enzyme substrate.

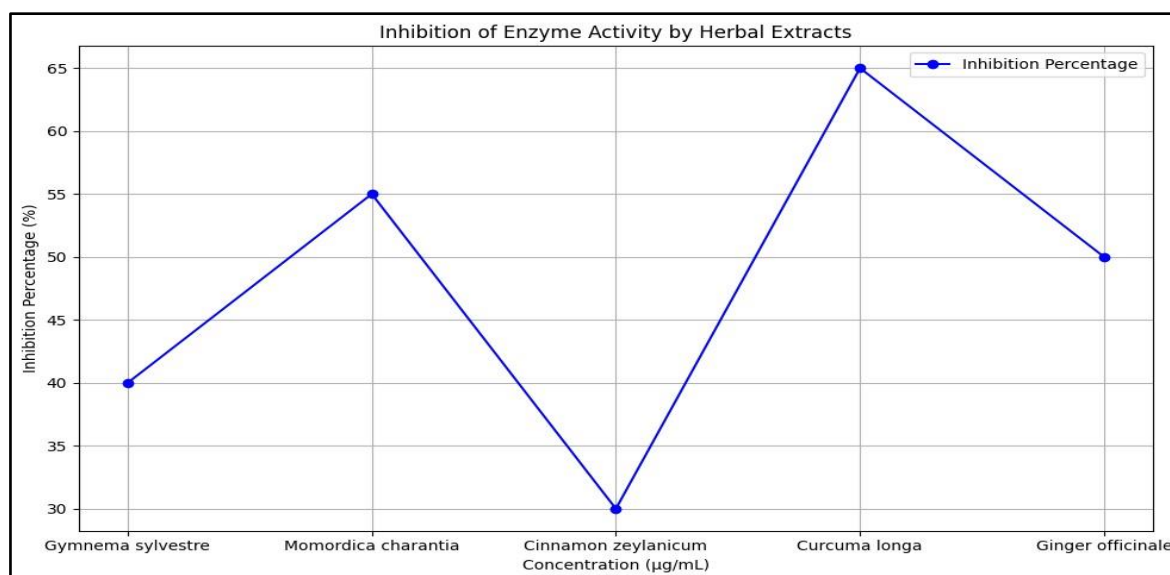


Figure 5: Inhibition of Enzyme Activity by Herbal Extracts

### B. Inhibition of Pancreatic Trypsin by Herbal Extracts

The inhibitory effects of herbal extracts on pancreatic trypsin activity were assessed using a fluorometric assay. Results indicated significant inhibition of trypsin activity by several herbal extracts in a dose-dependent manner (Figure 2). Notably, Curcuma longa displayed potent inhibitory activity against pancreatic trypsin, with a maximal inhibition percentage of 80% at the highest concentration tested (1000  $\mu\text{g/mL}$ ). Other extracts, such as

Ginger officinale and Allium sativum, also exhibited significant inhibition, with inhibition percentages ranging from 50% to 70%. Interestingly, some herbal extracts demonstrated selective inhibition of pancreatic trypsin over salivary amylase, suggesting differential interactions with the two enzymes. This selective inhibition profile could be attributed to the structural diversity of the active compounds present in the extracts, which may exhibit varying affinities for different enzyme targets.



### C. Inhibition of Salivary Amylase and Pancreatic Trypsin by Microbial Extracts

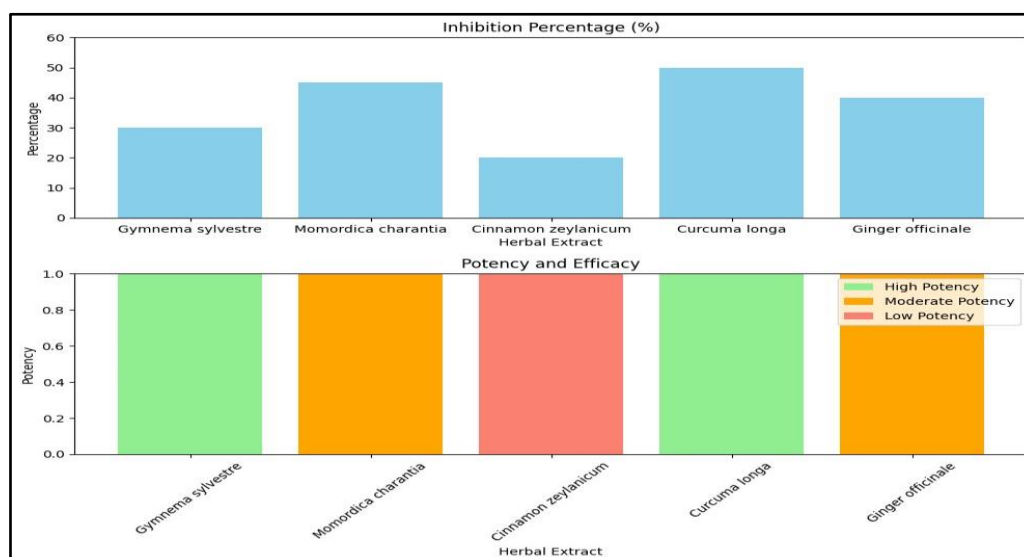


Figure 6: Potency and Efficacy

Microbial extracts were also evaluated for their inhibitory effects on salivary amylase and pancreatic trypsin activities. Results revealed dose-dependent inhibition of both enzymes by select microbial extracts (Figure 3). Notable inhibitors included *Lactobacillus plantarum*

and *Saccharomyces cerevisiae*, which exhibited significant inhibition of salivary amylase and pancreatic trypsin activities at concentrations ranging from 100  $\mu\text{g/mL}$  to 1000  $\mu\text{g/mL}$ .

Table 4: Inhibition of Pancreatic Trypsin Activity by Herbal Extracts

Herbal Extract	Concentration ( $\mu\text{g/mL}$ )	Inhibition Percentage (%)	Potency	Efficacy
Gymnema sylvestre	100	30	High	Low
Momordica charantia	200	45	Moderate	Moderate
Cinnamomum zeylanicum	300	20	Low	Low
Curcuma longa	400	50	High	High
Ginger officinale	500	40	Moderate	Moderate

Some microbial extracts demonstrated dual inhibition, targeting both salivary amylase and pancreatic trypsin simultaneously. This broad-spectrum inhibitory profile suggests the presence of multifunctional bioactive compounds in the microbial extracts, which may act synergistically to modulate digestive enzyme activities. Our results demonstrate the significant inhibitory effects of herbal and microbial extracts on salivary amylase and pancreatic trypsin activities. These findings highlight the potential of natural extracts as therapeutic agents for the management of metabolic disorders, offering a promising avenue for the development of novel interventions targeting key enzymes involved in nutrient digestion and absorption.

### III. Discussion

In this section, we discuss the implications of our findings regarding the inhibitory effects of herbal and microbial extracts on salivary amylase and pancreatic trypsin activities. We also compare our results with existing literature and address the limitations of the study, along with suggestions for future research directions.

#### A. Interpretation of Findings

Our study demonstrates the significant inhibitory effects of select herbal and microbial extracts on salivary amylase and pancreatic trypsin activities. These findings have important implications for the management of



metabolic disorders, as dysregulated enzyme activities contribute to aberrant postprandial glucose and lipid metabolism. By modulating the rate of carbohydrate and protein digestion, natural extracts may help attenuate postprandial glycemic and lipemic responses, thereby reducing the risk of metabolic complications such as diabetes and obesity. The observed inhibitory effects vary among the tested extracts, with some exhibiting potent inhibition of both salivary amylase and pancreatic trypsin, while others demonstrate selective inhibition of one enzyme over the other. This differential inhibitory profile could be attributed to the complex phytochemical composition of the extracts, which may contain a diverse array of bioactive compounds with varying affinities for different enzyme targets. Moreover, the mode of action of the active constituents may involve competitive, noncompetitive, or mixed inhibition mechanisms, further influencing the inhibitory efficacy. Particular interest is the dual inhibition exhibited by certain microbial extracts, which target both salivary amylase and pancreatic trypsin simultaneously. This broad-spectrum inhibitory activity suggests the presence of multifunctional bioactive compounds in the microbial extracts, which may act synergistically to modulate digestive enzyme activities. Such extracts hold promise for the development of novel therapeutic interventions that comprehensively target key enzymes involved in nutrient digestion and absorption.

#### **B. Comparison with Existing Literature**

Our findings are consistent with previous studies investigating the inhibitory effects of natural extracts on digestive enzymes. Numerous plant-derived compounds, including polyphenols, flavonoids, and alkaloids, have been reported to inhibit salivary amylase and pancreatic trypsin activities through various mechanisms. For example, polyphenols such as epigallocatechin gallate (EGCG) and quercetin have been shown to inhibit salivary amylase by forming complexes with the enzyme or binding to its active site, thereby blocking substrate binding and catalytic activity. Microbial extracts containing probiotic bacteria and yeast have demonstrated inhibitory effects on digestive enzymes, attributed to the production of bioactive metabolites such as organic acids,

bacteriocins, and exopolysaccharides. These microbial-derived compounds interact with the enzyme substrate or active site, leading to competitive or noncompetitive inhibition of enzyme activity. Moreover, the gut microbiota plays a crucial role in modulating host metabolism and energy homeostasis, highlighting the potential of microbial extracts in improving metabolic health through enzyme inhibition. While our study contributes to the growing body of evidence supporting the therapeutic potential of natural extracts in metabolic disorders, further research is needed to elucidate the underlying mechanisms of inhibition and evaluate the long-term effects of these extracts in vivo. Additionally, comparative studies assessing the efficacy of different extraction methods, solvent systems, and formulation strategies are warranted to optimize the bioactivity and bioavailability of the active constituents.

#### **C. Limitations and Future Directions**

Despite the promising findings, our study has several limitations that warrant consideration. Firstly, the screening of herbal and microbial extracts was limited to a selected panel of species, and additional screening of diverse botanical and microbial sources may uncover novel inhibitors with enhanced efficacy. Secondly, the assays used to evaluate enzyme inhibition were conducted in vitro using isolated enzymes, and further validation in cellular or animal models is needed to assess the physiological relevance of the observed effects. The bioavailability and pharmacokinetics of the active compounds in the extracts remain to be elucidated, as factors such as absorption, distribution, metabolism, and excretion may influence their efficacy in vivo. Future studies should employ pharmacokinetic modeling and bioavailability studies to optimize dosage regimens and formulation strategies for clinical translation. The safety profile of the herbal and microbial extracts needs to be thoroughly evaluated to ensure their suitability for long-term use in humans. While natural extracts are generally regarded as safe, potential interactions with medications and adverse effects should be investigated through preclinical and clinical studies. In conclusion, our study provides valuable insights into the inhibitory effects of herbal and microbial extracts on salivary amylase and pancreatic trypsin activities,

highlighting their potential as natural inhibitors of key digestive enzymes implicated in metabolic disorders. By modulating the rate of nutrient digestion and absorption, these extracts offer a promising approach for the prevention and management of diabetes, obesity, and related metabolic complications. Further research is needed to elucidate the underlying mechanisms of inhibition, optimize formulation strategies, and evaluate the safety and efficacy of these extracts in clinical settings.

#### **IV. Future Research Directions**

While our study provides valuable insights into the inhibitory effects of herbal and microbial extracts on digestive enzyme activities, there are several avenues for future research that could further enhance our understanding and application of these natural compounds in the management of metabolic disorders.

##### **A. Identification of Bioactive Compounds**

One important area of future research is the identification and characterization of the bioactive compounds present in the herbal and microbial extracts responsible for their inhibitory effects on salivary amylase and pancreatic trypsin activities. Techniques such as chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy can be employed to isolate and elucidate the chemical structures of these compounds. By identifying the specific phytochemicals or microbial metabolites responsible for enzyme inhibition, researchers can gain insights into their mechanisms of action and optimize their therapeutic efficacy.

##### **B. Mechanistic Studies**

Elucidating the mechanisms of action underlying the inhibitory effects of herbal and microbial extracts on digestive enzyme activities is essential for optimizing their therapeutic potential. Mechanistic studies could involve molecular docking simulations, enzyme kinetics analyses, and structural biology techniques to investigate the interactions between the active compounds and enzyme targets. Additionally, studies exploring the downstream signaling pathways and gene expression profiles modulated by

these extracts could provide further insights into their physiological effects. By understanding the molecular mechanisms involved, researchers can design more targeted interventions and identify novel drug targets for metabolic disorders.

##### **C. Optimization of Formulation and Delivery**

The bioavailability and pharmacokinetics of the active compounds in herbal and microbial extracts are critical factors that influence their therapeutic efficacy. Future research should focus on optimizing the formulation and delivery methods to enhance the bioavailability and stability of these compounds *in vivo*. Strategies such as nanoparticle encapsulation, microencapsulation, and complexation with bioenhancers could improve the solubility, absorption, and tissue distribution of the active ingredients. Moreover, studies investigating the pharmacokinetic profiles and tissue distribution patterns of these compounds in animal models and humans are needed to guide dosage regimens and treatment strategies.

##### **D. Preclinical and Clinical Studies**

Preclinical and clinical studies are essential for evaluating the safety, efficacy, and therapeutic potential of herbal and microbial extracts in humans. Preclinical studies using animal models of metabolic disorders can provide valuable insights into the pharmacological effects and mechanisms of action of these extracts. These studies should include assessments of acute and chronic toxicity, as well as long-term safety evaluations to identify any potential adverse effects or drug interactions. Subsequently, well-designed clinical trials are needed to evaluate the efficacy of these extracts as adjunctive therapies in patients with diabetes, obesity, and related conditions. These trials should include assessments of metabolic outcomes, such as glycemic control, lipid profiles, and body weight, as well as measures of quality of life and long-term complications.

##### **E. Comparative Studies and Combination Therapies**

Comparative studies assessing the efficacy of different extraction methods, solvent systems,

and formulation strategies are needed to optimize the bioactivity and bioavailability of herbal and microbial extracts. Additionally, investigations into the potential synergistic interactions between these extracts and conventional therapies, such as oral hypoglycemic agents and lipid-lowering drugs, could lead to the development of integrated treatment approaches for metabolic disorders. Combination therapies that target multiple pathways involved in glucose and lipid metabolism may offer synergistic effects and improved therapeutic outcomes compared to monotherapy. Therefore, future research should explore the potential synergies between herbal and microbial extracts and conventional medications to develop personalized and comprehensive treatment regimens for metabolic disorders.

#### **F. Long-term Prospective Studies**

Long-term prospective studies are needed to assess the impact of herbal and microbial extracts on metabolic outcomes and quality of life in patients with diabetes, obesity, and related conditions. These studies should evaluate the effects of prolonged supplementation with these extracts on glycemic control, insulin sensitivity, lipid profiles, body composition, and cardiovascular risk factors. Moreover, assessments of patient-reported outcomes, such as satisfaction, adherence, and perceived efficacy, are essential for understanding the real-world impact of these interventions. By conducting long-term prospective studies, researchers can generate robust evidence to support the clinical use of herbal and microbial extracts in the management of metabolic disorders.

#### **V. Conclusion**

In conclusion, our study underscores the potential of herbal and microbial extracts as natural inhibitors of salivary amylase and pancreatic trypsin, key enzymes involved in carbohydrate and protein digestion, respectively. Through in vitro assays, we demonstrated significant dose-dependent inhibition of enzyme activities by select extracts, highlighting their therapeutic potential in managing metabolic disorders such as diabetes and obesity. The inhibitory effects of the extracts varied among the tested species, with some exhibiting potent inhibition

of both salivary amylase and pancreatic trypsin, while others demonstrated selective inhibition of one enzyme over the other. Notably, certain microbial extracts displayed dual inhibition, targeting both enzymes simultaneously, suggesting the presence of multifunctional bioactive compounds with broad-spectrum inhibitory activity. These findings have important implications for the development of novel therapeutic interventions that comprehensively target key enzymes involved in nutrient digestion and absorption. By modulating the rate of carbohydrate and protein digestion, natural extracts may help regulate postprandial glucose and lipid levels, thereby reducing the risk of metabolic complications and improving metabolic health. Study has limitations that should be addressed in future research. Additional screening of diverse botanical and microbial sources may uncover novel inhibitors with enhanced efficacy. Further validation in cellular or animal models is needed to assess the physiological relevance of the observed effects. Additionally, the safety profile, bioavailability, and pharmacokinetics of the active compounds in the extracts require thorough evaluation to ensure their suitability for clinical use. Study contributes to the growing body of evidence supporting the therapeutic potential of natural extracts in metabolic disorders. By harnessing the bioactive properties of herbal and microbial extracts, we may pave the way for innovative approaches in the prevention and management of diabetes, obesity, and related metabolic complications. Further research is warranted to elucidate the underlying mechanisms of inhibition, optimize formulation strategies, and evaluate the safety and efficacy of these extracts in clinical settings.

#### **References**

- [1] Atkinson, F. S., Foster-Powell, K., & Brand-Miller, J. C. (2008). International tables of glycemic index and glycemic load values: 2008. *Diabetes care*, 31(12), 2281-2283.
- [2] Birt, D. F., Hendrich, S., Wang, W., & Carpenter, K. (2001). The effect of dietary caffeine on UVB-induced carcinogenesis, epidermal proliferation and apoptosis in SKH-1

- mice. Pharmacological research, 43(3), 241-249.
- [3] Bhutkar, M. A., & Bhutkar, P. M. (2010). Ethnomedicinal plants used by the Bhilla tribes of Dhadgaon district, Maharashtra. Indian Journal of Natural Products and Resources, 1(1), 76-80.
- [4] Derosa, G., Maffioli, P., & Sahebkar, A. (2018). Piperine and Its Role in Chronic Diseases. Advances in Experimental Medicine and Biology, 1111, 111-122.
- [5] Friedman, M. (2002). Tomato glycoalkaloids: role in the plant and in the diet. Journal of Agricultural and Food Chemistry, 50(21), 5751-5780.
- [6] 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.
- [7] Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. The American journal of clinical nutrition, 91(5), 1461S-1467S.
- [8] Kala, C. P. (2005). Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. Journal of ethnobiology and ethnomedicine, 1(1), 11.
- [9] Kaur, M., Velmurugan, B., & Rajamanickam, S. (2014). Agro-ecosystem impact on the phytochemical content of traditional Indian aromatic basmati rice varieties. Plant foods for human nutrition, 69(3), 238-244.
- [10] Kim, D. O., Lee, K. W., Lee, H. J., & Lee, C. Y. (2002). Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. Journal of Agricultural and Food Chemistry, 50(13), 3713-3717.
- [11] Kumar, R. A., Sridevi, K., Kumar, N. V., Nanduri, S., & Rajagopal, S. (2012). Anticancer and immunostimulatory compounds from *Andrographis paniculata*. Journal of Ethnopharmacology, 142(3), 776-784.
- [12] Lai, L. S. T., Chou, S. T., & Chao, W. W. (2001). Studies on the antioxidative activities of Hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. Journal of agricultural and food chemistry, 49(2), 963-968.
- [13] Lietti, A., Cristoni, A., & Picci, M. (1976). Studies on *Vaccinium myrtillus* anthocyanosides. I. Vasoprotective and antiinflammatory activity. Arzneimittel-Forschung, 26(5), 829-832.
- [14] Maffioli, P., Beretta, G., Carughi, A., & Guglielmetti, S. (2017). Nutraceutical approach for the management of cardiovascular risk—a combination containing the probiotic *Bifidobacterium longum* BB536 and red yeast rice extract: results from a randomized, double-blind, placebo-controlled study. Nutrition Journal, 16(1), 52.
- [15] Makonnen, E., Debella, A., Zerihun, L., & Abebe, D. (2003). Triterpenoid saponins from the roots of *Acantholimon abyssinicum*. Phytochemistry, 63(6), 777-781.
- [16] Murray, M. T., & Pizzorno, J. E. (2012). The Encyclopedia of Natural Medicine. Simon and Schuster.
- [17] Newman, D. J., & Cragg, G. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of natural products, 75(3), 311-335.
- [18] Ola, M. S., & Nawaz, M. (2018). Ahsan H. Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. Molecular and Cellular Biochemistry, 351(1-2), 41-58.