International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

In-Vitro Investigation of Polyherbal Granules Potency on Inhibition of Alpha: Amylase Enzyme

Shukla Priya[1], Gangani Hinal[2], Maury Shweta[3]

[1]Assistant Professor, Department of Pharmacology, SSR College of Pharmacy, Sayli
[2,3]M. Pharm Student, Department of Pharmacology, SSR College of Pharmacy, Sayli

Abstract
Diabetes is an increasingly prevalent global disorder, affecting approximately 10% of the world's population. While there are various treatment options available in allopathic medicine, many of them come with undesirable side effects and can be financially burdensome. In response to these challenges, traditional plant-based approaches have emerged as promising antidiabetic treatments. In this research, we explore the potential of certain natural plants with alpha amylase inhibitory activity to mitigate the side effects associated with conventional diabetes treatments and provide more affordable and accessible alternatives for individuals managing this condition. In this study, we assess the impact of polyherbal granules and standard acarbose on α-amylase inhibition. The findings reveal that polyherbal granules demonstrate a significant inhibitory effect on alpha-amylase.

Keywords: Diabetes mellitus, Polyherbal granules, α-amylase

1. Introduction

Diabetes mellitus (DM) represents a substantial and concerning category of metabolic disorders that have far-reaching implications on a global scale (1). The hallmark of diabetes is hyperglycemia, manifests as elevated blood sugar levels, primarily stemming from inadequate insulin production in the pancreatic islets (2). Insulin, a pivotal hormone essential for glucose regulation, encounters a deficiency arising from either insufficient secretion, diminished insulin sensitivity, or a confluence of both elements in DM (3). This disruption in the intricate interplay between insulin and glucose precipitates a spectrum of complications inherent to diabetes, ranging from vascular anomalies to neuropathies, nephropathies, and retinopathies (4). Simultaneously, the metabolic shifts linked to DM exert a profound impact on essential physiological components, encompassing sugars, fats, proteins, electrolytes, and fluid balance (5). The World Health Organization (WHO) reports that diabetes impacts 422 million individuals globally, with the majority residing in low- and middle-income countries. Annually, this condition directly causes 1.5 million deaths. In recent decades, there has been a consistent increase in diabetes prevalence (6). Approximately 77 million Indians aged 18 and above have received a diagnosis of type 2 diabetes; another 25 million are prediabetic, meaning they have a higher chance of getting the condition in the future. Alarmingly, more than half of the affected population is unaware of their diabetic condition, posing potential health complications if not identified and treated promptly (7).

The therapeutic strategy treatment of diabetes depends on addressing its underlying cause, with a primary classification into two categories based on pathogenesis: Insulin-dependent diabetes mellitus (DM), or type-1 diabetes, and non-insulin dependent DM, or type-2 diabetes (8). Type-1 diabetes is characterized by autoimmune processes leading to the destruction of pancreatic islet β-cells. In contrast, Type-2 diabetes primarily develops due to insufficient release of insulin and/or the onset of insulin resistance in fat, muscle, and liver cells. This classification highlights the diverse etiological pathways that necessitate tailored treatment approaches for each subtype of diabetes (9). Therefore, Among the treatment courses for diabetes is to lower post-prandial hyperglycemia (10). Attaining this objective involves inhibiting crucial carbohydrate-hydrolyzing enzymes, comprising α-amylase and α-glucosidase, pivotal in carb digestion. α-glucosidase enzymes, particularly α-amylase, are responsible for breaking down complex carbohydrates into simpler sugars, aiding their absorption in the intestines (11). Focusing on these enzymes offers a hopeful pathway for advancing the development of primary compounds for treating diabetes (12). While there are certain α-glucosidase inhibitors that are therapeutically beneficial, using them is linked to gastrointestinal problems, including symptoms like diarrhoea, flatulence, and stomach pain (13).

In contrast, traditional Indian medicine, with a rich history spanning thousands of years, highlights various anti-diabetic plants known for their effectiveness and safety profile. Despite their global use in diabetes treatment, the mechanisms underlying their efficacy remain incompletely understood (10). Consequently, scientific evaluation becomes imperative to confirm their anti-diabetic properties, provid+ing a pathway for further exploration and understanding in the realm of diabetes management (14).

In this research, we have meticulously selected specific natural plants (Syzygium cumini Linn, Psidium guajava, Tinospora cordifolia, Swertia chirata, Gymnemasylvestre) based on their traditional utilization, plant background and a thorough literature review as shown in table 1.1. These plants have been combined to formulate a polyherbal granules. Our objective is to evaluate their potential antidiabetic properties by assessing their α-amylase inhibition activity.

Table 1.1: Plant Background and Traditional Uses

Sr no.	**Parameter**	**Herbal plants**				
		Jamun Seeds (15,16)	**Guava Leaves** (17,18)	**Giloy Stem** (19–21)	**Chirata Leaves** (22,23)	**Madhunashini Leaves** (24–26)
1	Botanical name	Syzygium cumini Linn.	Psidium guajava	Tinospora cordifolia	Swertia chirata	Gymnema sylvestre
2	Kingdom	Plantae	Plantae	Plantae	Plantae	Plantae
3	Order	Myrtales	Dicotyledon	Ranunculales	Gentianales	Gymnemasylvestre
4	Family	Myrtaceae	Myrtaceae	Menispermaceae	Gentianaceae	Asclepiadaceae
5	Genus	Syzygium	Psidium	Tinospora	Swertia	Gymnema
6	Species	Cumini	Guajava	Cordifolia	Chirata	Sylvestre

7	Synonyms	Jambu, Jamuna, Jambul	Guava bush, Jamfal, Amrud	Guduchi, Amrita	Chirayata, East Indian balmony	Gurmar, Podapatri
8	Origin and distribution	India, East Indies, Thailand, Philippines, Madagascar and some other countries	Mexico, Central America or North/South America throughout the Caribbean region, subtropical and tropical Asia, South Asia, Southeast Asia, and Oceania	India, Myanmar, Thailand, Sri Lanka, China, Bangladesh, North Africa, South Africa, Indonesia, Philippines	It is found in India, Nepal and Bhutan. It grows in Kashmir, Meghalaya, Madhya Pradesh and Khasi hills.	India, Sri Lanka, Malaysia, Tropical Africa and Asia
9	Traditional medicinal uses	Gastroprotective, Antiulcerogenic, Antiinflammatory, Hypoglycemic, Hypolipidaemic activity, Anti-oxidant, Anti-allergic	Laxatives, problems associated with oral cavity, Antidiabetic, Antibacterial, antacid and ulcer protectant activity, wound healing etc.	Antidiabetic activity, Anticancer activity, immunomodulatory, Antimicrobial, Antioxidant	Anti-inflammatory, Antidiabetic, Anti-bacterial, Hepatoprotective, Antimalarial, hypoglycemic, Anti-microbial, etc.	Antidiabetic, Antiarthritic, treatment of dental caries, Antibiotic, Antimicrobial, Antiinflammatory, Antihyperlipidemic.
10.	Hypoglycemic properties	It triggered the activation of PPARγ and PPARα genes, leading to the suppression of NF-κB, COX, iNOS, TNF-α, and other inflammatory cytokines,	It will enhance AMPK activity, leading to increased levels of AMPK and ACC phosphorylation in the	Improve glucose metabolism in b-cells or activating enzyme systems that produce cyclic AMP or phospholipid-derived messengers	Adipogenesis-related improvements in PPAR- γ, GLUT-4, and adiponectin expression indicate that it is responsible for the	Antidiabetic effects achieved by the regeneration of β-cells.

		subsequently resulting in the upregulation of Nrf2.	diabetic liver. These changes can result in beneficial effects for individuals with diabetes.		antidiabetic effects.	

2. Material and Method

2.1. Plant material

All the herbal medications were acquired from the local market in Vapi and subsequently employed for experimental purposes.

2.2. Methods

2.2.1. Preparation of plant extract

Guava leaf extraction (27)

The dried guava leaves were meticulously cut into small pieces. The extraction process involved two cycles of using 75% ethanol and was carried out with the aid of a Soxhlet apparatus, maintaining the ethanol at its boiling point for a duration of 4 hours in each cycle.

Jamun seed (28)

Using 70% ethanol (EtOH), the jamun seed kernel powder was Soxhlet-extracted over the course of at least eight hours. Then, at 37°C, the ethanolic extract was concentrated while operating at lower pressure.

Giloy stem (29)

The dried stems of giloy were finely grind after drying in the shade. Subsequently, 20 g of this plant material was soaked in 100 mL of dichloromethane at room temperature, occasionally shaken for 15 hours. The hexane-soluble portion was separated, and A rotating evaporator was used to dry the residual liquid under vacuum at 40°C.

Madhunashini leaves (30)

The dried leaves that were collected were left to air dry at room temperature in the shade. Dry leaves were ground into a powder and then sieved through a fine muslin cloth. The finely powdered leaves were stored in a Soxhlet device containing 90% alcohol in order to extract the crude.

Chirata leaves (31)

S. chirata leaves that had been air-dried at room temperature were used in this investigation. Ethanol was used in the extraction procedure after these dried leaves were placed into a Soxhlet device.

2.2.2. Preliminary phytochemical screening for various extracts (32)

Using many qualitative tests, the extract was screened initially for phytochemicals to determine the existence of many phytoconstituent classes. Various tests were carried out, among them involving alkaloids, phenol, resin, steroids, terpenoids, cardiac glycosides, saponins, tannins, and flavonoids.

2.2.3. Formulation of granules

The method uses to formulate granules is wet granulation method. Transfer an equal amount of each of the five extracts into a mortar pastel. Add the lactose and starch paste as needed to form a dough mass. After placing the dough through a sieve no. 10, dry it at 80°C for 30 minutes. After drying, filter it through sieve number 20 to obtain small granules.

2.2.4. Evaluation of granules (33)

Standard procedures were used to assess granules' physicochemical properties.

Angle of repose (θ)

The funnel method was used to determine the coefficient of friction inside granules by measuring the angle of repose. A 1-cm-tall funnel was used to pour the granule formulation (h). The formula below was utilized to calculate the angle of repose based on the measured radius (r) of the pile.

$$\theta = \tan - 1\ (h \div r) \qquad (1)$$

Bulk Density (δb).

The bulk density was ascertained by addition of precisely weighed amount (M) of the granular blend to the measuring cylinder. The density was calculated using the following formula, and the volume that was obtained is known as bulk volume (Vb).

$$\delta b = \frac{M}{Vb} \qquad (2)$$

Tapped Density (δt)

After five hundred taps of the granules' bulk mass, the tapped density was computed using provided formula.

$$\delta t = \frac{M}{Vt} \qquad (3)$$

Carr's Index

Granule compressibility is measured using Carr's index. The provided formula was used to determine it.

$$\text{Carr's Index} = \left[\left(\frac{\delta t - \delta b}{\delta t}\right)\right] \times 100 \qquad (4)$$

Hausner Ratio (H).

The following formula was used to get Hausner's ratio: tapped density divided by bulk density

$$H = \frac{\delta t}{\delta b} \qquad (5)$$

Loss on drying

The test is carried out by drying weighed amount of product in an oven at 105°C till a persistent weight is observed.

2.2.5. α-Amylase Inhibition Assay (34)

In this experimental assay, plant extracts were evaluated at concentrations of 20, 40, 60, 80, and 100µg/ml Porcine pancreatic amylase (PPA) solution was combined with 100µl of each plant extract concentration. The PPA solution contained 27.5 mg of PPA in 100 ml of a 20 mmol phosphate buffer (pH 6.9) with 6.7 mmol sodium chloride. For twenty minutes, the mixture was incubated at 37 °C.

Subsequently, 100µl of a 1% starch solution (prepared by stirring 1g starch in 100 ml of 20 mmol phosphate buffer, pH 6.9, with 6.7 mmol sodium chloride) was added, and the incubation continued at 37 °C for an additional 10 minutes. To halt the reaction, 200µl of DNSA solution (composed of 1g 3,5 di-nitro salicylic acid, 30g sodium potassium tartarate, and 20ml 2N sodium hydroxide, adjusted to a final volume of 100 ml with distilled water) was introduced. Reaction mixture was then incubated in a hot water bath around five minutes. After that, 2.2 ml of water was added to the reaction mixture to dilute it, and the absorbance at 540 nm was measured.

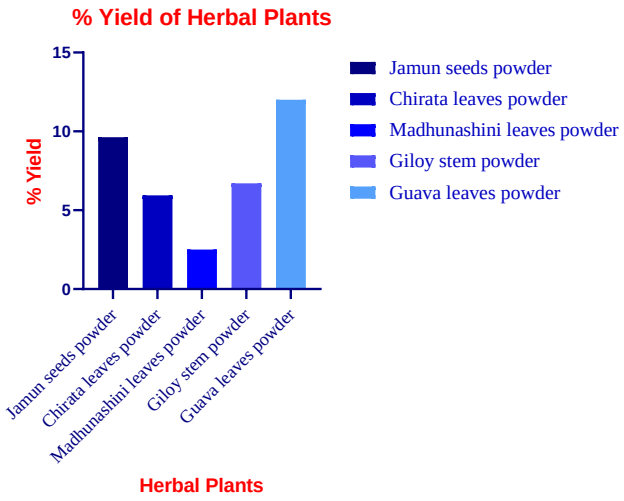
To create blank tubes, 200µL of distilled water was used in place of the enzyme solution for each concentration. Without the plant extract, a control sample with 100% enzyme activity was made in similar manner. The same experiment was conducted thrice.

3. Result

3.1. Percentage yield of extract

The results of the extraction process, expressed as percentage yields for the procured drugs,as shown in figure 1 are as follows: Jamun Seeds Powder exhibited a yield of 9.63%, Chirata Leaves Powder demonstrated a yield of 5.94%, Madhunashini Leaves Powder presented a percentage yield of 2.51%, Giloy Stem Powder resulted in a yield of 6.71%, and Guava Leaves Powder showed a notable yield of 12.00%.

Figure 1: Percentage Yield Obtained of Procured Drugs After Extraction.



3.2. Qualitative analysis of extracts

All the five extract was examined for basic phytochemical constitute present in it and was evaluated as shown in table no.3.2

Table 3.2: Phytochemical Constituents Present in Herbal Plant

Parameters	Jamun seed powder	Chirata stem powder	Madhunashini leaves powder	Giloy stem powder	Guava leaves powder
Alkaloids	+	+	+	+	+
Tannins	+	-	+	+	+
Terpenoid	+	+	-	+	+
Phenol	-	+	+	+	+
Resins	+	+	+	-	+
Saponins	-	-	-	-	+
Cardiac glycoside	-	+	-	-	-
Glycosides	-	+	+	+	+
Steroid	+	+	+	+	+
Flavonoids	+	+	+	+	+

3.3. Evaluation of poly-herbal granules

The primary parameters evaluation of the prepared poly herbal granules was doneand was been recorded for the following parameter:

Table 3.3: Evaluation Parameters and Observations

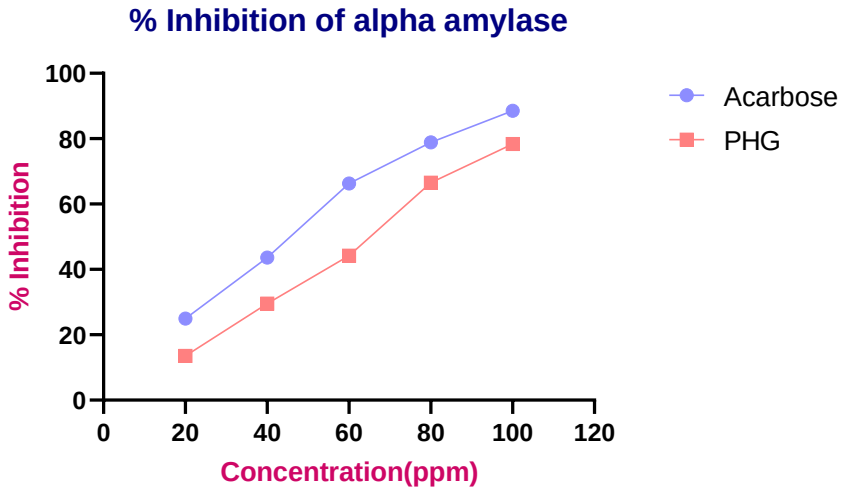
Sr.No.	Parameters	Observation
1.	Color	Dark brown
2.	Odour	Characteristic
3.	Taste	Astringent
4.	Loss on drying (% w/w)	3.63
5.	pH value (1% w/v solution)	5.22
6.	Angle of repose	28.4
7.	Bulk Density (g/ml)	0.35
8.	Tapped Density (g/ml)	0.49
9.	Hausner’s Ratio	1.08
10.	Compressibility index (%)	12

3.4. α-Amylase inhibition assay

Amylase stands as a pivotal enzyme in the intricate process of breaking down starch and glycogen during carbohydrate metabolism (35). Targeting the inhibition of amylase emerges as a viable strategy for addressing disorders related to carbohydrate uptake, including prevalent conditions like diabetes and obesity. Notably, amylase is a key player in carbohydrate metabolism, and hindering its activity holds promise for reducing postprandial blood sugar levels (36).Traditional medicinal practices, such as those in the Indian Ayurvedic system and various ethnomedicinal approaches, have long incorporated herbal

plants and their preparation in management of diabetes(37). These natural remedies harness the potent bioactive components found in these plants, showcasing both robust α-amylase inhibitory and antioxidant properties.

Figure 2: Percentage Inhibition of alpha Amylase



4. Discussion

Ayurveda has various poly-herbal compositions for medical care of diabetes mellitus. In market, polyherbal granules are readily available. A number of different sites are targeted, and side effects can be reduced leads to the idea of formulating, assessing and standardizing poly-herbal granules with anti-diabetic action. All of these herbs, namely jamun seed, chirata leaves, madhunashini leaves, giloy stem, and guava leaves, have anti-diabetic properties on individual basis, but polyherbal granules have been developed to improve the anti-diabetic affect by acting through multiple pathways. In this study, the taxonomy of jamun seed, chirata leaves, madhunashini leaves, giloy stem, and guava leaves has been investigated and their active compounds are extracted using certain solvents and procedures. These specific plants are subjected to phytochemical screening/chemical assays. All of these herbs are mixed to create poly-herbal granules. An experiment targeting alpha-amylase inhibition was conducted, resulting in the suppression of the enzyme. This experiment offers insights into the potency of the polyherbal granules intended for treating diabetes mellitus.

5. Conclusion

The research emphasizes the rising incidence of diabetes worldwide and the shortcomings of the allopathic treatments that are now available, which frequently have negative side effects and high costs. It highlights the potential of traditional plant-based methods as a means of controlling diabetes. In order to mitigate the negative effects of traditional diabetes therapies, the study carefully chooses particular natural plants that have been shown to have alpha-amylase inhibitory activity. The goal is to offer more accessible and reasonably priced options for those who are managing this condition. The goal of the research is to investigate the possibilities of combining herbs such as *Syzygium cumini Linn, Psidium guajava, Tinospora cordifolia, Swertia chirata*, and *Gymnema sylvestre* to mitigate diabetes through the creation of polyherbal granules. These herbs, which each have unique anti-diabetic qualities, are mixed in a way that multiplies their effectiveness.

In order to provide scientific evidence for the plants' usefulness, the study conducts chemical assays and phytochemical screening in addition to extracting the active compounds from the plants and looking into their taxonomy. The production of polyherbal granules and an experiment aimed at alpha-amylase inhibition, which shows the suppression of the enzyme. This trial is meant to be a first assessment of these polyherbal granules' efficacy in treating diabetes. Whilst, the research indicates that plant-based formulations with alpha-amylase inhibitory activity may be useful in treating diabetes, more thorough investigations especially clinical trials are necessary to confirm the effectiveness, safety, and wider applicability of these polyherbal granules in the management of diabetes mellitus.

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