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Development and Assessment of a Novel Polyherbal Syrup Formulated from *Syzygium cumini* Seeds and *Tinospora cordifolia* Stems for Hyperglycemia Management

Jubershaha Fakir[1]*, Rahul Gunjal[1], Chandrashekhar Patil[1], Kajal Pansare[1], Ganesh Sonawane[1], Vijayraj Sonawane[1], Deepak Somavanshi[2], Yogesh Ahire[2], Vishal Jadhav[3]

[1]Divine College of Pharmacy, Satana, Nashik-423301, India

[2]KBHSS Trust's, Institute of Pharmacy, Malegaon-423105, India

[3]GES's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nashik-422005, India

***Corresponding Author:** zubershaha95@gmail.com

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ABSTRACT

Diabetes mellitus is a persistent metabolic ailment characterized by elevated blood glucose levels and disruptions in the metabolism of carbohydrates, lipids, and proteins. This condition hinders body's capacity to regulate blood glucose, resulting in hyperglycemia, the hallmark of diabetes mellitus. The present study the development of an anti-diabetic polyherbal syrup formulated using an extract derived from the dried seeds of *Syzygium cumini L.* and *Tinospora cordifolia*. The resulting product was subjected to comprehensive evaluation. The evaluation encompassed phytochemical analysis to identify key bioactive compounds. 03 batches of Polyherbal syrup formulations were developed using extracts of *Syzygium cumini L.* and *Tinospora cordifolia.* Formulation F1 to F3 were prepared using Propylene glycol, Methyl paraben, Peppermint oil, Erythrosine and Saccharin sodium. Developed formulations were evaluated for various evaluation parameters for syrup such as Organoleprtic properties evaluation, Determination of pH, Viscosity Measurement, density and specific gravity. Results showed that, Formulation F2 showed good hypoglycemic effect in wistar rats compared to diabetic control group. F2 formulation was found to be stable compared to F1 and F3. It was discovered that there was a clear correlation between the extracts' ability to adsorb glucose, on the basis of this developed formulation shown hypoglycemic activity in STZ induced diabetic wistar rats as compared. It is needed to be scaled up on pilot scale; also clinical trials are necessary to carry out the successful launching of Java plum syrup formulation for management of diabetes mellitus.

Keywords: Java plum, Guduchi, Anti-diabetic, Anti-microbial, Syzygium cumini seeds, Tinospora cordifolia stems, etc.

1. INTRODUCTION

In the modern world, metabolic diseases are considered major health problems. The prevalence

of metabolic disorders is rising daily as a result of people adopting unbalanced lifestyle patterns, which ultimately increases the metabolic syndrome (MS) health burden on society [1]. It is a medical condition that is defined by visceral obesity, insulin resistance, elevated blood pressure, and abnormal cholesterol levels, according to the World Health Organization (WHO) [2]. The most common MS biomarker is diabetes. It is referred to as the "third killer" of humanity and affects about 10% of all native people on the planet today. Due to oxidative stress and inflammation brought on by hyperglycemia, it is among the top 10 causes of death worldwide, taking the lives of almost 1.6 million individual's year [3]. The development and course of type 2 diabetes are mostly associated with inflammation and oxidative damage brought on by hyperglycemia. Persistent low-grade inflammation has been related in multiple studies to an increased risk of type 2 diabetes [4]. Alternatively, this underlying inflammation leads to insulin resistance, which is linked to symptoms of multiple sclerosis, including hyperglycemia. Hyperglycemia, the hallmark of type 2 diabetes (T2DM), is a chronic endocrine metabolic disorder resulting from altered protein, lipid, and glucose metabolism. One such promising molecule of Jamun (*Syzygium cumini*) shows an antihyperglycemic activity compared to metformin, a conventional antidiabetic medication [5].

The tropical fruit jamun (*Syzygium cumini L.)* has a purple peel. It belongs to the family Myrtaceae. Jamun fruits are also referred to as Indian blackberries, jambul, java plum, pompozia in Egypt, and black plum in other regions of the world [6]. *Tinospora cordifolia* commonly named as "Guduchi" in Sanskrit belonging to family *Menispermaceae* is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude. The jamun fruit is small and fragile. Due to the lack of systematic farming, it is one of the underutilized crops [7]. This fruit is highly perishable, with a shelf life of only one or two days in normal conditions [8]. Jamun fruits were widely used in traditional medicine to cure a variety of diseases and physiological issues [9]. It is used to treat a variety of ailments, including diarrhea, headaches, sore throats, allergies, gastric ulcers, viral infections, spleen disorders, and cancer [10–12]. This fruit is said to aid in blood cleansing and diabetes management. The jamun fruits' substantial iron content serves as a blood purifier and promotes a rise in hemoglobin count [13,14]. Diabetics can take this fruit or its juice without experiencing an increase in blood sugar levels [13,15–17]. Research conducted over the past 20 years has shown that jamun has a good complex of antioxidant chemicals that are found naturally [18]. According to recent research, jamun fruit and seeds are a notable source of

antioxidant chemicals, including flavonoids, anthocyanins, and phenolic acids. These bioactive substances aid in the prevention of certain metabolic disorders [19]. Because of its many bioactive ingredients, jamun seed powder or extract works well as an anti-inflammatory to lessen both acute and chronic inflammation [20]. Unrefined extracts from jamun seeds have been demonstrated to have antimicrobial properties against drug-resistant enzyme lactamase, which is generated by isolated bacteria [21]. The present research was carried out to formulate and evaluate the antihyperglycemic properties of jamun seed syrup. Antidiabetic syrup formulated from jamun seeds extract is that the bioactive compounds present in the seeds may have a positive impact on regulating blood sugar levels. The bioactive compounds present in jamun seeds, such as flavonoids and polyphenols, may have the ability to improve insulin sensitivity, regulate blood sugar levels, and potentially contribute to better glycemic control in individuals with diabetes. It is hypothesized that these compounds may have beneficial effects on blood glucose regulation. Some compounds in jamun seeds might have insulin-mimetic properties, meaning they could act like insulin in promoting the uptake of glucose by cells. This could help lower blood sugar levels in individuals with diabetes. It is possible that jamun seeds can enhance insulin sensitivity in peripheral tissues, allowing cells to respond more effectively to insulin and uptake glucose from the bloodstream. Conducting clinical studies to evaluate the effects of jamun seed extracts on blood glucose control and glycemic parameters in individuals with diabetes will be necessary to test the validity of these hypothesis. The present research was carried out to evaluate the phytochemical, formulation and preclinical evaluation of Polyherbal syrup (PHS).

2. MATERIALS AND METHODS

2.1. Materials

Syzygium cumini L. seed and *Tinospora cordifolia* stem were purchased from local market of Nashik, Maharashtra. The plant materials were authenticated by Department of Botany, MVPS KAANMS Arts, commerce and Science College, Satana, Dist. Nashik, Maharashtra, (Authentication No. KAANMS/2023-24/56/Herbarium 2). Propylene glycol, Methyl paraben, Peppermint oil, Erythrosine and Saccharin sodium were obtained from Divine College of Pharmacy, Nashik. All other chemicals used were of analytical grade.

2.2. Animals

The Wister rats weighing between 150-200 gm were procured from Animal house of Name of college, and maintained under constant conditions (temperature 25± 2C, Humidity 40-60%, 12 h light/ 12 h dark cycle). During maintenance the animals received a diet of food pellet supplied from animal house and water ad libitum. These experiments were approved by the Institutional Animal Ethics Committee (Reg. No. 1566/Po/Re/S/11/CPCSEA).

2.3. Methods

2.3.1. Preparation of Plant Extract (by decoction)

Syzygium cumini L. seed and *Tinospora cordifolia* stem were dried in the sun for three days. The dried seeds and stem were crushed separately finely ground. A sieve no. 2 was used to sieve the finely powdered particles. Mix of Indian black jamun seed powder and Guduchi powder in 500 ml of filtered distilled water. Boil the mixture until the volume reaches 14 of the initial volume, then cool it and filter it through filter paper was carried out and evaluated for various phytochemical screening in previous study by authors. The cooked mixture's filtrate is utilized to make the final PHS.

2.3.2. Preparation of Flavor Solution

Amount of peppermint oil in concentration of propylene glycol was prepared separately.

2.3.3. Preparation of simple syrup with sodium saccharin

To make a concentrated solution, combine sodium saccharin with 10 ml distilled water in a mixing vessel.

2.3.4. Preparation of PHS

Filtrate was taken and added to a mixing vessel containing simple syrup, and stirred thoroughly before adding excipients such as methyl paraben, flavor solution, and finally a coloring agent erythrosine, and finally making up the value to 50 ml with distilled water.

Table 1. Composition of Polyherbal Syrup (50 ml)

Code	ASSC (g)	ASTC (g)	Propylene glycol (ml)	Methyl paraben (g)	Peppermint oil (ml)	Erythrosine (ml)	Sachharin sodium (g)	Distilled water (50ml)
F1	8	2	1	0.02	1.5	0.3	0.2	q.s.
F2	6	4	1.5	0.02	1	0.4	0.25	q.s.
F3	5	5	2	0.02	2	0.5	0.3	q.s.

ASTC: Aqueous Stem Extract of *Tinospora cardiofolia*

ASSC: Aqueous Seed Extract of *Syzygium cumini L.*

2.4. Evaluation of PHS

2.4.1. Organoleptic Properties

5 mL of the resulting syrup was placed in a watch glass and exposed to light, and the colour was seen with the naked eye. 2 ml of final PHS was smelled individually and then the odor can be detected. To identify the taste, a pinch of finished syrup was placed on the tongue's taste bud.

2.4.2. Determination of pH

The pH of each PHS formulation was measured using pH meter. The pH meter was calibrated with standard buffer solution at pH 4, 7 and 9 before it's use. Before taking the reading, for 10 min the electrode was inserted in the sample at room temperature and then reading of pH were noted.

2.4.3. Determination of Viscosity

The viscosity of PHS formulation was assessed for viscosity using An Ostwald viscometer at room temperature (25±2°C). The Ostwald viscometer is cleaned extensively with chromic acid or acetone. The viscometer should be mounted vertically on a suitable platform. Fill the dried viscometer halfway with water. Take note of the time it takes for water to flow from mark A to mark B. To achieve an accurate reading, repeat the operation three times. Wash the viscometer and fill it with herbal syrup, then record the time it takes for the syrup to flow from mark A to mark B.

2.4.4. Determination of Density and Specific Gravity

Pycnometers were used to determine the density of PHS. Rinse the pycnometer (specific gravity bottle) with filtered water after cleaning it with chromic acid and nitric acid. Take note of the weight of the empty dry bottle (w1). Weigh the pycnometer after filling it with 10 ml of water (w2). Finally, take note of the weight of the bottle with 10 mL of syrup (w3).

2.4.5. Selection of Satisfactory Formulation of PHS

Among 03 PHS formulation satisfactory formulations were selected on the basis of color, odor, taste, pH, viscosity, density and specific gravity.

2.5. Stability Studies

The main objective of the stability testing is to provide evidence on how the quality of the drug product varies with time under the influence of temperature and humidity. The stability study for the syrup formulation was done as per ICH guidelines in a stability chamber for a period of 6 months. The selected syrup formulation F2 was placed in a humidity chamber at 25°C ± 2°C/60% RH ±5% RH, 32°C ± 2°C/60% RH ± 5% RH and 40°C ± 2°C/75% RH ± 5% RH. Samples were withdrawn at an initial, first, second, third and sixth months and evaluated for change in homogeneity, pH and viscosity.

2.6. In Vivo Evaluation of Satisfactory Formulation

2.6.1. Repeated-Dose Toxicity Studies

The effect of oral administration of PHS at a concentration of 100, 200, and 400 mg/kg of body weight per rat per day for a period of 28 days on body weight gain and morphological changes was measured to assess the toxic effect of PHS [22].

2.6.2. Experimental induction of diabetes

The animals were fasted for 18 hours, and diabetes was induced by a single intravenous injection of a freshly prepared solution of STZ (55 mg/kg of body weight) in 0.1 M citrate buffer (pH 4.5) [23]. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats were injected with citrate buffer alone. At 24 hours after STZ injection their fasting blood glucose levels were estimated. STZ-treated animals were considered as diabetic when the fasting blood glucose levels observed was above

250 mg/dL**.**

2.6.3. Experimental Design

The rats were divided into two sets each comprising six groups (n= 6 in each group), one for the analysis of biochemical parameters and the other for the evaluation of glucose tolerance test: Group I, control rats receiving 0.1 M citrate buffer (pH 4.5); Group II, diabetic controls; Group III, diabetic rats given Polyherbal syrup (100 mg/kg of body weight/day) in aqueous solution orally for 28 days, Group IV, diabetic rats given glibenclamide (600 g/kg of body weight/day) in aqueous solution orally for 28 days. The body weight gain and fasting blood glucose of all the rats were determined at regular intervals during the experimental period. After 28 days of treatment, the rats were fasted overnight and sacrificed by cervical decapitation, and the blood was collected using EDTA as anticoagulant. The whole blood was used for the estimation of glucose and urea. The plasma was used for the assay of cholesterol, proteins, Liver Glycogen. A portion of wet liver tissue was used for the estimation of glycogen content.

2.6.4. Glucose Tolerance Test

After 28 days of treatment, a fasting blood sample was taken from all the groups of rats. Four more blood samples were collected at 30, 60, 90, and 120-minute intervals after administration of glucose at a concentration of 2 g/kg of body weight. All the blood samples were collected with potassium oxalate and sodium fluoride solution for the estimation of glucose.

2.7. Statistical Analysis

All the grouped data were statistically evaluated, and the significance of various treatments was calculated using Student’s t test. All the results were expressed as mean SD values.

3. RESULT AND DISCUSSION

3.1. Evaluation of PHS

It was discovered that all three PHS formulations were uniform, well-preserved, and had a reddish-brown colour, aromatic scent, and mildly bitter taste.

3.2. Determination of pH

The pH values of all the formulations were in the close range of neutral pH (6.22-6.86) and

hence it good for medical purpose.

3.3. Viscosity Measurement

Viscosity of all formulations was found to be between 0.035 to 0.651 Pa.s. for all these formulation. Among all formulations; F2 PHS showing excellent viscosity of 0.045 Pa.s.

3.4. Density and specific gravity Measurement

All of these formulations' densities were found to range from 1.36 to 1.47 kg/m3. F2 PHS has the best density of 1.37 kg/m3 out of all the formulations. 0.00132 to 0.00134 was determined to be the specific gravity.

Table 2. Evaluation Parameters

Sr. No.	Evaluation Parameters	Observation
1	Color	Reddish Brown
2	Odor	Aromatic
3	Taste	Lightly Bitter
4	PH	6.3
5	Viscosity	0.045
6	Density	1.36
7	Specific Gravity	0.00132

3.5. Stability Studies

In order to ensure the quality of PHS formulation throughout the shelf life, stability study was performed as per ICH guidelines on F2 formulation as it exhibited better quality characteristics. Negligible change in homogeneity, pH and viscosity was observed after 0,1,2,3 and 6 months of stability testing. Results of the study clearly revealed that the formulated Polyherbal syrup F2 is found to be stable.

3.6. In Vitro glucose bound test

Figure 1 illustrates the results of the chosen Polyherbal extract (PHE) capacity for glucose adsorption. The research' findings regarding the ability of PHE to adsorb glucose demonstrated this ability. It was discovered that there was a clear correlation between the PHE ability to adsorb glucose and the concentration of glucose. Additionally, it was discovered that the PHE were efficient in adsorbing glucose at concentrations of 5 and 100 mmol L-1 glucose, which were employed in the investigation. The molar concentration of glucose was found to be directly proportional to the PHE capacity to adsorb glucose.

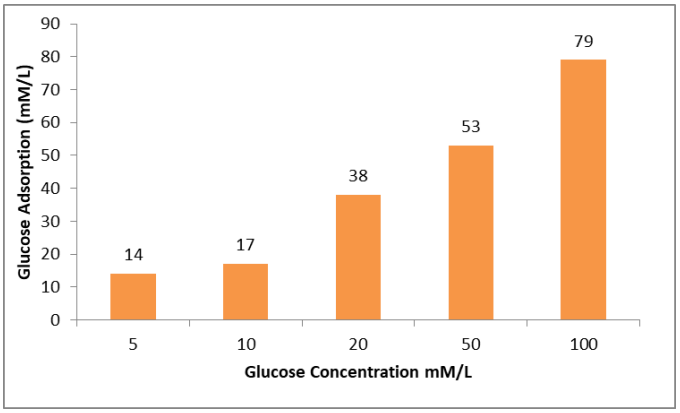

Figure 1. *In Vitro* glucose bound test

3.7. Toxicity Studies

The acute toxicity studies revealed no comparable weight loss after 28 days, and the experimental treatments were shown to be non-toxic as monitored by survival outcome. At the end of 4 weeks no deaths or significant changes in general behavior or other physiological activities were observed at any point in the present study. Clinical signs of neither toxic nor adverse effect were noted throughout the study. Figure 2 are shown that changes in body weight gain at regular intervals during the experimental period were seen. A marked decrease in body weight was observed in the diabetic group of rats when compared with the control group of rats. Oral treatment with glibenclamide resulted in a notable increase in body weight gain when compared with PHS group of rats.

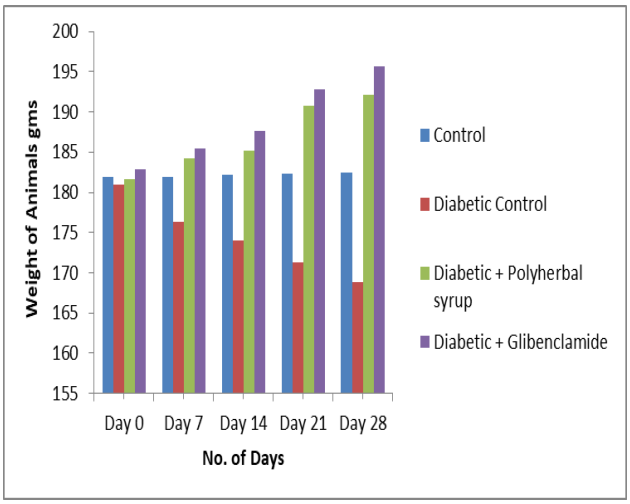

Figure 2. Change in body weight.

3.8. Hypoglycemic effect in rats

Table 3 shown that blood glucose levels of control and experimental groups of rats after oral administration of glucose. The blood glucose value in the control rats rose to a peak value 60 minutes after glucose load and decreased to near normal levels at 120 minutes. In diabetic control rats, the peak increase in blood glucose concentration was observed after 60 minutes and remained high over the next 60 minutes. PHS and glibenclamide treated diabetic rats showed significant decreases in blood glucose concentration at 60 and 120 minutes compared with diabetic rats. The levels of blood glucose, total proteins, cholesterol, and liver glycogen of the control and experimental groups of rats are shown in table 4. In experimental rats there was a significant elevation in the level of blood glucose and cholesterol during diabetes, while the levels of plasma proteins and liver glycogen decreased when compared with control group of rats. Administration of PHS and glibenclamide decreased the levels of blood glucose and cholesterol and increased the levels of plasma proteins and liver glycogen as compared with the diabetic group of rats.

Table 3. Effect of Polyherbal Syrup and Glibenclamide on Blood Sugar Level In Glucose-Loaded Diabetic Rats

Group	**Blood Sugar Level mg/dL**				
	Fasting	30 Minutes	60 Minutes	90 Minutes	120 Minutes

Control	76.2±4.2	145.2±5.2	179.1±3.7	129.3± 5.2	84.1±2.5
Diabetic control	256±11	319.5±23.5	390±20.8	344±14.5	308±10.6
Diabetic+PHS	100.1±3.8	144±3.2	189.5±2.6	156± 2.9	109±3.2
Diabetic+Glibenclamide	80.7±2.7	142.3±3.8	179.1±3.7	130.2± 4.5	93.4±3.9

Diabetic control group were compared with control group: P< .001, PHS and Glibenclamide treated diabetic group were compared with diabetic control group: P< 0.00, Values are given as mean ± SD for groups of 06 animals in each group

Table 4. Levels of Blood Glucose, Cholesterol, Total Proteins, and Liver Glycogen in Control and Experimental Groups

Group	**Blood Sugar Level mg/dL**			
	Fasting Glucose, mg/dL	**Cholesterol, mg/dL**	**Protein, g/dL**	**Liver Glycogen, mg of glucose/g wet tissue**
Control	81.6±5.8	81.8±4.6	6.5±0.2	54.2 ± 2.5
Diabetic control	251.1±23.7	197.6±8.6	6.1±0.3	22.3±4.2
Diabetic+PHS	180.5±17.2	100.7±5.7	6.9±0.9	31.4±5.2
Diabetic+Glibenclamide	101.5±12.1	89.1±2.7	7.2±0.3	50.8±4.6

Diabetic control group were compared with control group: P <0.00, PHS and Glibenclamide treated diabetic group were compared with diabetic control group: P<0.01, Values are given as mean ± SD for groups of 06 animals in each group.

4. CONCLUSION

Herbal remedies are used by 50% of the world's population because of their greater acceptability and compatibility with humans. It has fewer side effects than synthetics do. Using an Indian Black jamun seed extract and Guduchi stem extract, we created PHS anti-diabetic syrup for this investigation. According to the literature review, these herbs have a strong

anti-diabetic impact. The prepared syrup is evaluated using a variety of metrics, and its value is found to be within accepted bounds. Prepared PHS has been the subject of in vitro and anti-microbial research for anti-diabetic activity, and the results indicate a strong antidiabetic effect. The need for herbal treatment has grown in the modern era. People may be more receptive to herbal medicines because of their fewer adverse effects**.** The syrup could potentially help regulate blood sugar levels, leading to improved glycemic control in individuals with diabetes. Bioactive compounds in the syrup might enhance the body's sensitivity to insulin, allowing for more efficient use of glucose by cells. Compounds present in the PHE could act as antioxidants, helping to reduce oxidative stress that is often elevated in diabetes. The syrup could have potential benefits on lipid metabolism, leading to improvements in cholesterol and triglyceride levels. The syrup's components might have a positive impact on the function of insulin-producing cells in the pancreas, potentially aiding in insulin secretion. Conducting rigorous clinical trials to validate the efficacy and safety of the syrup in managing diabetes is crucial. These trials would provide evidence of its benefits and establish appropriate dosages. Conducting long-term studies to assess the syrup's effects on preventing diabetes-related complications and improving overall quality of life.

In conclusion, because *Syzygium cumini L*. seed and *Tinospora cordifolia* stem contain high concentrations of hypoglycemic active principles, oral treatment of PHS demonstrated hypoglycemic effect in STZ-induced diabetes in experimental rats. In-depth chemical and pharmacological research is required to clarify the precise mechanism underlying PHS hypoglycemic action.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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None

Availability of data and materials

The datasets used and/or analyzed during the current study were available from the

corresponding author on reasonable request.

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