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Original Article

Formulation and *in vitro* Evaluation of Tablet Dosage form of Unani Anti-diabetic Powder Containing *Gymnema sylvestre* R. Br, *Syzygium cuminii* Linn. and *Zingiber officinale* Rosc.

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ABSTRACT

Background and Objectives: Diabetes Mellitus (DM) is one of the leading causes of end stage renal disease, adult blindness etc. The side effects and resistance of conventional medicines for DM had put forward a need to develop an effective antidiabetic formulation with the aid of traditional medicine. Modification of dosage form in traditional system of medicine for palatability and portability is the need of hour. The antidiabetic *safoof*(powder) formulation containing Gurmar buti(*Gymnema sylvestre* R.Br), Jamun (*Syzygium cuminii* Linn.) and Zanjabeel (*Zingiber officinale* Rosc.) is having inconvenience to follow the accurate dose, palatability and stability issues due to surface area of powder. To rectify this problem the *Safoof* is modified into tablet dosage form with documentation of standard manufacturing procedure, its physicochemical analysis and *in vitro* antidiabetic evaluation. **Materials and Methods:** For process standardization total 10 batches were generated for the optimum working process related to the powder size, quantity of binder, wetting, granulation, time for drying and compression by trial and error. Ideal batch was selected on the basis of set parameter (friability, hardness and disintegration time), its physicochemical standards including HPLC as well as α-amylase and α-glucosidase inhibition activity were established. **Results:** With excipients 5% Gum acacia, 5% CMC, 1% SSG, 0.5% Mg stearate and 0.5% Talc, the mean value of the hardness, friability and disintegration time of tablet were found to be 4.83 ± 0.17, 0.29 ± 0.01 and 14.24 ± 0.05 respectively. Physico-chemical data with HPLC quantification of Gymnemagenin, 6-Gingerol, 8-Gingerol, 6-Shogaol and 10-Gingerol were set in. The heavy metals and microbial contaminations are within permissible limit. Antidiabetic activity through α-amylase and α-glucosidase inhibition were 0.00 ± 0.00 at the 2500 µg/ml and 84.28 ± 0.58% at the 3000 µg/ml concentration respectively. **Conclusion:** Classical powder is modified effectively in tablet dosage form with *in vitro* evaluation of its antidiabetic activity particularly α-glucosidase inhibition of crude powder and standardization data was also set in.

Keywords: Physico-chemical standardization, Diabetes mellitus, Tablets, Powder, α-glucosidase.

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INTRODUCTION

Contemporary world is facing various metabolic disorders due to stress and sedentary lifestyle. Diabetes Mellitus (DM) is one among them and is defined as a group of chronic metabolic conditions, all of which are characterized by increased blood glucose levels resulting from the body's inability to produce insulin or resistance to insulin action, or both.[1] This heterogeneous disease which runs an insidious course may result from a complex interaction of metabolism, environmental, genetic factors, etc.[2] DM is one of the leading causes of end stage renal disease, blindness and lower extremity amputations of non-traumatic origin. It also predisposes to cardiovascular disease with an increasing incidence worldwide, DM will be likely a leading cause of mortality and morbidity in the future.[3] DM a dreadful lifestyle metabolic disorder with recent studies estimated increase of people with diabetes to 366 million by 2030.[4,5] There are many side effects of conventional therapies while treating DM, Sulfonylureas are known to cause weight gain and hypoglycemia, Biguanides has low risk of lactic acidosis especially among the elderly and the most common side effects of metformin are nausea, cramps and diarrhea.[6] Vitamin B deficiency has been reported in approximately 7% of patients on metformin for approximately one year of treatment.[7] Since 2007, Rosiglitazone, a thiazolidinedione, has raised concerns due to increased risk of myocardial infarction and cardiovascular mortality.[8] It is a need of hour to develop an effective antidiabetic formulation with multidimensional approach. Unani medicine possesses many single drugs as well as formulations used as *Dafe Dhayabitus*

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(anti-diabetic). Several formulations are used in different dosage form such as *Safoof* (powder), *Habb* (pills), *Joshanda* (Decoction) etc. Out of these, many such formulations have palatability and compliance issues which can be rectified by improving formulation and change of dosage form. *Safoof* are fine dried powder formulation but it has many disadvantages. Due to high dose there is difficulty in ingestion of *Safoof,* it is also inconvenient to follow accurate dose. Besides this there are palatability and stability issues due hygroscopic nature and more surface area of powder.[9] These factors become very important while dealing with anti-diabetic medications, owing to chronicity and complications of the disease. For better compliance, *Safoof* particularly used in diabetes can be converted into tablet because of frequency of doses required to maintain the desired therapeutic response. It is being convenient and safe way of drug administration, easy to swallow, having better physical and chemical stability, precise and low content variability due to unit dose.[10,11] Keeping the above mentioned facts in mind, one such powder formulation used in DM is selected for its modification in respect of dosage form *i.e.* from powder to tablet dosage form with standardization of formulated tablet and *in vitro* evaluation of antidiabetic activity by α-amylase and α-glucosidase inhibition models.-amylase inhibitors are also called as starch blockers because they prevent dietary starch from being absorbed by the body and can be useful in the treatment of obesity and DM. They put forth their blood glucose lowering effect through the inhibition of an enzyme such as salivary and pancreatic amylase.[12] α-glucosidase which is a membrane bound enzyme situated at the epithelium of the small intestine has the property of enhancing the breakage of glucose from disaccharides. Thus, the retardation of the action of α-glucosidase may be one of the most effective approaches to control diabetes.[13] Since carbohydrate intake influences obesity, α-glucosidase inhibition may be useful in obesity.[14] In diabetic patients the α-glucosidase inhibitors has also the ability of lowering the postprandial hyperglycemia by completely and reversibly inhibiting α-glucosidase in the intestine. This inhibition reduces glucose absorption through extended digestion time and delayed carbohydrate digestion.[15] Standardization is also a vital process for maintaining and assessing the quality and safety of the polyherbal formulation. It not only reduces batch to batch variation but also assures safety, efficacy, quality and acceptability. The formulation selected for study contains Gurmar buti (*Gymnema sylvestre* R. Br.), Jamun (*Syzygium cuminii* Linn.) and Zanjabeel (*Zingiber officinale* Rosc.).[16]

MATERIALS AND METHODS

In the present study, a formulation of antidiabetic *Safoof* (powder) was selected for conversion into modified dosage form tablets. It was formulated by preparing multiple batches and final batch was selected on the basis of ideal post compression parameters viz. Hardness, friability and disintegration time, and standard manufacturing process was also developed. This final selected batch was further subjected to standardization (physico-chemical) and *in vitro* anti-diabetic activity evaluation.

Procurement and identification of the drugs: Gurmar Buti was procured from Kolkata, Jamun was procured from Jumerati area Bhopal and Sonth was procured from Avenue Road, Bangalore. All the three drugs are identified by expert Pharmacognosist, Senior Asst. Prof. Noorunnisa Begum, at FRLHT (Foundation for Revitalization of Local Traditions) Bangalore and the accession numbers of the drug identified as Gurmar Buti (*Gymnema sylvestre* R.Br.), Sonth (*Zingiber officinale* Rosc.) and Jamun (*Syzygium cuminii* Linn.) were 5096, 5097 and 5098 respectively. A specimen of each plant material used was deposited in the drug museum of National Institute of Unani Medicine, Bangalore, and voucher specimen number 57/IS/Res./2019, was obtained for future reference.

Ingredients of antidiabetic Safoof[16]

The ingredients of this formulation are leaves and stems of Gurmar Buti (*Gymnema sylvestre* R.Br.), kernels of Jamun (*Syzygium cuminii* Linn.) and rhizomes of Sonth (*Zingiber officinale* Rosc.). The quantity of these drugs in the formulation is 24 gm Gurmar Buti, 12 gm Jamun and 12 gm Sonth which are in the proportion of 50%, 25% and 25% respectively.

Preparation of tablets of antidiabetic Safoof

First the entire drugs were cleaned from foreign matter and dried. Then powdering and sifting of crude drug was done through sieve # no. 80 as per UPI specification. Then weighing of powdered drugs as per their proportion present in the formulation was performed, weighing of excipients used was also done in different proportions in different batches. The mixing of excipients to the antidiabetic *safoof* was carried out and wetting was done by adding distilled water to it, in order to obtain the damp mass. The granules were made after passing the damp mass through sieve # no. 16 and drying of granules was done in hot air oven on 60°C for 1 hr. Later on, these granules were subjected to compression in a 20-station rotary press tablet making machine with fixed calibration of 16 mm feeder depth size, 8 mm die size and 6 tons of compression pressure.

Different batches were prepared with same particle size of drugs, amount of water used in wetting, and same compression pressure but different percentage of excipients. Time and temperature for drying of granules was kept constant *i. e*. 60 minutes and 60°C respectively. Total 10 batches of granules were prepared; the final batch among these was selected on the basis of ideal post-compression parameters, viz., hardness, friability, and disintegration time. All the batches prepared are depicted in Table 1.

Table 1: All batches of antidiabetic tablets prepared from *safoof* (powder).

Batch		Powder	G A	CMC	SSG	PVP	Starch Soluble	Mg. Sterate	Talc	Hardness (Kg)	Fraibility (%)	Disintegration time
1.	Gm	60	6	-	-	-	-	0.33	0.33	3	1.84	8 min
	%	90	10	-	-	-	-	0.5	0.5			
2.	Gm	60	4.5	-	-	-	-	0.32	0.32	2	23.37	4min 30 sec
	%	92.1	7.5	-	-	-	-	0.5	0.5			
3.	Gm	60	6.3	-	-	-	3	0.35	0.35	6	0.17	20 min
	%	85.7	10	-	-	-	5	0.5	0.5			
4.	Gm	60	-	6	-	-	-	0.33	0.33	3	0.75	1 min 15 sec
	%	90	-	10	-	-	-	0.5	0.5			
5.	Gm	60	6.3	3	-	-	-	0.35	0.35	6	0.41	25 min
	%	85.7	10	5	-	-	-	0.5	0.5			
6.	Gm	60	6.3	-	-	3	-	0.35	0.35	4	7.2	23 min
	%	85.7	10	-	-	5	-	0.5	0.5			
7.	Gm	60	-	2.5	-	2.4	-	0.33	0.33	3	2.73	2 min 30 sec
	%	91.5	-	4	-	4	-	0.5	0.5			
8.	Gm	60	4.61	1.5	-	-	-	0.33	0.33	6	0.22	22 min
	%	89.8	7.5	2.5	-	-	-	0.5	0.5			
9.	Gm	60	3.15	3	-	-	-	0.33	0.33	5	0.28	19 min
	%	89.8	5	5	-	-	-	0.5	0.5			
10.	Gm	60	3.15	3	0.66	-	-	0.33	0.33	5	0.27	14 min 30sec
	%	88.9	5	5	1	-	-	0.5	0.5			

Techniques used for Selection of ideal batch and Process Standardization

Friability test

Friability test apparatus Roche´s friabilator (Labinda mod. no. 1020) was used for determination of friability of tablet. Test was done as per procedure mention in Theory and Practice of Industrial Pharmacy. This procedure was repeated three times for mean friability value.[17,18]

Hardness test

Randomly three tablets in a batch were picked up and they were individually tested for the hardness by Monsanto hardness tester (Shital Scientific Industries, S.no. 11012010) in term of kg/cm. The hardness of 4 kg is considered to be minimum for a satisfactory tablet.[9,17]

Disintegration test

Disintegration testing apparatus manufactured as per USP (TAB machine mod. no. TD 20S) was used for determination of disintegration time. Test was done as per procedure mention in Theory and Practice of Industrial Pharmacy and protocol for testing. Uncoated USP tablets have disintegration time standard minimum 5 min, majority of the tablets have maximum disintegration time of 30 min. [17][19]

Physico-chemical Parameters for antidiabetic tablets

Organoleptic evaluation: The organoleptic evaluations refer to the evaluation of the antidiabetic tablet formulation by color, odor, taste, appearance, particle size and texture.[19]

Uniformity of diameter: Three tablets were picked up randomly to perform test for determination of uniformity of diameter of tablet by using a Vernier calliper (UTTAR, IME type 6 inch/15 cm) and diameter of each tablet was measured individually and reading is expressed in mm.[20]

Weight variation: Randomly selected 20 tablets from final batch were weighed individually and average weight was calculated. Individual weight was compared to average weight not more than 2 tablets should fall outside the permissible percentage difference range given by USP.[20]

Granule characterization: The granule characterization like angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio were done.[9,17,21]

Loss on drying at 105°C

An accurately weighed 3 g of antidiabetic tablet was taken in apetri dish. The drug was heated at 105°C in an oven till a constant weight and percentagemoisture content of the sample was calculated concerning the weighed antidiabetic tablet sample.[22]

Ash values: Determination of total ash, acidinsoluble ash, and water soluble ash is done as perprotocol for testing of ASU drug and UPI.[19,22]

Determination of pH: 1% and 10% solution of antidiabetic tablet was prepared in distilled water(w/v), and pH was determined by using digital pH meter.[23]

Extractive values: The extractive values like water soluble extractive, alcohol soluble extractive, successive and non-successive extractive values were done as per UPI and physicochemical standardization of Unani formulations.[22,23]

Qualitative estimation: Qualitative estimation of antidiabetic tablet for organic constituent's *viz.* alkaloids, glycosides, tannins, flavonoids, terpenoids, saponins, phenols, carbohydrates and steroids were done as per method mentioned in Physicochemical standardization of Unani formulations.[23]

Heavy metal: Heavy metal was done at Merieux Nutrisciences Bangalore Pvt. Ltd. The method used was Inductively Coupled Plasma Mass spectrometry (ICP-MS) for the quantitative analysis of toxic metals like Lead, Cadmium, Mercury and Arsenic.[24]

Microbial screening: Microbial load and specific organism study was done at Merieux Nutrisciences Bangalore Pvt. Ltd. as per USP. Microbial screening carried out for the safe use of the individual plant as well as the mixed formulation and checked for prescribed limits of total aerobic count, total yeast and mould count.[25,26]

HPLC analysis: The HPLC fingerprinting and quantitative analysis of antidiabetic tablet was done at Natural Remedies Pvt. Ltd., Estimation of Gymnemagenin of Gurmar Buti and Gingerol (6-gingerol, 8-gingerol, 6-shogaol and 10-gingerol) of Sonth in methanolic extract of antidiabetic tablet was performed.[27]

Gymnemagenin HPLC Analysis

Mobile phase preparation: Dissolve 0.136 g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) in 900 ml of HPLC grade water and add 0.5 ml of Orthophosphoric acid. Make up to 1000 ml with water, filter through 0.45 µ membrane and degas in a sonicator for 3 min. The gradient conditions of Buffer concentration (Solvent A) and Acetonitrile concentration (Solvent B) at time (min) 0.01, 20, 25, 30, 35, 40 are 75, 45, 40, 40, 75, 75 and 25, 55, 60, 60, 25, 25 respectively.

Instrument details: HPLC, LC2010cHT from Shimadzu; Column: Hibar, Prepacked column, LiChrospher 100, RP-18e (5 µm) (Merck) Phenomenex- Luna 5µ C-18(2) Size: 250x 4.60mm,; Detector: Photo diode array detector or UV Detector; Wave length:205 nm; Flow rate:1.5 ml/min; Injection volume:50 µl.

Standard preparation: 10.0 mg of Gymnemagenin was accurately weigh to a 50 ml volumetric flask and dissolved in 20 ml of HPLC Methanol by sonicating for 5 minutes and cooled, to make up to 100 ml with Methanol.

Extract: 1.000 gms of given sample (Callus sample) was accurately weighed to 100 ml round bottom flask and then dissolved in 20 ml of 50% v/v ethanol by sonicating for 5 minutes. 2 ml of 12% w/v KOH was added and reflux on a boiling water bath for 1 hour. After reflection, solution was cooled and5.5 ml 4N HCl was added and reflux on a boiling water bath for 1 hour. After cooling adjust the pH to 7.5 - 8.5 with 12%w/v KOH. After adjusting the pH, transfer the solution to 100 ml volumetric flask. Wash the round bottom flask with 50% v/v ethanol, transfer to the same volumetric flask and make up the volume to 50 ml with 50%v/v ethanol. Filter through paper and subject to HPLC analysis.

Calculation

$$\frac{\%\ \text{Gymnemic acid} = \text{wt. of std.} \times \text{dilution of sample} \times \text{peak area of sample} \times \text{purity of std.} \times 100}{\text{Dil. of std. wt. of sample peak area of std. } 100 - \text{LOD}}$$

Gingerol HPLC Analysis

Mobile phase preparation: 0.136 g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) was dissolved in 900 mL of HPLC grade water and 0.5 ml of Orthophosphoric acid was added to make up to 1000 ml with water, then filtered through 0.45 µ membrane and degas in a sonicator for 3 minutes (Solvent A) and Acetonitrile (Solvent B).

Mobile phase: Solvent A (45): Solvent B (55), Instrument details: HPLC, LC2010cHT from Shimadzu, Column: Hibar, Prepacked column, LiChrospher 100, RP-18e (5 µm) (Merck) Phenomenex-Luna 5µ C-18(2) Size: 250x 4.60 mm, Detector:Photo diode array detector or UV Detector, Wave length: 278 nm, Flow rate:1.3ml/min, Injection volume:20 µl Standard preparation: 0.1 mg/ml of 6-Gingerol reference standard in HPLC Grade Methanol. Sample preparation: 20.0 mg/ml of Polyherbal Tablets (Powdered) in HPLC Grade Methanol.

Procedure: Instrument was set as per the chromatographic condition prescribed above. 20 µl of standard preparation was injected and the chromatogram was recorded. Injected 3times and the mean area and RSD was calculated. The RSD should not be more than 2.0%. 20 µl of sample preparation was injected and the chromatogram was recorded.

Calculation

$$\% \text{ Gingerol} = \frac{\text{wt. of std.} \times \text{dilution of sample} \times \text{peak area of sample} \times \text{purity of std.} \times 100}{\text{Dil. of std.} \times \text{wt. of sample} \times \text{peak area of std.} \times 100 - \text{LOD}}$$

In vitro Antidiabetic activity

In vitro evaluation of antidiabetic activity of antidiabetic tablet of *Safoof* of *Dhayabitus* was done by α-amylase and α-glucosidase inhibition models at Natural Remedies Pvt. Ltd. Bengaluru.[28,29]

Alpha amylase inhibitory activity: The sample polyherbal tablet, a sample stock solution of 3880 µg/ml was prepared. Briefly, 38.8 mg of sample was dissolved and made upto 10ml with phosphate buffer. The sample was found to be approximately 60% soluble upon visual inspection. For calculation purposes the sample was considered to be 100% soluble. The sample was filtered through syringe filter 25mm/0.2µm and the filtrate was used in the assay. Further dilution of the filtrate was made as required with phosphate buffer.

For acarbose, a stock solution of 1000µg/ml was prepared. Briefly, 2mg of acarbose was dissolved in 2ml of phosphate buffer to get a stock of 1mg/ml. Further dilutions were made,as required,with phosphate buffer.

The assay was performed as per Gella *et al*., with modifications. In brief, a pre- incubation mixture contained phosphate buffer/ positive control/test sample at various concentrations and α-amylase enzyme. The plate was mixed and pre-incubated. Following pre-incubation, substrate (CNP-G3) was added and the plate was mixed and incubated. The absorbance was measured at 405 nm in a micro-plate reader.

Alpha glucosidase inhibitory activity: The sample polyherbal tablet, a sample stock solution of 3600 µg/ml was prepared. Briefly, 36 mg of sample was dissolved and the volume was made up to 10 ml with phosphate buffer. The sample was found to be approximately 70% soluble upon visual inspection. The sample was filtered and the filtrate was used in the assay. For calculation purposes the sample was considered to be 100% soluble.

For acarbose, a stock solution of 1000 µg/ml was prepared. Briefly, 2 mg of acarbose was dissolved in 2 ml of phosphate buffer to get a stock of 1mg/ml. Further dilutions were made, as required, with phosphate buffer.

The assay was performed as per *Vogel and Vogel* with modifications. In brief, the pre-incubation mixture contained phosphate buffer/ positive control/test sample at various concentrations and enzyme. The reaction mixture was mixed and pre incubated. Following pre-incubation, substrate (sucrose) was added and the reaction mixture was mixed and incubated. The reaction was arrested by keeping in boiling water bath and then cooled to room temperature. Finally, glucose reagent was added to the reaction mixture then mixed and incubated. The absorbance was measured at 510 nm in a micro-plate reader.

The % inhibition of the alpha amylase and alpha glucosidase enzyme was calculated as follows

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)} \times 100}{\text{Absorbance (control)}}$$

RESULTS

The pre-compression parameters of granules of final batch of antidiabetic tablet like bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose is depicted in Table 2. The mean values of hardness, friability and disintegration time of batch 10 (final selected batch) of antidiabetic tablets were found to be 4.83 ± 0.17, 0.29 ± 0.01 and 14.24 ± 0.05 respectively. The organoleptic properties of antidiabetic tablet showed circular, slight biconvex and hard appearance; greenish brown color; characteristic odor; astringent with slightly bitter in each batch Figure 1. The mean value of uniformity of diameter and weight variation for antidiabetic tablet batch was found to be 13 ± 0.00 and 1.85 ± 0.14 respectively. The mean value of pH determined at 1% and 10% solution and mean percentage values of the total ash, acid insoluble ash, water soluble ash, sulphated ash and loss of weight on drying at 105°C of antidiabetic tablets is depicted in Table 3. The mean percentage values of alcohol and water soluble extractives of antidiabetic tablet were found to be 8.79 ± 0.48 and 15.36 ± 0.4 respectively. Successive and Non-Successive extraction, Phytochemical Screening, Heavy metal analysis and Microbial contamination analysis of antidiabetic tablet are depicted in Table 4, Table 5, Table 6 and Table 7 respectively. The HPLC Quantification of constituents and fingerprinting are depicted in Tables 8, 9, 10 and in Figures 2, 3, 4, 5. and *in vitro* antidiabetic activity of tablet i.e. α-amylase and α-glucosidase inhibition activity is depicted in Table 11.

Table 2: Granule characterization / flow property of formulated antidiabetic tablet.

Sl. No.	Parameters	Percentage mean (*n*=3) ± SEM
1.	Bulk density (gm/ml)	0.56 ± 0.02
2.	Tapped density (gm/ml)	0.6 ± 0.02
3.	Carr's index (%)	9.23 ± 0.01
4.	Hausner's ratio	1.13 ± 0.01
5.	Angle of Repose (θ)	30.60 ± 0.54

Table 3: pH Value of 1% and 10% solution, ash values and loss on drying at 105°C.

Sl. No.	Parameters		Percentage mean (*n*=3)± SEM
1.	pH	1% sol.	6.63 ± 0.02
		10% sol.	5.46 ± 0.02
2.	Ash values	Total ash (%)	6.9 ± 0.1
		Acid insoluble ash (%)	1.85 ± 0.12
		Water soluble ash (%)	2.68 ± 0.12
		Sulphated ash (%)	3.89 ± 0.04
3.	Loss on Drying (%)		8.05 ± 0.15

Table 4: Successive and Non- Successive extractive values.

Sl. No.	Parameters		Percentage mean (*n*=3)± SEM
1.	Successive extractive values	Pet. Ether (%)	3.64 ± 0.1
		Benzene (%)	1.10 ± 0.03
		Chloroform (%)	0.90 ± 0.03
		Ethanol (%)	8.64 ± 0.05
		Water (%)	16.21 ± 0.08
2.	Non-successive extractive values	Pet. Ether (%)	3.55 ± 0.11
		Benzene (%)	4.43 ± 0.07
		Chloroform (%)	5.12 ± 0.03
		Ethanol (%)	9.66 ± 0.07
		Water (%)	27.22 ± 0.12

Table 5: Qualitative analysis of antidiabetic tablets.

Sl. No.	Test		Positive/Negative
1.	Alkaloids	Dragendroff's test	+ve
		Hagers test	+ve
		Mayer's test	+ve
		Wagner's test	+ve
2.	Glycosides		+ve
3.	Tannins	Ferric chloride test	+ve
		Lead acetate test	+ve
4.	Flavonoids		+ve
5.	Carbohydrates	Fehling's test	+ve
		Anthrone test	+ve
		Benedict's test	+ve
6.	Phenols	Ferric chloride test	+ve
		Lead acetate test	+ve
7.	Steroids		+ve
8.	Saponins		+ve

Table 6: Heavy Metal analysis of antidiabetic tablet.

Sl. No.	Name of metal	Qty. in antidiabetic tablet	Permissible limit (ppm) as per WHO
1.	Lead	< 0.5	Not more than 10.0 ppm
2.	Cadmium	< 0.1	Not more than 0.3 ppm
3.	Mercury	< 0.25	Not more than 1.0 ppm
4.	Arsenic	0.9	Not more than 3.0 ppm

Table 7: Estimation for microbial contamination and specific of tablet.

Sl. No.	Parameters	Results	Limits
1.	Total bacterial count	Less than 10 CFU	Not more than 100000 CFU
2.	Total fungal count	Less than 10 CFU	Not more than 1000 CFU
3.	*E. coli*	Absent	Absent
4.	*Salmonella*	Absent	Absent
5.	*Staphylococcus aureus*	Absent	Absent
6.	*Pseudomonas aeruginosa*	Absent	Absent

Table 8: HPLC Quantification of gymnemagenin, 6,8,10-Gingerol & 6-Shogaol of tablet.

Name of the sample	Gymnemagenin (%w/w)	6-gingerol (%w/w)	8-gingerol (%w/w)	6-shogaol (%w/w)	10-gingerol (%w/w)
Antidiabetic tablet	1.43	0.074	0.011	0.021	0.013

Table 9: Peaks of antidiabetic tablet in HPLC Fingerprinting at 254 nm.

Peak	Ret. Time	Area	Area%
1.	2.724	224722	2.901
2.	4.662	866163	11.181
3.	5.612	26401	0.341
4.	7.096	80116	1.034
5.	8.069	74278	0.959
6.	8.338	36242	0.468
7.	8.610	43122	0.557
8.	9.068	53213	0.687
9.	9.492	296560	3.828
10.	9.939	104415	1.348
11.	10.200	16950	0.219
12.	10.494	16050	0.207
13.	10.828	445671	5.753
14.	11.181	114569	1.479
15.	11.976	4702209	60.697
16.	12.412	156983	2.026
17.	12.606	489399	6.317
Total		7747062	100.000

Table 10: Peaks of antidiabetic tablet in HPLC Fingerprinting at 205 nm.

Peak	Ret. Time	Area	Area%
1.	2.725	660908	3.855
2.	3.685	120866	0.705
3.	4.661	2765386	16.129
4.	4.961	67751	0.395
5.	5.369	43984	0.257
6.	5.605	68246	0.398
7.	6.097	87945	0.513
8.	7.085	162023	0.945
9.	8.058	109967	0.641
10.	8.345	96324	0.562
11.	8.660	68623	0.400
12.	9.062	128405	0.749
13.	9.319	53527	0.312
14.	9.482	314682	1.835
15.	9.946	68556	0.400
16.	10.203	80611	0.470
17.	10.826	215434	1.257
18.	11.045	43217	0.252
19.	11.348	64890	0.378
20.	11.607	7060	0.041
21.	11.831	306206	1.786
22.	11.973	2284180	13.322
23.	12.382	458854	2.676
24.	12.605	1007328	5.875
25.	17.779	176185	1.028
26.	19.626	157870	0.921
27.	20.177	709949	4.141
28.	20.807	508463	2.966
29.	21.245	17396	0.101
30.	21.754	143449	0.837
31.	22.362	236149	1.377
32.	22.826	59401	0.346
33.	23.196	239025	1.394
34.	23.441	1799999	10.498
35.	23.908	275285	1.606
36.	25.707	59060	0.344
37.	27.670	296486	1.729
38.	28.120	103572	0.604
39.	28.363	37735	0.220
40.	28.620	585173	3.413
41.	28.959	5168	0.030
42.	29.749	1918984	11.192

Peak	Ret. Time	Area	Area%
43.	30.457	39541	0.231
44.	30.858	407108	2.374
45.	31.977	84332	0.492
Total		17145307	100.000

Table 11: IC_{50} data of tested sample of antidiabetic tablet and reference inhibitor in α-amylase and α-glucosidase inhibition assay.

Name of the Inhibition Assay	Sample	Concentration (µg/ml)	% Inhibition (Mean ± SEM)	IC_{50} (µg/ml) (95% Confidence Interval)
α-amylase	Acarbose (Reference inhibitor)	0.1	18.88 ± 2.00	0.39 (0.32 - 0.60)
		0.25	40.49 ± 1.17	
		0.5	52.85 ± 2.81	
		21.0	73.10 ± 1.03	
	Polyherbal Tablet	100	0.00 ± 0.00	**
		250	0.00 ± 0.00	
		500	0.00 ± 0.00	
		1000	0.00 ± 0.00	
		2500	0.00 ± 0.00	
α- glucosidase	Acarbose (Positive Control)	0.25	17.61 ± 0.74	1.40 (1.30 - 2.16)
		0.5	28.68 ± 1.30	
		1	43.65 ± 0.57	
		2	47.33 ± 0.25	
		4	68.93 ± 0.40	
	Poly Herbal Tablet	50	8.27 ± 1.08	503.51 (420.27 - 603.44)
		100	19.09 ± 0.66	
		250	36.23 ± 0.52	
		500	52.14 ± 0.73	
		1000	68.46 ± 0.28	
		2000	76.01 ± 0.14	
		3000	84.28 ± 0.58	

2. Note: ** IC_{50} cannot be calculated since the sample did not exhibit at least 50% inhibition even at the highest concentration tested

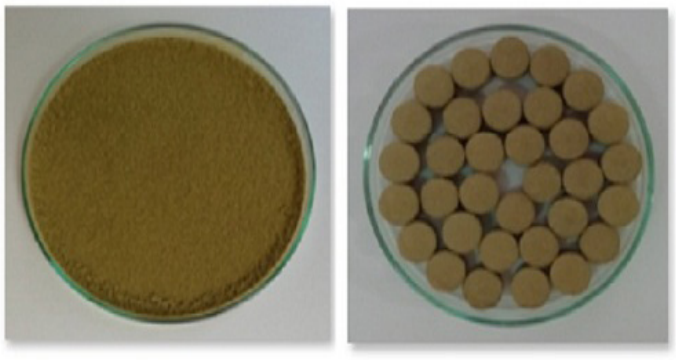

Figure 1: Antidiabetic *safoof* and tablet of final selected batch.

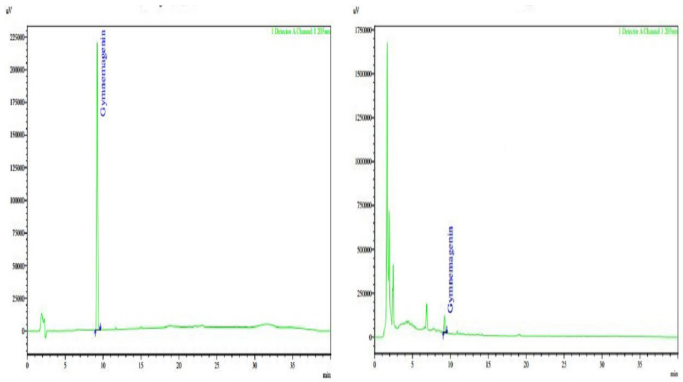

Figure 2: HPLC of gymnemagenin in standard and antidiabetic tablet.

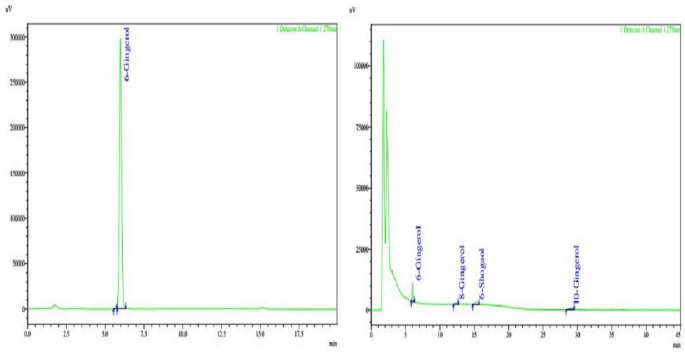

Figure 3: HPLC of 6-gingerol in standard and 6,8,10-gingerol & 6-shogaol inantidiabetic tablet.

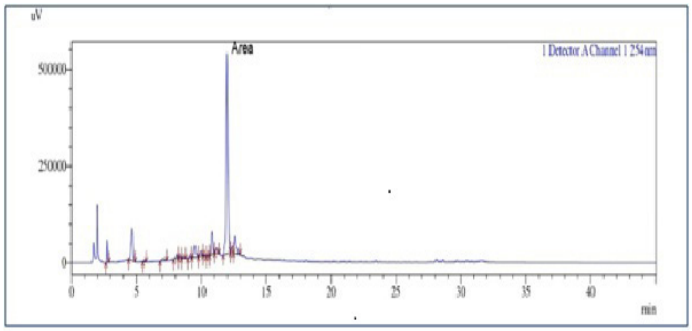

Figure 4: HPLC Fingerprinting of antidiabetic tablet at 254 nm in solvent system.

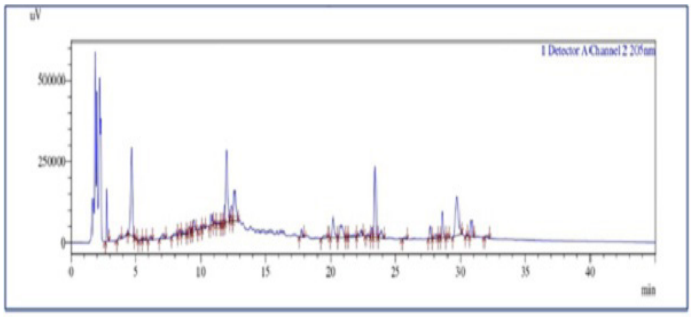

Figure 5: HPLC Fingerprinting of antidiabetic tablet at 205 nm in solvent system.

DISCUSSION

The organoleptic characteristics like appearance, color, odor, taste and touch of antidiabetic tablet were found to be slightly biconvex, circular, brownish green, astringent with slight bitter taste and hard in texture. The organoleptic properties of any product are important parameters for drug identification and necessary for patient compliance.[30]

The post compression parameters like hardness, friability and disintegration time were found to be within acceptable limit *i. e.* 4.83 ± 0.17, 0.29 ± 0.01 and 14.24 ± 0.05 respectively for final batch. The hardness and friability parameters are important because it indicates the wear and tear during handling and transportation. Disintegration is the first physical change observed for a drug when it enters into the body and this test helps in knowing the Active Pharmaceutical Ingredient's solubility in the gastric fluids of the digestive system.[31] Bulk quantity of tablets was prepared by following ideal batch procedure for further physico-chemical and *in vitro* anti-diabetic activity study.

The pH of 1% and 10% solution was found to be 6.63 ± 0.02 and 5.46 ± 0.02 respectively. Acidic pH indicates better absorption from stomach. Both 1% and 10% were found slightly acidic near to neutral pH. The correlation between the pH and α-glucosidase inhibition activity was studied by Nguyen *et al.* and suggested that the acidic pH favours stable environment for good potential activity of α-glucosidase inhibition.[32]

Loss of weight on Drying (LOD) at 105ºC indicates the loss of volatile substances along with the water.[30][33] Moisture content level affects quality and efficacy of drugs and is also an indication of adulteration. If any drug has more moisture level then this becomes ideal medium for growth of different types of bacteria and fungi which affect the purity, quality and efficacy of drug.[34] The loss of weight on drying at 105ºC of antidiabetic tablet were found to be 8.05 ± 0.15 respectively. The loss on drying should not be more than 10 per cent when drying for 2 hours.[35] The ash value is an important parameter in the quality control of herbal drugs. A high ash value depicts the contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing. The acid insoluble ash value represents the amount of silica or calcium oxalate in a drug.[33] The total ash, water soluble ash, acid insoluble ash and sulphated ash were found to be 6.9 ± 0.1, 1.85 ± 0.12, 2.68 ± 0.12 and 3.89 ± 0.04 respectively.

Extractive value is an index for evaluating the quality, purity and adulteration of the drugs. The extractive values will be high in particular solvent in which the drug constituents aremaximally soluble. For establishing the standards of any drug these extractive values play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one.[30,34,36]The successive and non successive extractive values of antidiabetic tablet in petroleum ether, benzene, chloroform, ethyl alcohol, water were

found to be 3.64 ± 0.1, 1.10 ± 0.03, 0.90 ± 0.03, 8.64 ± 0.05, 16.21 ± 0.08 and 3.55 ± 0.11, 4.43 ± 0.07, 5.12 ± 0.03, 9.66 ± 0.07, 27.22 ± 0.12 respectively. These values suggest that antidiabetic tablet formulation is highly soluble in water. It means that the antidiabetic tablet have very good solubility, dissolution and gastrointestinal permeability that helps in control rate and extent of drug absorption and its bioavailability.[37]

Qualitative chemical test indicated the presence of for alkaloids, saponins, terpenoids, flavanoids, phenols, glycosides, tannins and carbohydrates. It is a valuable step in the detection of the bioactive principles present in herbal plants that may lead to drug discovery and development.

The toxicity of heavy metal in human body results in deterioration of central nervous functions, thereby affecting mental health, it can damage the blood composition, lowers the energy levels and can damage the vital organs.

The persistent exposure of these heavy metals may also result in slowly progressing neurological degenerative processes impending Alzheimer's disease, Parkinson's disease, Muscular dystrophy and Multiple sclerosis.[38] The heavy metals like Lead, Cadmium, Mercury and Arsenic were found less than 0.5 ppm, less than 0.1 ppm, less than 0.25 ppm and 0.9 ppm respectively. This is under permissible limit.[22] Total fungal and total bacterial count/g were found to be less than 10 CFU for both of these tests which is under permissible limit. Specific pathogens such as *E. coli, Salmonella* spp., *S. aureus, P. aeruginosa* were found to be absent. It indicates that the drug is safe for use. The uptake of contaminated drug by human resulted in health trouble, and the World Health Organization (WHO) has suspended all such products from the list of suppliers until there has been official confirmation that the medications are not contaminated.[39]

In HPLC quantification gymnemagenin (% w/w) in 1 g of antidiabetic tablet was found to be 1.43%, while 6-gingerol, 8-gingerol, 6-shogaol, and 10-gingerol in 20 mg of antidiabetic tablet formulation (% w/w) were found to be 0.074%, 0.011%, 0.021%, and 0.013%, respectively.

No. of peaks in HPLC chromatogram of antidiabetic tablet at 205 nm and 254 nm were 45 and 17 respectively. The fingerprinting of antidiabetic tablet at 254 nm shows maximum area percentage of 60.697% in 15th peak whereas fingerprinting of antidiabetic tablet at 205 nm shows maximum area percentage of 16.129% in 3rd peak. Tables 8-11 and in Figures 2-5. In this study the HPLC helps in identification of those constituents which are compared with reference standards, but others cannot be identified. Qualitative presence of other unknown constituent was confirmed by fingerprinting data. HPLC can also help in chemical separation, purification, tablet dissolution, shelf-life determination, forensic analysis, quality of the drug and also helps in to set the data of the amount of the particular constituent presents in the drug.[40] The *in vitro* antidiabetic activity was done with α-amylase and α-glucosidase inhibition model. α-amylase inhibition activity of antidiabetic tablet at the concentration of 100, 250, 500 and 1000 µg/ml were found to be0.00 ± 0.00, 0.00 ± 0.00, 0.00 ± 0.00and 0.00 ± 0.00 respectively while as α-glucosidase inhibition activity of antidiabetic tablet at the concentration of 50, 100, 250, 500, 1000, 2000 and 3000 µg/ml were found to be 8.27 ± 1.08, 19.09 ± 0.66, 36.23 ± 0.52, 52.14 ± 0.73, 68.46 ± 0.28, 76.01 ± 0.14 and 84.28 ± 0.58 respectively. The α-amylase and α-glucosidase inhibition activity of antidiabetic tablet was compared with the standard control drug "acarbose". These results indicate that this antidiabetic tablet acts only by α-glucosidase inhibitory mechanism. But there are various researches pointing out that *Gymnema sylvestre* and *Syzygium cumini* in extract forms shows α-amylase inhibition activity.[41-43] Furthermore owing to α-amylase activity in single drugs, it can be promising in this regard for this formulation. There may be α-amylase inhibition activity in antidiabetic tablet made from extracts of Gurmar, Jamun and Zanjabeel. Further studies are required in this formulation to find out the *in vitro* antidiabetic activity in different / appropriate extract combination. In the present study the solubility of crude powder may be the hindrance in screening the α-amylase activity of the formulation. Besides *in vitro* hypoglycemic activity these drugs also have antioxidants, hypolipidemic and other supportive activity related to disturbance in carbohydrate-metabolism.

Further detailed study on pharmacokinetic of polyherbal antidiabetic tablet is needed to establish the most appropriate kinetic property, mechanism of action of this formulation through further *in vitro, in vivo* study and future sophisticated tests. The clinical trial is also needed to prove the antidiabetic activity and its synergistic effect of the combination to establish full efficacy of the studied formula.

CONCLUSION

Classical powder formulation composed of *Gymnema sylvestre* R.Br, *Syzygium cuminii* Linn. and *Zingiber officinale* Rosc. was modified effectively in tablet dosage form with *in vitro* evaluation of its anti-diabetic activity and standardization data was also set in as a monograph for future evaluation and reference.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DM: Diabetes mellitus; **UPI:** Unani Pharmacopeia of India; **HPLC:** High Performance Liquid Chromatography; **CFU:** Colony Forming Unit; **gm:** Gram; **USP:** United state pharmacopeia; **KOH:** Potassium hydroxide; **GA:** Gum Acacia.

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