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Phytopharmaceutical formulation of *Ocimum sanctum* tablets: Evaluation of hypoglycemic effects

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Abstract
Historically, phytotherapeutic approaches have demonstrated efficacy in the management of diabetes. Due to the higher prevalence of adverse effects associated with conventional allopathic treatments, it is imperative to prioritize research into plant-derived remedies. In India, approximately 60% of diabetes patients rely on traditional herbal medicine. *Ocimum sanctum* Linn. (Commonly referred to as Tulsi), a prominent member of the Lamiaceae family, holds a distinguished place in Ayurveda for its antidiabetic properties. In this study, a tablet formulation was developed using the hydroalcoholic extract of *Ocimum sanctum*. The tablets were produced via the direct compression technique, and both pre-compression and post-compression parameters were evaluated in accordance with established pharmaceutical guidelines. The formulations conformed to all required physical and pharmaceutical quality standards. The antidiabetic potential of the formulation was assessed through alpha-glucosidase inhibition studies. The tablet blend exhibited potent alpha-glucosidase inhibitory activity, with an IC50 value of 3.58 µg/mL.

Keywords: *Ocimum sanctum*, tablet, formulation, alpha glucosidase

Introduction
Through ages, traditional Indian physicians were aware of diabetes mellitus, also known as Madhumeha [1]. Diabetes is treated by Ayurvedic practitioners utilizing a multifaceted approach that includes yoga, breathing techniques, herbal remedies, Panchkarma, and diet modification. Many plants, such as shilajit, turmeric, neem, Ivy gourd, amalaki, triphala, bitter gourd, rose apple, bilva leaves, cinnamon, gymnema, fenugreek, bay leaf, and aloe vera, are used against diabetes. Among the powders (Churana) utilized are Naag Bhasma, Haldi powder, and Amalaki Churna. Chandraprabhavati and Vasanta Kusumakar Ras, two Ayurvedic medicines, are thought to reduce blood sugar levels [2, 3].

Research has demonstrated that oral administration of *Ocimum sanctum* (OS) extract significantly lowers blood glucose levels in normal and glucose-fed hyperglycemic diabetic rats [4,5]. In a crossover, single-blind, randomized, placebo-controlled clinical trial, fasting and postprandial blood glucose levels were reduced by 17.6% and 7.3%, respectively, accompanied by a similar reduction in urinary glucose levels. Additionally, OS exhibits aldose reductase inhibitory activity, which may contribute to mitigating the risk of diabetes-related complications such as cataracts and retinopathy [6].

In experimental studies, OS leaves have been reported to exhibit both hypoglycemic and antihyperglycemic properties [7, 8]. The antidiabetic effects of the leaf extracts may be attributed to their stimulatory influence on physiological pathways involved in insulin secretion. Furthermore, OS has shown potential in mitigating corticosteroid-induced diabetes mellitus, suggesting its ability to prevent the onset of diabetes associated with corticosteroid use [7].

Previous studies have also highlighted its significant inhibitory activity against the alpha-glucosidase enzyme. Building on this, the present study developed three tablet formulations of OS using the direct compression technique and evaluated their antidiabetic potential through alpha-glucosidase enzyme inhibition. Among the active biomolecules in OS, Oleanolic acid is a known alpha-glucosidase inhibitor, as reported in earlier studies [9, 10]. Consequently, the study included alpha-glucosidase inhibition analysis for oleanolic acid and performed HPLC analysis to confirm its presence in the extract.

Materials and Methods

Raw material

Ocimum Sanctum leaves were procured from Noor Nihal, Bengaluru, and Karnataka.

Solvents and Chemical

Methanol, Acetonitrile, Potassium dihydrogen Phosphate, Ethanol, Iso-propyl alcohol, Red Iron oxide, Titanium oxide, Dimethyl chloride, Hydroxypropyl methyl cellulose 6 cps IP, Polyethylene glycol 4000IP and Purified talc IP were of analytical grade. Oleanolic acid, Purity ≥ 97.5% was procured from Tokyo Chemical Industries, Tokyo Japan.

Preparation of plant extract

The dried plant material of OS was grounded and extracted by soaking powdered raw material (100g) in, 50% ethanol at ambient temperature. The mixture was refluxed at temperature 70 °C in reflux condenser without stirring for 3 cycles to get maximum extract. The extract was filtered through polypropylene cloth. The extract was concentrated to dryness using a vacuum rotary evaporator with the water bath set at 50 °C.

Pre formulation studies

- **Organoleptic Properties:** The extract prepared was evaluated based on primary senses.
- **UV spectrum:** A UV spectrum of hydro alcoholic extract of OS was constructed by preparing different concentrations in the range of 5ppm to 100ppm and measured in the range of 200nm to 400nm. A stock solution was prepared by dissolving accurately weighed 10mg of extract in 0.1N HCl was prepared and volume adjusted to 10 mL with further addition of 0.1N HCl. From the stock solution, appropriate aliquots were prepared to get concentrations of 5, 15, 25, 50, 100 ppm.

Preparation of tablets

Tablets of OS extract was prepared by direct compression method. The required quantities of microcrystalline cellulose (Avicel 102), Crosscarmellose sodium, Aerosil 200 were weighed. The above ingredients were passed through mesh 40 and blended for 10minutes. Three different formulations were prepared by variation in proportion of these ingredients as seen in Table 1. Magnesium stearate was added to the above blend as a lubricant, passed through a mesh size of 60 and allowed to blend for another 3 minutes. The blend was characterized for its precompression parameters. Round and biconvex shaped tablets, with average weight of 200mg were compressed using a Rotary tablet punching machine and evaluated for post compression parameters [9, 11].

Table 1: Composition of the three different formulations of Ocimum sanctum extract

Sr. No.	Ingredients	F01	F02	F03
1	*Ocimum sanctum* extract	100mg	100mg	100mg
2	Microcrystalline Cellulose (Avicel PH 102)	90mg	80mg	86mg
3	Crosscarmellose sodium	6mg	16mg	10mg
4	Aerosil 200	2mg	2mg	2mg
5	Magnesium Stearate	2mg	2mg	2mg
Coating solution			**(gm)**	
Hydroxypropyl Methyl cellulose 6cps IP			16.04	
Polyethlene glycol 4000 IP			1.60	
Titanium Dioxide IP			0.32	
Purified Talc			0.32	
Color red oxide Iron NF			1.72	
Isopropyl alcohol IP+BP			190.73	
Methyl Chloride IP			190.73	

Pre-compression parameters of the blend:

- **Bulk density:** A known quantity of powder was poured into the measuring cylinder. Powder was levelled without compacting and read the unsettled apparent volume, V0, to the nearest graduated unit. Bulk density, in gm per ml was calculated by the formula [12],
- Bulk Density = weight of the powder / apparent volume
- **Tapped density:** A known quantity was poured into measuring cylinder. Powder was levelled and tapped 100 times and read the apparent volume.
- Tapped density = weight of the powder/tapped volume of the packing
- **Compressibility index:** The compressibility index of the granules was determined by Carr's compressibility index [12],

  Carr's index (%) = Tapped Density – Bulk Density x 100/Tapped Density

Post compression parameters

- **Physical characteristics**: The overall elegance, its visual identity and appearance of the tablets is important for consumer acceptance. Size, shape, and organoleptic characters was evaluated of the tablets. Vernier caliper was used for measuring the thickness and diameter of the tablets [13].
- **Weight variation / Uniformity of weight**: Randomly from each batch twenty tablets were selected and weighed individually on a digital weighing balance. Individual tablets weight was noted down, and average weight was calculated. The individual weight was compared with the average weight. The individual weight of not more than two tablets must deviate the average weight by ±7.5% [13].
- **Hardness**: It is the force required to break a tablet across the diameter. Monsanto Hardness tester was used for measuring the hardness. Ten tablets were taken from each batch and average hardness values obtained were expressed in Newton.
- **Disintegration time**: Disintegration time was determined using Distilled water at 37±0.5 °C. The disintegration time of six individual tablets was recorded [12].

HPLC Analysis

High Performance Liquid Chromatography (HPLC) (Alliance) was used for the analysis of extract and tablet formulation. The conditions during HPLC fingerprinting: UV-

Vis detector (200-254 nm), Inertsil BDS 3V 250 × 4.6 (ADL 304) mobile phase was KH2PO4: MeOH (10:90), flow rate was 0.5ml/min, injection volume 20µl. The compounds were detected at a wavelength of 214nm. For sample and standard preparation, Oleonolic acid standard solution was prepared by dissolving 10mg standard in methanol and volume was made upto 10 mL with further addition of methanol. The stock solution of OS extract were prepared by dissolving 100 mg of extract into a 20 mL volumetric flask add solvent and sonicate for 15 min. make up the volume with diluent then filtered through 0.45µ filter and used for the analysis. For HPLC of tablets, 20 tablets were triturated. About 100 mg of the powder was taken dissolved in 20 mL volumetric flask add solvent and sonicate for 15 min. Then volume was made up with diluent then filtered through 0.45µ filter and used for the analysis.

Stability studies

All the prepared batches / formulations were subjected to stability study as per ICH guidelines for three months at 2-8 ºC and 40 °C / 75% RH. At the end of one month all the formulations were evaluated for critical parameters like physical appearance, hardness, disintegration time and drug content [11].

Alpha glucosidase assay

A colorimetric assay method was used to evaluate the inhibitory effect of the extract against α-glucosidase enzyme obtained from *Saccharomyces cerevisiae* [14]. The chromogenic substrate p-nitrophenyl glucopyranoside (PNPG) was used and 0.1M sodium phosphate buffer with pH 6.8 was used as a medium to carry out the assay. Stock solution of enzyme was prepared by dissolving 1mg of enzyme in the phosphate buffer and further diluted by 100x in the same buffer. Substrate solution was prepared by dissolving 1.5mg of substrate in 1mL of buffer. The assay mixture for these experiments contained 20µL PNPG and 160 µL phosphate buffer. The extract 10mg was dissolved in 1mL of buffer to make further dilutions before adding the substrate. Blank sample contained whole test mixture and the extract without enzyme solution [10].

Test samples of Oleanolic acid were prepared concentration at 10ppm, 5ppm, 2.5ppm, 1.25ppm, and 0.625ppm to determine the IC_{50} values of compound. For OS extract test samples in concentration of 200 ppm, 100 ppm, 50 ppm, 25 ppm, 12.5 ppm, 6.25 ppm, 3.125 ppm were taken to determine its α-glucosidase activity. In 96 well plate 10µL of enzyme, 10 µL of inhibitor solution was added and preincubated at temperature 37 °C for 10 min.

After the addition of reaction mix, the plate was incubated for 30 min at 37 °C. Initial absorbance and final absorbance was recorded at 405 nm before and after incubation of the plate. The α-glucosidase activity was determined by measuring the yellow-colored p-nitrophenol (PNP) released from PNPG, using a microplate reader.

The α-glucosidase inhibitory activity was expressed as% inhibition and calculated as follows:

$$\% \text{ Inhibition} = (\text{Abs control} - \text{Abs sample} \div \text{Abs control}) \times 100$$

Where Abs stands for Absorbance

Results and Discussion

Preformulation studies

Preformulation studies are imperative for making tablets because they help determine the comportment and properties of tablets during and after manufacturing.

- **Organoleptic Properties:** The appearance and palatability of pharmacological dosage forms are determined by their organoleptic qualities; a patient would strive to avoid using a medicine if its color, flavor, or taste are unacceptable [12]. Thus, the color and flavor of the extract was determined to be dark brown in color with aromatic fragrance of Indian holy basil leaves.
- **UV spectrum:** Herbal extracts can be analysed using the ultraviolet-visible (UV-Vis) spectroscopy technique for a number of reasons, such as quality control of herbal medications and product identification and authenticity. Also, certain bioactives' with known absorption maxima can be detected by taking UV absorption spectra. UV absorption spectra of OS extract obtained can be seen as in Figure 1. Oleanolic acid shows maximum absorption at 214nm (λmax at 214nm. Data not shown here). Thus the peak seen in the extract at around 214nm in Figure 1. confirms the presence of Oleanolic acid in the extract. The presence of Oleanolic acid in the extract, being one of the potent bioactive agents assures the quality of the prepared extract.

Evaluation of physical properties of powder blend of all the three formulations as precompression parameters

The precompression parameters are critical properties of the blend that must be evaluated prior to tablet formulation, as they provide insights into the powder's flowability, compressibility, and packing behavior. Inadequate flow characteristics can result in dose uniformity issues and manufacturing defects such as capping or lamination.

- **Bulk density and Tapped density:** Both loose bulk density (LBD) and tapped bulk density (TBD) results are shown in Table 2. The LBD and TBD for all the formulations varied from 0.49 gm/cm^3 to 0.53 gm/cm^3. The values obtained lies within the acceptable range and no large differences found between LBD and TBD. This result helps in determining the percentage compressibility of the powder.
- **Hausner's ratio:** The Hausner ratio is a reliable and efficient parameter for predicting the flow behavior of powders, playing a vital role in maintaining consistent product quality and regulatory compliance. It assists manufacturers in optimizing material flow properties, thereby enhancing production reliability and efficiency. A high Hausner ratio indicates poor flowability, which can result in uneven tablet filling, while a ratio below 1.25 signifies optimal flow characteristics. Hausner's ratio of the powder was determined from the LBD and TBD and lies within the acceptable range as 0.9 to 1.06 as seen in Table 2.

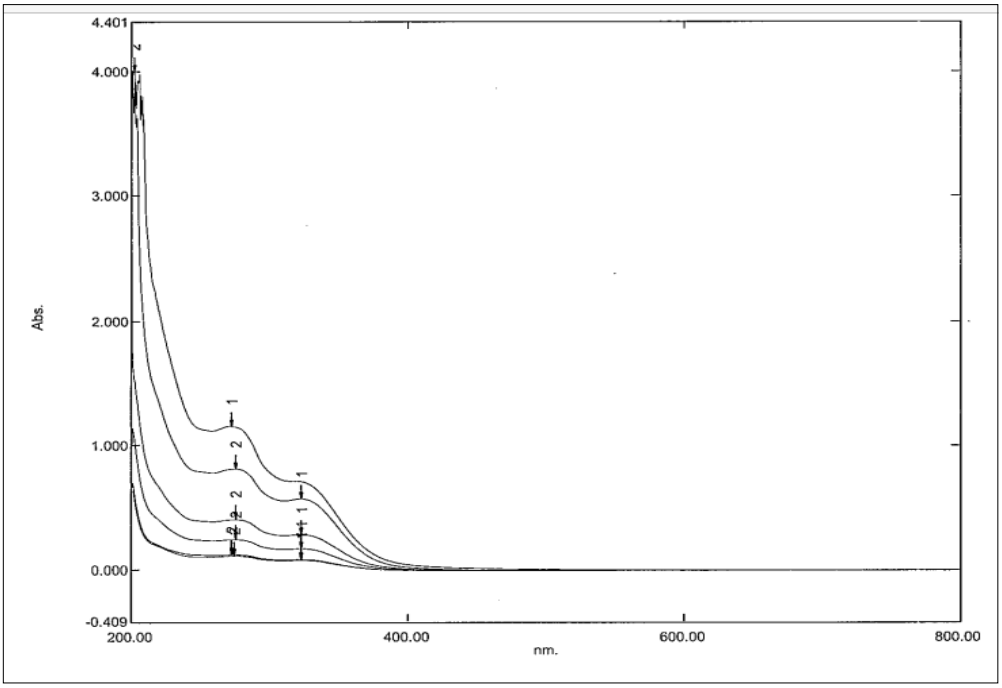

Fig 1: UV Spectrum of *Ocimum Sanctum* extract at various concentrations.

Percentage compressibility: The compressibility index, also referred to as the Carr index, quantifies the extent to which a powder can be compressed under applied pressure. This parameter is crucial for identifying potential challenges related to flow and compaction during tablet production. It aids in determining the necessity of glidants to enhance flowability and contributes to ensuring the quality of the final tablet. Lower compressibility index values indicate superior flow properties, while higher values suggest poor flow behavior. An index below 15% is generally regarded as indicative of good flowability, whereas values exceeding 25% are considered indicative of poor flow characteristics. The percent compressibility of powder mixture was determined as shown in Table 2. The percent compressibility for all the three formulations is less than 15% indicating superior flow properties.

Table 2: Evaluation of Pre-compression parameters

Sr. no.	Parameters	F01	F02	F03
1	Bulk density	0.49	0.48	0.50
2	Tapped density	0.52	0.50	0.53
3	Hausner's ratio	0.90	1.04	1.06
4	Compressibility Index	5.30%	6.00%	5.83%

Post-compression parameters: All the formulations were subjected for Organoleptic, physical and chemical evaluations. In-vitro disintegration time, shape and color, thickness, hardness, weight variation and HPLC assay was carried out.

- ***In vitro* disintegration time:** The internal structure of tablets that is pore size distribution, water penetration into tablets and swelling of disintegration substance are suggested to be the mechanism of disintegration. The disintegration time of a tablet refers to the duration required for its active pharmaceutical ingredients to be fully released from the tablet matrix or coating. This parameter is influenced by the properties of the coating and the tablet formulation. For tablets with simple coatings, the disintegration time should not exceed 60 minutes. It was determined as per Indian Pharmacopoeia for all the formulations. All the three formulations showed a disintegration time of less than 8min (Table 3) which falls well within the time limit for coated tablets**.**
- **Shape and Color of the Tablets:** The shape of the tablets was circular convex and were dark brown in color. Since the tablets contained blend of excipients which are white in color and consists of herbal extract; tablets appeared to look mottled but the mottled appearance is not due to defect but due to the tablet contents.
- **Thickness Test**: The thickness of the tablets was measured by using Vernier caliper by picking the tablets randomly. The mean values are shown in Table 3. The values are almost uniform in all formulations. Thickness was found in the range from 2.7 mm to 3.5 mm.
- **Hardness test:** Tablet hardness is a quality control parameter that evaluates a tablet's resistance to fracturing or breaking. It reflects the tablet's ability to endure mechanical stresses encountered during manufacturing, packaging, and transportation. The hardness of tables was maintained within 90N as seen in Table 3. Hardness test was performed by Monsanto hardness tester.
- **Weight variation test:** The percent weight variation for all the formulation is tabulated in Table 3. It was found to vary from 185 mg to 206 mg. This is due to good flow property and compressibility of the formulation.
- **HPLC Assay:** The content uniformity was performed for all the three formulations and results are shown in Table 3. Three tablets from each formulation were analyzed by HPLC. The drug content of the tablets was found between 90.3% and 100.3% of Oleanolic acid. The

results showed that the oleanolic acid was present in all the formulations as seen in Figure 2.

Based on the post compression parameters F03 was selected for the further study and analysis as the F03 formulation did not show much variation and showed better acceptance criteria for disintegration time, hardness, thickness, weight variation and assay as compared to F01 and F02.

Table 3. Evaluation of Post compressional parameters of different formulations

Sr. No.	Parameters	F01	F02	F03
1	Disintegration time in water(min)± SD	6±1.01	8:20±1.20	7:34±1.30
2	Hardness (N) ± SD	54±0.21	87.21±0.06	90.21±0.16
3	Thickness (mm) ± SD	2.7±0.08	3.4±0.06	3.5±0.04
4	Weight variation (mg) ± SD	185±7.3	190±8.2	206±7.4
5	HPLC Assay (%)	90.3%	98.3%	100.3%

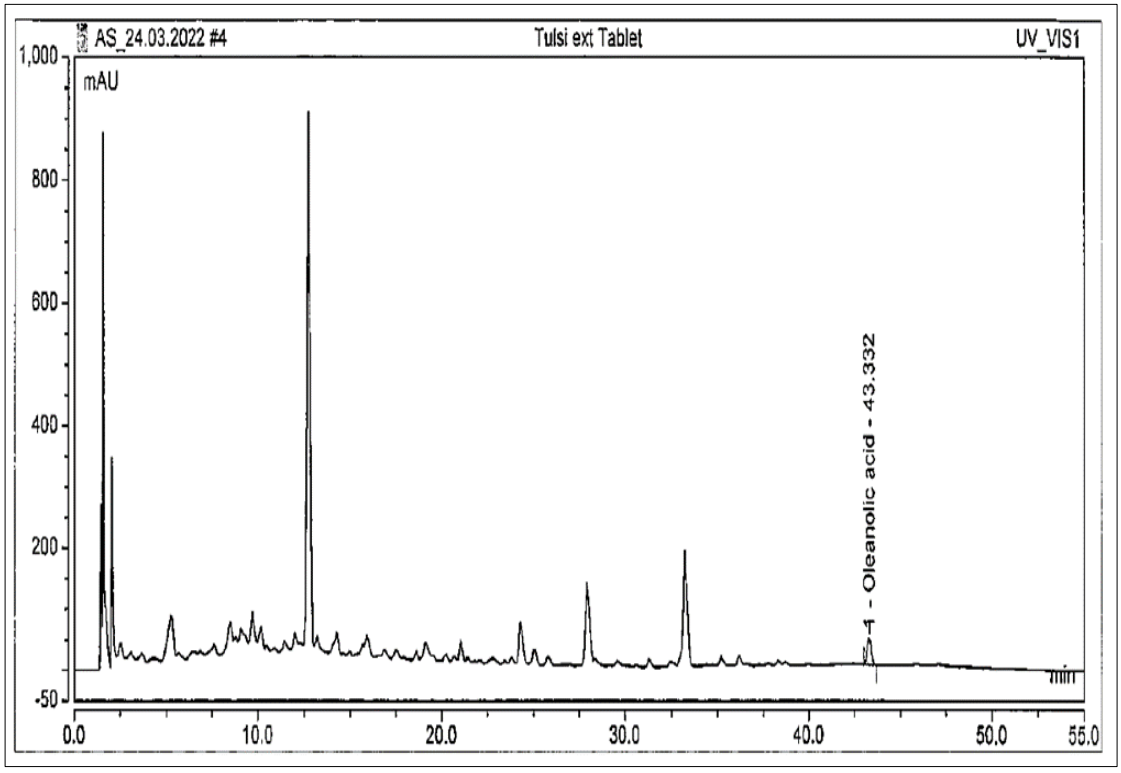

Fig 2: HPLC chromatogram of *Ocimum sanctum* extract Tablet

Coating of the tablets

The formulation F03 was selected for coating as sticking, capping and slight weight variation was observed in F01 and F02 batch whereas F03 was nonsticking and showed very less weight variation. The coated tablets were evaluated for the disintegration time, hardness, thickness, weight variation and HPLC Assay. The results indicate that not much variation is observed in the parameters as seen in Table 4.

Table 4: Evaluation of coated tablets formulation (F03)

Sr. no.	Parameters	Result
1	Disintegration time in water (min)	9.56±1.32
2	Hardness (N)	93±0.22
3	Thickness (mm)	3.6±0.09
4	Weight variation (mg)	205±7.5
5	Assay (%)	101.2%

Stability studies: The stability studies were carried out at 2-8 ºC and 40 ºC/75%RH for the selected formulation F03 up to 30 days. After 30 days' time interval the tablets was analyzed for drug hardness, in-vitro disintegration time and percent drug release. This formulation did show not much variation in any parameter. Table 5. Shows that, formulation F03 is stable and retained its original properties.

Table 5: Stability studies of coated tablets F03 stored at 2-8 ºC and 40 ºC/75%RH

Sr. no.	Parameters (F03)	2-8ºC	40ºC/75%RH
1	Disintegration time in water (min)	8.32±1.42	8.52±1.22
2	Hardness (N)	95±0.12	90±0.12
3	Thickness (mm)	3.7±0.09	3.5±0.09
4	Weight variation (mg)	204±7.6	204±7.3
5	Assay (%)	98.2%	99.2%

Alpha glucosidase activity

Alpha glucosidase activity of Oleanolic acid and tablet blend was carried out prior to formulation. Figure 3. Illustrates that oleanolic acid is a strong inhibitor, with an IC_{50} value of 0.82 (ppm) µg/ml. With an IC_{50} value of 3.58 (ppm) µg/ml, the *Ocimum sanctum* extract was also discovered to be a potent inhibitor, as shown in Figure 4.

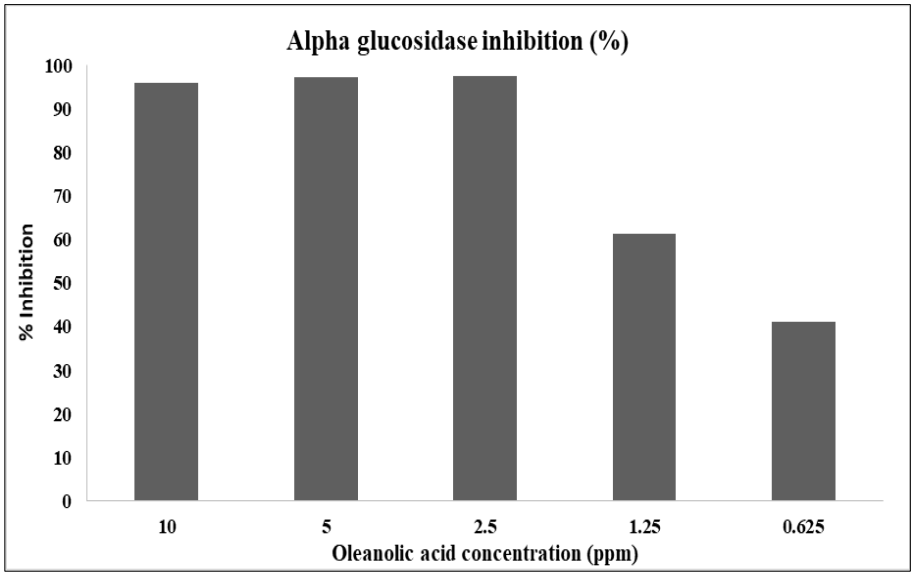

Fig 3: Inhibition of alpha glucosidase enzyme by Oleanolic acid

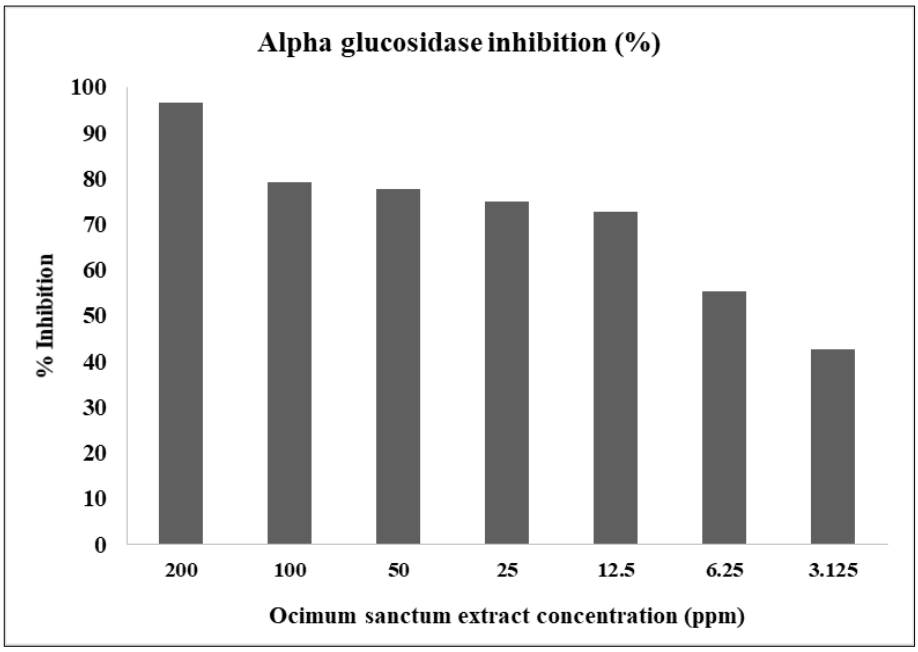

Fig 4: Inhibition of alpha glucosidase enzyme by *Ocimum sanctum* extract

Conclusion

The scientific research on *Ocimum sanctum* L. suggests a huge biological potential of its antidiabetic activity. The global burden of diabetes is increasing worldwide as it is a costly disease for developing economies of the world. Herbal formulation can be an advantage over synthetic drugs as it does not cause any side effects. In the present study we have used OS, an easily available and well-known herb for antidiabetic activity. The pre-compressional parameters of tablet i.e.; Angle of repose, Bulk density, tapped density, Compressibility index, Hausner's ratio were studied and found to be in satisfactory limits indicating that the physical mixtures of the formulations were suitable to formulate the tablets. Post compressional parameters of tablet i.e.; Weight variation, Hardness, Friability, Drug content, swelling index were evaluated and the results obtained were satisfactory. The F03 formulation had maximum percentage HPLC assay and less weight variation when compared to other formulations.

Alpha-glucosidase inhibitors (AGIs) play a crucial role in diabetes management by lowering blood glucose levels and reducing the risk of diabetes-related complications. AGIs are effective in preventing or delaying the onset of type 2 diabetes and improving the metabolic profile, thereby mitigating long-term complications associated with hyperglycemia. They are particularly beneficial for patients with comorbidities such as renal, cardiovascular, or hepatic disorders. Plant-derived bioactives, such as oleanolic acid, are recognized as potent AGIs. Oleanolic acid, a major component of *Ocimum sanctum*, has demonstrated an IC50 value of 0.82 µg/mL, while the herbal formulation exhibited an IC50 value of 3.58 µg/mL. These findings highlight the therapeutic potential of the *Ocimum sanctum* tablets formulated in this study for managing type 2 diabetes. Further research is planned to evaluate the one-year stability of these tablets and conduct clinical trials to validate their use as health supplements, with a proposed dosage of twice daily to achieve the desired antidiabetic effects.

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Disclosure Statement of Interest
The authors declare that there is no conflict of interest

Author contributions
The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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