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# Formulation and *In vitro* Characterization of *Syzygium Cumini* Chitosan (Poly-[D-glucosamine]) Nanoparticles for Diabetes.

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**ABSTRACT**

The study aims to develop chitosan encapsulated nanoparticles for the leaf extract of *Syzygium cumini* targeting effective management of diabetes. Traditionally, *Syzygium cumini* (commonly known as jamun) is well known for its ability to control diabetes. To increase its effectiveness as a remedy, special nanoparticles were created from Syzygium cumini extract with the goal of enhancing its absorption and overall therapeutic benefits. The particle size of these nanoparticles were analyzed using methods such, as Dynamic light scattering (DLS) and their morphology was scanned through electron microscopy (SEM). The particle size of nanoparticles were found to be 355 nanometers and their stability was measured through zeta potential (+24.65 millivolts). Anti diabetic activity were evaluated using enzyme inhibition tests that focused on alpha amylase, alpha glucosidase and beta glucosidase. Which, enzymes critical, in metabolism. The nanoparticles displayed inhibition levels; 75 % for alpha amylase 74 % for alpha glucosidase and 78 %, for beta glucosidase enzymes. The results indicate that nanoparticles derived from Syzygium cumini show improved effectiveness, in managing diabetes. Could be an approach for diabetes treatment going forward suggesting the need for additional research to ensure their safety and effectiveness over time, in practical experiments.


**Keywords**
Chitosan, *Syzygium cumini*, Enzymatic study, in vitro anti-diabetic activity

**INTRODUCTION**

Diabetes is a persistent metabolic condition characterized by elevated blood glucose levels due resistant or insufficient insulin production and also no insulin production. This condition is a leading global health concern and results in the development of cardiac diseases, peripheral neuropathies, amongst others. As the number of diabetic patient increases, it is imperative to find safer and better treatment regimens for diabetic patients [1]. Traditional therapies including insulin injections and tablets are exhibiting many drawbacks. Side effects of these fundamental treatments and reduced effectiveness in the long-term prompt the search, for relief.

Of all these alternatives, the natural plant extracts have hogged the limelight in relation to diabetes management. Traditional uses of *Syzygium cumini: Syzygium cumini* is recognized in Ayurveda, Unani and other state systems of medicine for its antidiabetic property. Flavonoids, tannins, and alkaloids had been identified in the plant and they all possess hypoglycemic and antioxidant effects that recommend the plant for management of diabetes [2]. However, one of the limitations in the usage of Syzygium cumini is that the bioavailability and stability of the active principles are low limiting its clinical application.

Nanotechnology provides one solution to enhance the delivery and effectiveness of these natural compound . The solubility and stability of the compound enhances by encapsulating into nanoparticles. This nanoparticles by conjugation with aptamers will specifically target the site of action. [3]. Chitosan is a polysaccharide obtained from chitin, which is biocompatible, biodegradable and bioactive polymer, which gained much interest by the researchers towards nanoparticle synthesis [4, 18].

There has been reported of antidiabetic activity associated with chitosan, brought about by several factors. Khan et al have reviewed that chitosan polymer and their drug delivery applications. Many researchers has reported antihyperglycemic effect of chitosan, because it suppresses the activity of alpha-amylase and alpha-glucosidase, enzymes which are responsible for the degradation of carbohydrates in the intestines [5]. Also chitosan improves the insulin sensitivity and also enhance the lipid metabolism which lead to increase the anti diabetic activity [6]. Thus chitosan polymer for the preparation of nanoparticles have great potential in drug delivery systems, mainly for augmentation of antidiabetic activity through plant extracts.

To improve and enhance the anti diabetic activity using plant based therapy, *Syzygium cumini* extract was taken into consideration. Here we planned to incorporate the extract into chitosan nanoparticles. The prepared nanoparticles were evaluated for its particle size and stability to ensure its effectiveness. Moreover, the anti-diabetic potential of the formulation was studied using the enzyme inhibitory model, which focuses on enzymes related to carbohydrate metabolic system.

## METHODS

### Collection of plant material and Authentication

The fresh leaves of *Syzygium cumini* were collected from Malumichampatti, Coimbatore district Tamil Nadu in the month of August 2023. The type of plant was certified by the BSI at the Tamil Nadu Agricultural University at Coimbatore, India. A voucher specimen (No. BSI/SRC/5/23/2023/TECH-806) has been deposited in the institution's herbarium as of November 30, 2023. Only healthy and mature leaves were selected for subsequent experiments.

### Preparation of *Syzygium cumini* leaves extract

The collected *Syzygium cumini* leaves were washed, air-dried to constant weight and ground into homogenized coarser powder. Extraction was done using right solvents including ethanol, methanol or water (hydroalcoholic) through maceration technique. Therefore extraction solution was filtered and filtrate was frozen at -50°C and lyophilized to get a powdered form of the leaf extract. The powdered extract was kept in an airtight container in low light, low temperature and dry environment to minimise degradation [7].

### Preparation of Chitosan Nanoformulation

Ionic gelation method was followed for the preparation of chitosan nanoparticles with the help of cross linking agent sodium tripolyphosphate (STPP). 1 percent Acetic acid solution was used to dissolve chitosan and allowed to stand at room temperature for 20-24 hours to make it clear. Chitosan solutions were prepared in different concentrations (0.05% to 0.5 %w/v) and add Tween 80 to minimize particle agglomeration. The pH was also maintained at 4.6 – 4.8 using 1 N sodium hydroxide. The STPP solution was added drop wise into the polymer solution, both chitosan and STPP solution were simultaneously stirred at 800 rpm. The obtained suspension was centrifuged at 12,000 rpm for 30 minutes, the pellet was washed by water and then resuspended in water for freeze-drying for further examination [8,9].

### Drug Loaded Chitosan Nanoformulation

A 50ml of chitosan solution was measured and placed in a beaker and then followed the addition of 5ml of the prepared plant extract solution while stirring. To the STPP solution, was added drop wise with stirring set at 1000 rpm until the solution became opalescent. The solution was then transferred to centrifuge tube and keep the solution on centrifuge for about 15mins at 3000 rpm. The pellets formed were washed with distilled water and used for lyophilization the next conducted experiments [10,17].

## CHARACTERIZATION

### Particle size Analysis

The size of the chitosan nanoparticles was evaluated by the Malvern Zeta Sizer Nano ZS90 at 25.1°C, after dilution in distilled water, and the particle size was 192.7 nm.

### Zeta potential

The chitosan nanoparticles were characterized for its zeta potential using a zeta sizer. Samples were afterwards diluted with 0.1 mL of distilled water and placed in the electrophoretically formed cell under an applied electrical field of 15.5V/cm All estimations were repeated three times for each sample to minimize variability [6].

### Fourier-transform infrared spectroscopy (FTIR)

All the characterizations were performed on the synthesized chitosan nanoparticles using; portable ATR-FTIR spectrometer from A2 Technologies where the collected data is presented below. Reference spectra were recorded in absorbance mode with 10 scans using the mid-IR region, 4000 $cm^{-1}$ – 400 $cm^{-1}$, 4 $cm^{-1}$ resolution, and at room temperature. In this case, around 1 mg of each sample was deposited on the sensor and experimental spectra were compared to standard chitosan and STPP [12].

### Scanning Electron Microscopy

The shape and the size of the dried chitosan nanoparticles was determined with a Quanta 400 ESEM/EDAX (FEI). The samples were prepared and mounted on SEM stub by using the double sided adhesive tape then dried under vacuum. In addition, photomicrography at acceleration voltage 20kV was used to study the surface topography [13].

**Evaluation for Antidiabetic Property**

***Alpha-Amylase Enzyme Inhibition Assay***

The control group consisted of phosphate buffer (pH 7), which was mixed with various concentrations of the samples and alpha-amylase (10 µl). This mixture was incubated at 37°C for 10 minutes. Then, starch solution was added to the mixture. After an hour, 0.1 ml of 1% iodine solution was added, followed by 5 ml of distilled water. The optical density (OD) was then measured at a wavelength of 565 nm. [14].

***Alpha-Glucosidase Enzyme Inhibition Assay***

Sample was prepared by mixing 225 µl of 80 mM phosphate buffer (pH 7.0) (control) , and various concentrations of test samples, which was then mixed with alpha-glucosidase. This mixture was pre-incubated at 37°C for 30 minutes. The reaction was stopped by keeping it in a water bath for 2 minutes. After cooling, 250 µl of glucose reagent was added. The mixture was incubated at room temperature for 10 minutes. After that optical density was measued the at 510 nm. [15].

***Beta- glycosidase inhibitory assay***

The enzyme assay involved adding 0.5 ml of enzyme to preincubated salicin substrate solution. The samples were prepared and incubated at 50°C for 30 minutes before the reaction mixture underwent heat inactivation at 100°C for 15 minutes. The amount of glucose released in the reaction mixture was measured using PGM, with the activity defined as the amount (µmol) of β-glucosidase that catalyzed the hydrolysis of salicin to produce 1 µmol of glucose per minute under the specified conditions. [16].

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control- Absorbance of Sample}}{\text{Absorbance of Control}}$$

**RESULT AND DISCUSSION**

**Formulation of Chitosan Nanoformulation**

The chitosan nanoparticles were prepared in this study using ionic gelation technique that entails the interaction of chitosan molecules with poly-anions. This gelation process occurs due to the formation of both inter and intra molecular cross linkages through the poly-anions. As for the nanoparticles preparation, a solution of sodium tripolyphosphate (STPP) with a negative charge was introduced into a chitosan solution which bears a positive charge under vigorous magnetic stirring at room temperature. In order to gain the most efficient synthesis method of the nanoparticles, precursory tests were made to identify the correct ratio of chitosan to STPP. This ratio was important for getting small nanoparticle size and size distribution as well.

After optimization, addition of the Syzygium cumini leaves extract were carried out, the mixture became from clear solution to an opalescent suspension implying that nanoparticles had been formed. This visual alteration supports evidential evidence regarding the effective encapsulation of the bioactive compounds existing in the leaf extract into the chitosan polymer.

**Particle Size Analysis**

The Z-Average size of the nanoparticles was **355.8** nm with a polydispersity index (PI) of 0.5894. This Z-Average measurement reflects the mean size of the particles, with a minimal deviation, suggesting a relatively homogeneous sample size distribution. There are two significant peaks observed in the intensity distribution. The first peak has a mean size of 639.2 nm and accounts for 71.32% of the area, indicating it is the dominance in the sample. The second peak has a mean size of 123.4 nm and comprises 26.44% of the area, representing a smaller portion of the particle sample. These results suggest the presence of two distinct sizes within the sample. The zeta size of the extract loaded chitosan nanoparticles (CNPs) illustrated in figure 1.

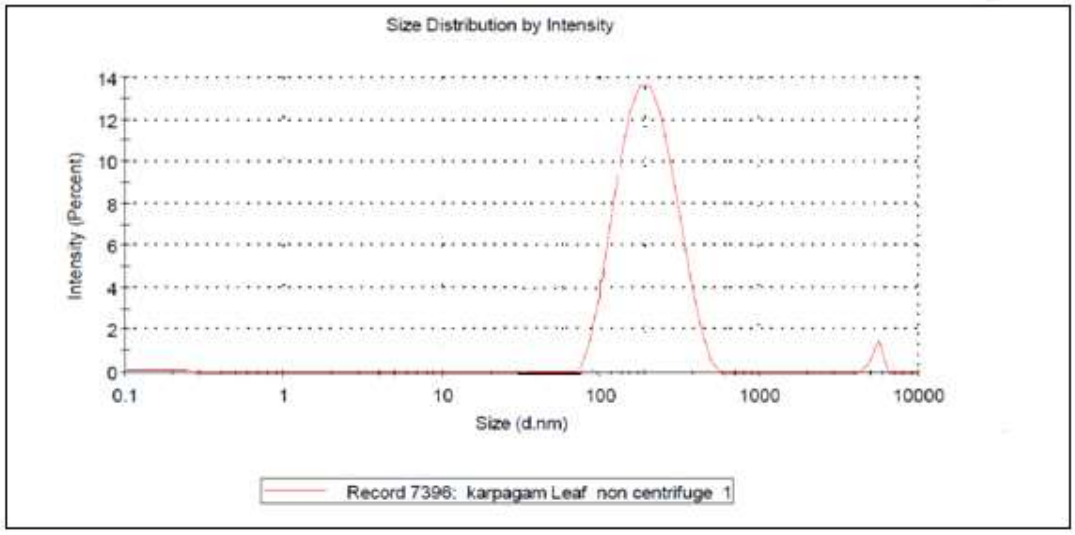


Figure 1: Size distribution of chitosan *Syzygium cumini* nanoparticles

**Zeta Potential**

The mean Zeta Potential was identified as **24.65 mV** (Figure: 2), demonstrating a consistent trend with no observed standard deviation. This lack of variance indicates a highly stable environment throughout the study, ensuring precision and reliability in the measurements. These findings offer valuable insights into the system under investigation, highlighting the uniformity of the Zeta Potential across the assessed conditions.

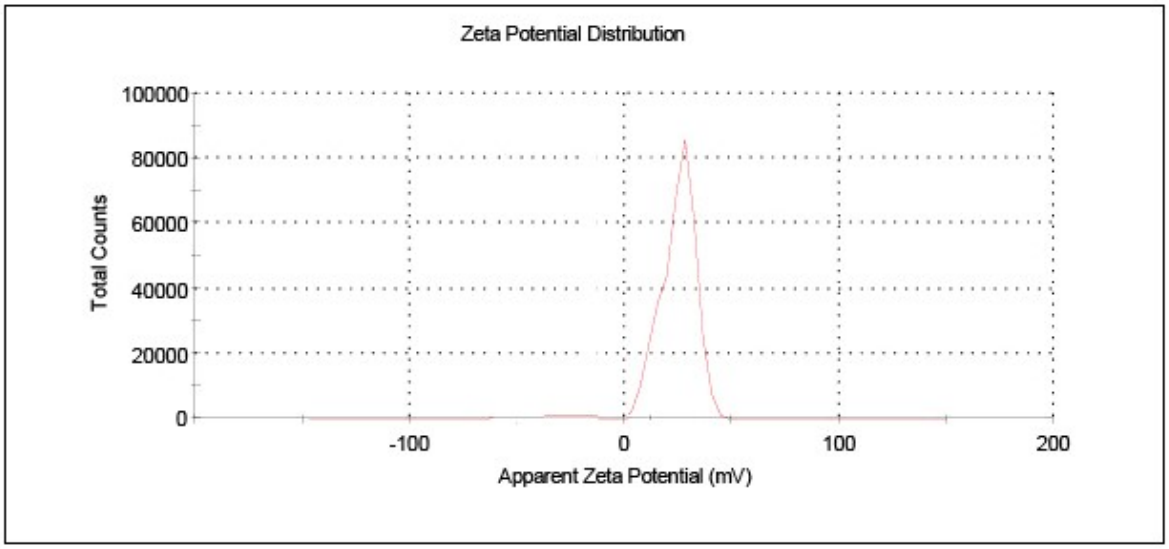


**Figure 2**: Zeta Potential Distribution of chitosan *Syzygium cumini* nanoparticles

**Fourier-transform infrared spectroscopy (FTIR)**

FTIR analysis of chitosan nanoparticles usually shows distinct absorption bands, such as a broad peak in the range of 3478 $cm^{-1}$ (O-H) stretching, 2856 $cm^{-1}$(C-H). 1650 $cm^{-1}$(C=O stretching), 1550 $cm^{-1}$ (N-H bending), 1323 $cm^{-1}$ (C-N stretching), and 1150 $cm^{-1}$ (C-O-C stretching). These specific peaks indicate that there is no incompatibility between the chitosan polymer and the plant extracts (Figure: 3).

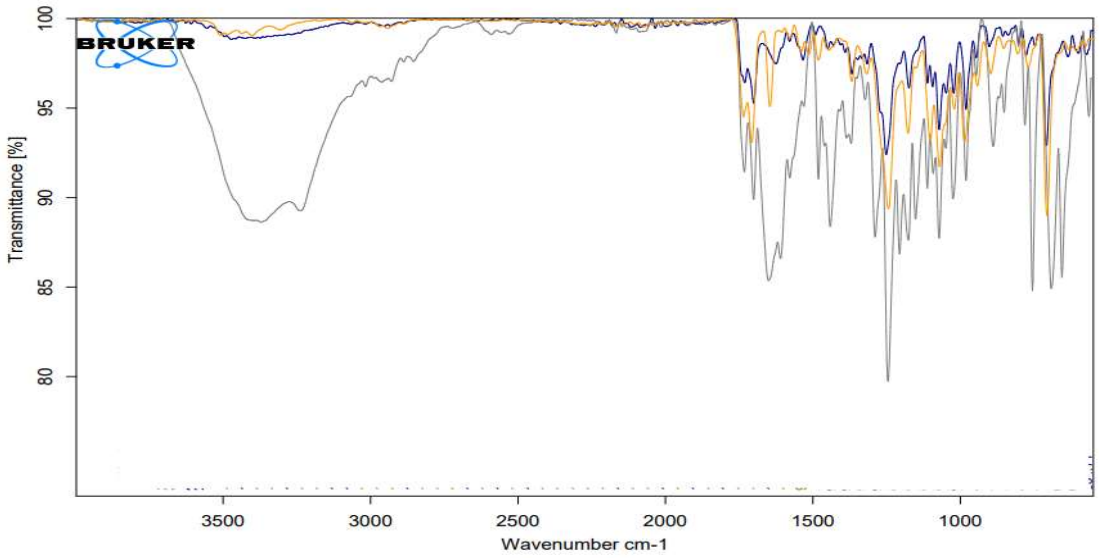


**Figure 3**: Stacked FTIR Spectra of chitosan, *Syzygium cumini,* and nanoparticles

**Scanning Electron Microscopy (SEM)**

The SEM analysis of chitosan nanoparticles confirmed their morphological characteristics, with most particles exhibiting a spherical shape and smooth surface texture. Some agglomeration was observed, which can be attributed to the nanoparticles' tendency to cluster due to their surface properties and interactions. The uniformity in shape and size suggests a controlled synthesis process. The absence of significant surface irregularities or fractures indicates good nanoparticle stability.

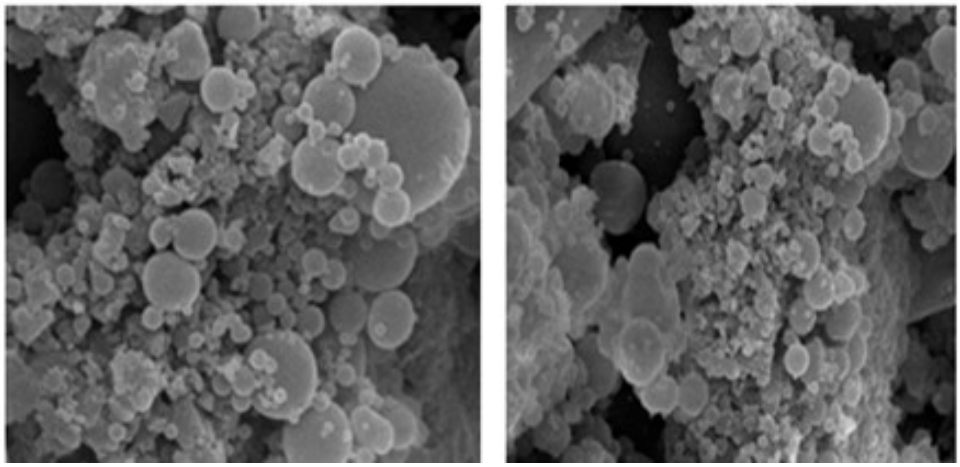

Figure 4: SEM images of nanoparticles

**Anti-Diabetic Assay**

***Alpha-amylase enzyme inhibition assay***

The inhibitory effects of Acarbose at various concentrations on both the extraction and formulation process. At a concentration of 10 µl, Acarbose exhibited inhibition rates of 32% in the extraction process and 25.00% in the formulation process, this increased marginally to 29.00% at 20 µl. Further increases in concentration resulted in higher inhibition rates for both processes, with 30 µl of Acarbose showing inhibition rates of 55% and 48.10% for extraction and formulation, respectively. At 40µl concentration, inhibition rates rose to 72% for extraction and 55.30%for formulation. The highest concentration tested, 50 µl, demonstrated the greatest inhibitory effects, with rates reaching 87% for extraction and 69.00% for formulation, slightly increasingto75.30% in the

formulation process. These results suggest a dose-dependent relationship between Acarbose concentration and inhibition efficiency in both extraction and formulation processes (Figure 5).

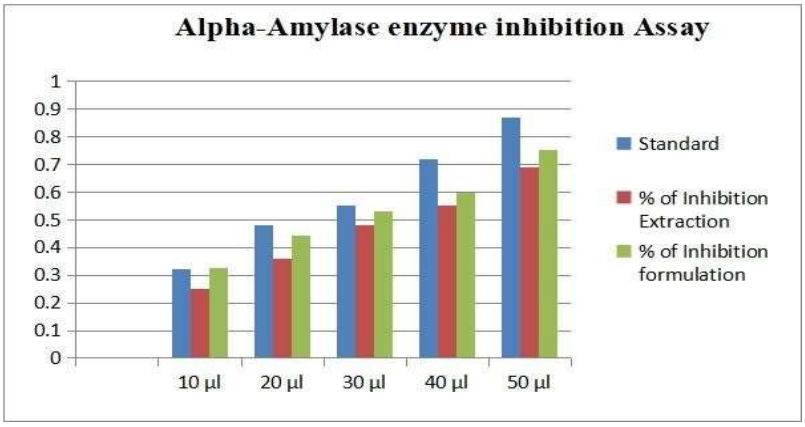


Figure 5: Alpha Amylase Enzyme Inhibition Assay

***Alpha-glycosidase enzyme inhibition assay***

The impact of varying concentrations of Acarbose on both the extraction and formulation processes. At a concentration of 10 µl, Acarbose demonstrated a 30% inhibition rate in the extraction process and 23.50% in the formulation process, which slightly increased to 28.50% at 20 µl. As the concentration increased to 30 µl, the inhibition rates rose to 58% for extraction and 48.50% for formulation. Further escalation to 40 µl concentration led to inhibition rates of 73% for extraction and 54.30% for formulation. Finally, the highest concentration tested, 50 µl, exhibited 81% inhibition in the extraction process and 70.00% in the formulation process, which further increased to 74.20%. These findings illustrate a clear concentration-dependent relationship between Acarbose concentration and inhibition efficiency in both extraction and formulation processes (Figure 6).

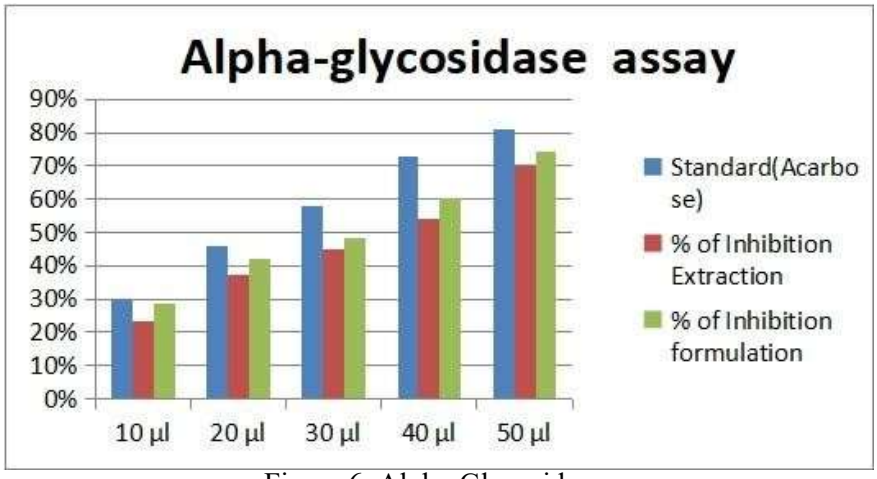


Figure 6: Alpha-Glycosidase assay

***Beta-glucosidase inhibitory***

The data illustrates the inhibitory effects of varying concentrations of Metronidazole on both the extraction and formulation processes. Starting with a concentration of 10 µg/ml, Metronidazole displayed a 17% inhibition rate in the extraction process and 14.30% in the formulation process, which increased to 18.50% at 20 µg/ml. As the concentration rose to 30 µg/ml, inhibition rates surged to 55% for extraction and 48.20% for formulation. Further escalation to 40 µg/ml concentration led to inhibition rates of 74% for extraction and 62.10% for formulation. Finally, at the highest concentration tested, 50 µg/ml, Metronidazole exhibited 85% inhibition in

the extraction process and 73.00% in the formulation process, which further increased to 78.50%. These findings demonstrate a concentration-dependent relationship between Metronidazole concentration and inhibition efficiency in both extraction and formulation processes (Figure 7).

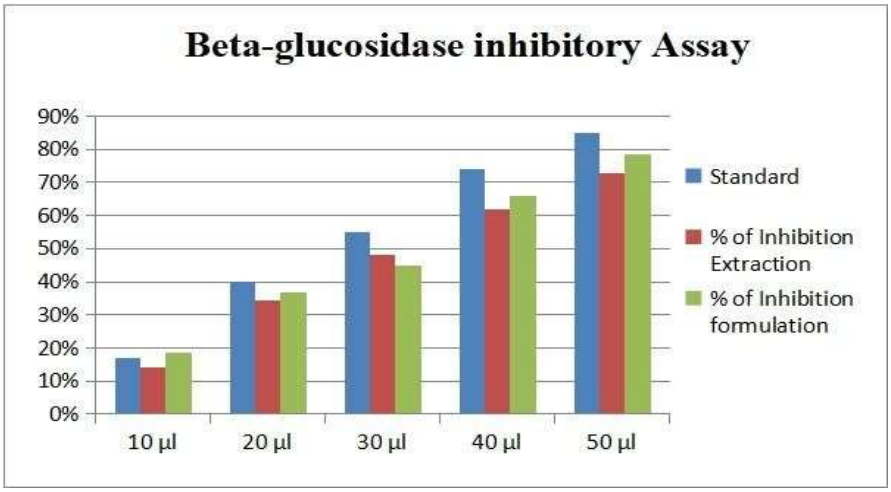


**Figure 7: Beta Glucosidase Inhibitory Assay**

## CONCLUSION

Therefore, the current study on *Syzygium cumini* using biodegradable nanoparticles has shown enhanced anti-diabetic activity of the plant bioactive compounds. The efficiency compared to the conventional extract was achieved by integrating the active principle in nanoparticles, which improved its formulation parameters: bioavailability, stability, and therapeutic effectiveness. In vitro tests depicting higher inhibitory effect on α-amylase and increased glucose uptake in cells, indicated better glycemic control and improved insulin responsiveness. *Syzygium cumini* nanoparticles can potentially be used in diabetes treatment ad management; there are certain challenges that plant extracts have that have prevented their use, some of which includes bad solubility and bioavailability. These results open avenue for future research where our model can be used to conduct further in vivo study and clinical trials to establish their effectiveness in human and also to understand more about their mode of action. Thus, the investigated nanotechnology might provide a better, less hazardous way for diabetes patients to get plant therapies.

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