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Research Paper

Formulation And In-Vitro Evaluation of Microbeads Containing Syzigium Cumini Leaf Extract

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ABSTRACT

Diabetes remains a massive global health hurdle, and while nature offers powerful tools like Jamun (Syzygium cumini) leaves, turning those leaves into reliable medicine is tricky. The raw extracts often break down too quickly in the body or aren't absorbed well.To solve this, our study developed a specialized "slow-release" system. We used a gentle technique called ionotropic gelation to tuck the leaf's natural antioxidants—specifically its phenolic acids and flavonoids—inside tiny sodium alginate microbeads.Why this matters:Protection: These microbeads act like a protective shield, keeping the active ingredients safe from harsh stomach acids.Precision: Instead of a sudden spike, the beads release the extract gradually. Effectiveness: The formulation successfully blocked α-amylase and α-glucosidase (the enzymes responsible for sugar spikes) while keeping its full antioxidant power intact.Ultimately, this isn't just about better chemistry; it's about creating a smarter, plant-based supplement that is easier for patients to take and more effective at managing blood sugar over the long term.

INTRODUCTION

The Global Burden of Diabetes Mellitus Diabetes Mellitus has emerged as a massive global health hurdle and one of the most pressing metabolic challenges of the modern era. The condition involves a persistent state of hyperglycemia caused by insufficient insulin production or cellular insulin resistance. Over time, erratic glucose levels trigger systemic damage, leading to renal complications, vision loss, cardiovascular disease, and neuropathy. While lifestyle modifications are effective, maintaining them over

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decades creates an "adherence gap," driving the search for easier-to-manage, plant-derived nutraceuticals that offer sustainable glycemic control without the harsh side effects of synthetic drugs.The Botanical Powerhouse: *Syzygium cumini* (Jamun) Leaf Extract *Syzygium cumini*, commonly known as Jamun, is an evergreen tropical tree renowned in traditional Ayurvedic and Unani medicine. The leaves of this plant are a botanical powerhouse, incredibly rich in complex phytochemicals including gallic acid, ellagic acid, tannins, flavonoids, jamboline, betulinic acid, and quercetin. These natural antioxidants exert a potent antihyperglycemic effect by inhibiting crucial enzymes like α-amylase and α-glucosidase (which are responsible for dietary sugar spikes), shielding the body from oxidative stress, and improving the body's overall insulin response.

Principles and Reasons for Microencapsulation Microencapsulation is defined as the process of enclosing an active agent or core material within a microscopic shell or embedding it into a matrix structure, typically ranging from 3 to 800 micrometres in diameter. For pharmaceutical purposes, this technology is focused on creating new drug delivery systems (DDS) with optimised therapeutic profiles. The primary reasons for microencapsulation include:

- Sustained Release: To provide a steady, prolonged release of the active drug over an extended period.
- Taste and Odor Masking: It effectively hides organoleptic properties, such as the bitter and astringent taste of crude herbal extracts, improving patient compliance.
- Physical Transformation: Liquid drugs or sticky extracts can be converted into free-flowing powders.
- Environmental Protection: It protects sensitive phytochemicals from destructive environmental factors such as moisture, light, oxidation, and harsh stomach acids.
- Volatility and Incompatibility Prevention: It prevents the vaporization of volatile plant constituents and physically separates reactive core materials to prevent chemical incompatibilities.
- Targeted Absorption: It alters the site of absorption by protecting the drug in the stomach and delivering it directly to the intestines, thereby reducing GI irritation and toxicity.

Materials for Microencapsulation:

The formulation of microparticles relies on selecting highly compatible core and coating materials:

- Core Materials: The core is the specific material to be coated. A liquid core can include dispersed active extracts, solvents, or oils. A solid core consists of the active pharmaceutical constituents, stabilizers, diluents, and release-rate retardants.
- Coating Materials (Polymers): The coating serves as the protective shell and must be satisfactory for gastrointestinal use. Common materials include water-soluble resins (gelatin, starch, polyvinylpyrrolidone), water-insoluble resins (ethyl cellulose, polyamide), waxes and resins (paraffin, beeswax), and enteric resins (cellulose acetate phthalate).

Excipient Profile: Sodium Alginate and Calcium Chloride For this formulation, specific hydrogel-forming excipients were chosen:

- Sodium Alginate: A naturally occurring, non-toxic, biodegradable, and biocompatible polysaccharide extracted primarily from brown seaweeds (*Laminaria* and *Macrocystis*). It is a linear copolymer composed of mannuronic acid (M) and guluronic acid (G) units. It is highly valued for its ability to form viscous solutions and heat-stable gels.

- Calcium Chloride ($CaCl_2$): A highly soluble, inorganic, hygroscopic salt that releases calcium ions (Ca^{2+}) upon dissolution. In pharmaceutical industries, it acts as a crucial cross-linking agent. When sodium alginate is dropped into a calcium chloride solution, the calcium ions bind with the polyanionic guluronic acid blocks of the alginate, creating an "egg-box" network through a process called ionotropic gelation, resulting in firm, stable gel spheres.

Microbeads: Ideal Characteristics and Advantages Microbeads are nearly spherical, free-flowing multiparticulate carriers (0.5 to 1000 µm) that allow for multiple release profiles. Ideal characteristics include:

- The ability to incorporate reasonably high concentrations of the drug.
- Stability of the preparation after synthesis with a clinically acceptable shelf life.
- Controlled particle size and good dispersibility.
- Biocompatibility with controllable biodegradability. Advantages of microbeads over single-unit dosage forms include:
- Providing a constant and prolonged therapeutic effect, which helps maintain a constant drug concentration in the blood.
- Protecting the drug from enzymatic and photolytic cleavage.
- Distributing more uniformly in the gastrointestinal tract, which improves overall bioavailability, reduces local irritation, and limits drug fluctuations within the therapeutic range.
- Decreasing dosing frequency, which vastly improves patient compliance.

Drug Release Kinetics and Mechanisms:

- Release Kinetics: The ultimate goal of a sustained-release microbead system is to deliver a drug at a specific rate to maintain constant blood levels. Ideally, the rate of drug delivery should be independent of the amount of drug remaining in the dosage form (zero-order kinetics). To achieve prompt therapeutic levels that are maintained over time, controlled systems often act in two parts: an initial loading dose released immediately, followed by a maintenance dose released at a slow, controlled rate equivalent to the drug's elimination rate.
- Dissolution Controlled Systems: In these systems, the rate-controlling step is dissolution. The drug is either coated with a slow-dissolving substance (Encapsulation), where the release depends entirely upon the solubility and thickness of the coating, or the drug is embedded in a slow-dissolving matrix (Matrix/Monoliths), which controls dissolution by regulating the rate of fluid penetration.
- Diffusion and Erosion Systems: In diffusion-controlled systems, water uptake causes the polymer chains to swell, creating pores through which the dissolved drug escapes the microbead. In erosion systems, the polymer matrix is designed to gradually degrade and erode with time, releasing the trapped drug as the microstructure breaks down.

Jamun Leaf Extract (*Syzygium cumini*)

- Properties & Phytochemistry: The leaves of Jamun are considered a botanical powerhouse rich in a complex array of natural secondary metabolites. Key bioactive compounds include flavonoids, phenolic acids, tannins, jamboline, ellagic acid, quercetin, gallic acid, betulinic acid, and anthocyanins.

- Therapeutic Effects:
  - Antidiabetic & Antihyperglycemic Action: The extract exerts potent blood-sugar-lowering

effects. It works by blocking enzymes like α-amylase and α-glucosidase (which are responsible for dietary sugar spikes) and by stimulating the surviving pancreatic beta cells to release more insulin.

- Antioxidant & Protective Effects: The high concentration of natural antioxidants helps shield the body from oxidative stress, a common complication of diabetes, and can even reduce radiation-induced DNA damage.
- Traditional & Systemic Uses: Beyond diabetes, Jamun leaf extract has pharmacological activities that include antibacterial, antifungal, antiviral, anti-allergic, and anticancer properties. It is traditionally used to treat diarrhoea, dysentery, liver and digestive ailments, bronchitis, asthma, mouth ulcers, and to accelerate wound healing.

Fig.1: Jamun Leaf Extract

Sodium Alginate

- Properties: Sodium alginate is a naturally occurring, non-toxic, biodegradable, and biocompatible polysaccharide extracted primarily from brown seaweeds such as *Laminaria* and *Macrocystis*. It is the sodium salt of alginic acid, consisting of a linear copolymer of mannuronic acid (M) and guluronic acid (G) units. It is highly water-soluble, stable across a wide pH range, and has excellent film-forming abilities. Its most crucial property for this project is its ability to undergo ionotropic gelation—forming viscous solutions and thermo-irreversible, heat-stable hydrogels when it interacts with divalent cations.

- Therapeutic Effects & Biomedical Applications:
  - Drug Delivery: It acts as a hydrophilic carrier and protective polymer matrix, encapsulating active drugs to mask taste, prevent degradation in stomach acid, and provide sustained, controlled release of the medication.
  - Clinical Applications: Beyond encapsulation, sodium alginate possesses its own antioxidant and anti-inflammatory potential and is widely used in wound healing, tissue engineering, and antacid/reflux treatments.

Fig.2: Sodium Alginate

Calcium Chloride ($CaCl_2$)

- Properties: Calcium chloride is a white, crystalline, inorganic salt composed of calcium and chlorine. It is highly soluble in water and hygroscopic (capable of absorbing water from the environment), making it an excellent desiccant. Dissolving it in water causes an exothermic reaction (releasing heat). Crucially, it readily releases calcium ions (Ca^{2+}), which have a strong affinity for

binding with polyanionic polymers like sodium alginate.

- Therapeutic Effects & Biomedical Applications:
  - Cross-linking Agent: In the formulation of microbeads, calcium chloride serves as the essential curing bath. The Ca^{2+} ions trigger cross-linking of the alginate chains, instantly forming stable, firm, and structurally supported gel-like beads.
  - Biomedical Uses: Its high biocompatibility and safety make it highly valuable for drug encapsulation, controlled delivery systems, and regenerative medicine or tissue engineering.

Fig.3: Calcium Chloride ($CaCl_2$)

1. Literature review

2.1 Pharmacognosy and Phytochemistry of Jamun: Detailed pharmacognostic evaluations are essential for the quality control of herbal drugs. The microscopic profile of *Syzygium cumini* leaves features wavy epidermal cells, anisocytic stomata, bordered pitted reticulate vessels, and abundant rosette and prismatic crystals of calcium oxalate. Kumari et al. (2023) highlighted that these leaves contain secondary metabolites like quercetin, tannins, and myricetin glycosides, which act as potent antioxidants and antidiabetic agents.

2.2 *In-Vivo* Antidiabetic Efficacy: Mulkalwar et al. (2021) investigated the antihyperglycemic activity of *Syzygium cumini* in diabetic rats. The administration of the extract caused a statistically significant reduction in fasting blood sugar levels and HbA1C. Notably, when Jamun extract was administered alongside Metformin, the resulting glycemic control was significantly superior to either drug administered alone. Patel P. et al. (2023) also confirmed its promise as an antidiabetic drug through extensive biochemical investigations.

2.3 Advancements in Microbead Drug Delivery: The formulation of multi-particulate microbeads via ionotropic gelation is an environmentally benign method ideal for encapsulating fragile biomolecules. Bhupathyraaj et al. (2021) established that these systems provide steady, prolonged therapeutic effects, maintain constant drug concentrations in the blood, and reduce dosing frequency. According to BenchChem's technical data, the structural stability of alginate microbeads relies on the "egg-box" model, highly dependent on the concentration of the calcium chloride cross-linking bath and the guluronic acid blocks within the polymer.

3. Aim and Objective

Aim: To successfully formulate, characterise, and evaluate sustained-release polymeric microbeads loaded with *Syzygium cumini* (Jamun) leaf extract utilising the ionotropic gelation method.

Key Objectives:

1. To standardise the raw plant material through pharmacognostic and physicochemical evaluation.
2. To extract the bioactive phytoconstituents from dried Jamun leaves via ethanolic maceration.
3. To formulate spherical microbeads using sodium alginate as the polymer matrix and calcium chloride as the cross-linking agent.

4. To evaluate the micromeritic flow properties of the microbeads.
5. To determine the encapsulation efficiency of the active compounds and the swelling index of the hydrogel.
6. To study the *in-vitro* sustained release profile of the bioactives and verify chemical compatibility using FTIR spectroscopy.

4. Plan of Work

4.1. Raw Material Sourcing & Pharmacognostic Standardization:

- **Procurement:** Fresh leaves of *Syzygium cumini* (Jamun) will be carefully collected, verified, and authenticated to serve as the source of the active pharmaceutical compounds.
- **Standardization:** The raw leaves will undergo macroscopic and powder microscopic evaluation to confirm their identity and purity. Essential physicochemical parameters such as moisture content (loss on drying) and total ash values will be measured to establish baseline quality standards before formulation begins.

4.2. Preparation of the Botanical Extract:

- **Drying and Milling:** The fresh leaves will be washed and shade-dried to prevent the thermal degradation of fragile phytochemicals (such as flavonoids and essential oils). Once dried, they will be ground into a coarse powder to increase the surface area for maximum solvent extraction.
- **Maceration:** The bioactive compounds will be extracted using 70% ethanol via a cold maceration process.
- **Filtration and Concentration:** The resulting liquid will be filtered to separate solid plant residue and concentrated using evaporation at a controlled temperature to yield a thick, solvent-free extract.

4.3. Preliminary Phytochemical Screening (Qualitative Testing):

- **Crucial Testing Phase:** Immediately following the extraction process, and *before* encapsulating the extract, the concentrated ethanolic extract will be subjected to rigorous preliminary phytochemical screening.
- **Purpose:** This step is performed to qualitatively confirm the presence of the desired secondary metabolites that drive the antidiabetic activity.
- **Methods Used:** The extract will be treated with specific reagents—such as Ferric chloride (for Phenols), Lead acetate (for Tannins), Sodium hydroxide (for Flavonoids), and shaken with water (for Saponins)—to verify the biochemical integrity of the batch prior to formulation.

4.4. Selection of Excipients and Microbead Formulation:

- **Excipient Preparation:** Sodium alginate will be prepared as the hydrophilic polymer matrix (the internal phase), and calcium chloride will be prepared as the cross-linking curing bath (the external phase).
- **Ionotropic Gelation Procedure:** The concentrated, pre-screened Jamun extract will be uniformly dispersed into the viscous sodium alginate solution. This active mixture will be extruded drop-by-drop through a 22-G syringe into the calcium chloride bath under constant magnetic stirring. The calcium ions will instantly cross-link the alginate chains, trapping the extract inside newly formed, spherical hydrogel microbeads.

4.5. Physicochemical Characterisation and Evaluation:

- **Micromeritic (Flow) Properties:** The dried microbeads will be evaluated for Bulk density, Tap density, Carr's index, and

Hausner's ratio to ensure they possess excellent flowability, which is essential for pharmaceutical processing and capsule filling.

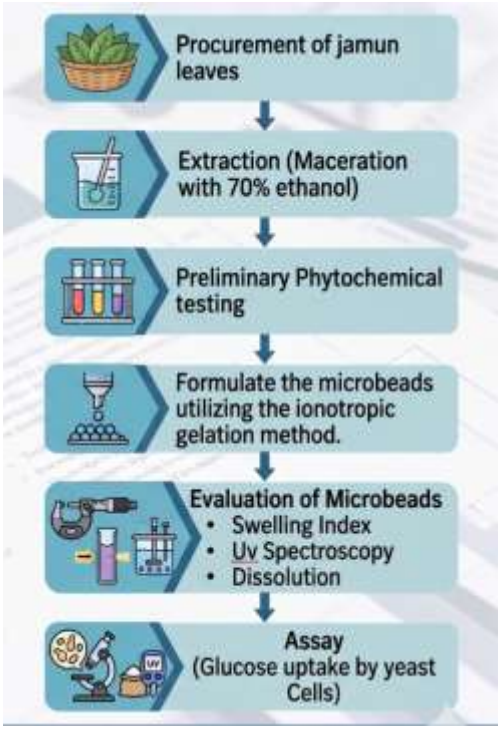
- **Formulation Efficiency:** The Entrapment Efficiency (EE%) will be calculated to determine the exact amount of herbal extract successfully encapsulated inside the polymer, and the Swelling Index will be measured to assess the matrix's hydration capacity.

4.6. *In-Vitro* Release Kinetics and Biological Assay:

- **Dissolution Testing:** The microbeads will undergo *in-vitro* drug release studies over a timed period to evaluate their sustained-release profile and verify that the matrix provides a reliable barrier.
- **Efficacy Testing:** An assay measuring glucose uptake by yeast cells will be performed to benchmark the *in-vitro* hypoglycemic activity of the formulated extract against a standard drug (Metformin).

4.7. Compatibility Analysis:

- **FTIR Spectroscopy:** Fourier Transform Infrared analysis will be conducted to verify that the active Jamun phytoconstituents did not chemically react with or degrade within the alginate polymer during the encapsulation process, ensuring complete chemical compatibility of the physical mixture.



5.Plant Profile

Here is the comprehensive plant profile for **Jamun** (*Syzygium cumini*), detailing its botanical, physical, chemical, and therapeutic characteristics based on the project's sources:

Botanical Classification & Nomenclature

- **Botanical Name:** *Syzygium cumini* (L.) Skeels.
- **Synonyms:** *Calyptranthes jambolana* MOON., *Eugenia jambolana* LAM., *Syzygium jambolana* DC., and *Syzygium jambolanum* DC.
- **Common Names:** Jamun (Hindi), Jambu (Sanskrit), Indian blackberry, Java plum, and Black plum.
- **Family:** Myrtaceae.

Geographical Source & Habitat

Syzygium cumini is a long, highly branched, evergreen tropical tree. It is native to India and Indonesia, and is widely distributed throughout all tropical and subtropical regions, excluding desert areas.

Macroscopic (Physical) Characteristics of Leaves

The leaves of the Jamun plant possess distinct morphological features:

- **Shape:** Simple, opposite, oblong-oval, elliptic, and acuminate with a blunt or tapering apex and an entire margin.
- **Size:** Typically measures 5 to 18 cm in length and 2.5 to 8 cm in width, with a slender stalk measuring 0.7 to 2.2 cm long.
- **Colour:** When fresh, the upper surface is shining and dark green, while the lower surface is light green. Dried leaves turn brownish-green.
- **Odour:** Aromatic and turpentine-like.
- **Taste:** Slightly astringent.
- **Texture:** Leather-like to the touch.

Microscopic (Anatomical) Characteristics

Under powder microscopy and cross-sectional evaluation, the leaves display several key diagnostic structures:

- A single layer of wavy epidermal cells covered with a striated cuticle.
- **Anisocytic stomata** present on the lower epidermis.
- **Abundant rosette and prismatic crystals of calcium oxalate**.
- Large, oval-to-round collenchymatous cells and spongy parenchyma.
- Bordered pitted reticulate vessels and spiral thickenings.

Phytochemistry (Active Chemical Constituents)

Jamun leaves are considered a botanical powerhouse packed with a complex array of secondary metabolites and natural antioxidants.

- **Key Bioactive Markers:** Quercetin, jamboline, ellagic acid, gallic acid, betulinic acid, mallic acid, and anthocyanins.
- **General Phytochemical Classes:** Highly rich in flavonoids, phenolic acids, tannins, essential oils, glycosides, and saponins.

Traditional & Therapeutic Uses

While traditional Indian systems of medicine (like Ayurveda and Unani) use various parts of the plant as blood purifiers and general tonics, the leaves specifically offer profound medicinal benefits:

- **Antidiabetic & Antihyperglycemic:** The primary focus of this project. The phytochemicals effectively lower blood glucose, inhibit sugar-spiking enzymes like α-amylase and α-glucosidase, and stimulate surviving pancreatic beta cells to release insulin.
- **Antioxidant & Cellular Protection:** It shields the body against oxidative stress and reduces radiation-induced DNA damage.
- **Gastrointestinal & Systemic Healing:** Traditionally used to treat diarrhoea, dysentery, stomach-ache, constipation, liver and digestive ailments, and to inhibit blood discharges in faeces.
- **Additional Properties:** Acts as an antibacterial, antifungal, antiviral, anti-allergic, anti-inflammatory, and anticancer agent, while also being used for bronchitis, asthma, dermopathy, and wound healing.

6.Experimental work

6.1 Materials and Equipment:

- **Plant Material:** 1 kg of fresh *Syzygium cumini* leaves (reduced to 50 g of fine coarse powder).

- **Excipients:** 2 g of Sodium Alginate (hydrophilic matrix), 5 g of Calcium Chloride (cross-linking agent), and 500 ml of 70% Ethanol.
- **Equipment:** 22G Syringe, optical microscope, magnetic stirrer, UV-visible spectrophotometer, and FTIR spectrophotometer

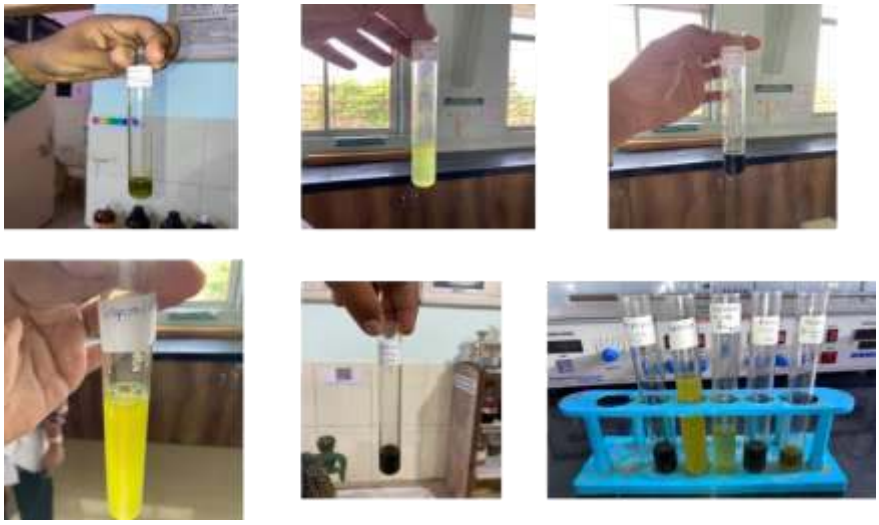


Fig.5: Preliminary Phytochemical Screening

6.2 Extraction & Preliminary Phytochemical Screening: Fresh Jamun leaves were washed and shade-dried to preserve fragile elements like flavonoids from thermal degradation. Bioactives were extracted via maceration in 500 ml of 70% ethanol, filtered, and evaporated to yield a concentrated paste. This extract was then subjected to preliminary phytochemical tests using specific reagents (FeCl3, Lead acetate, NaOH) to confirm the presence of active compounds.

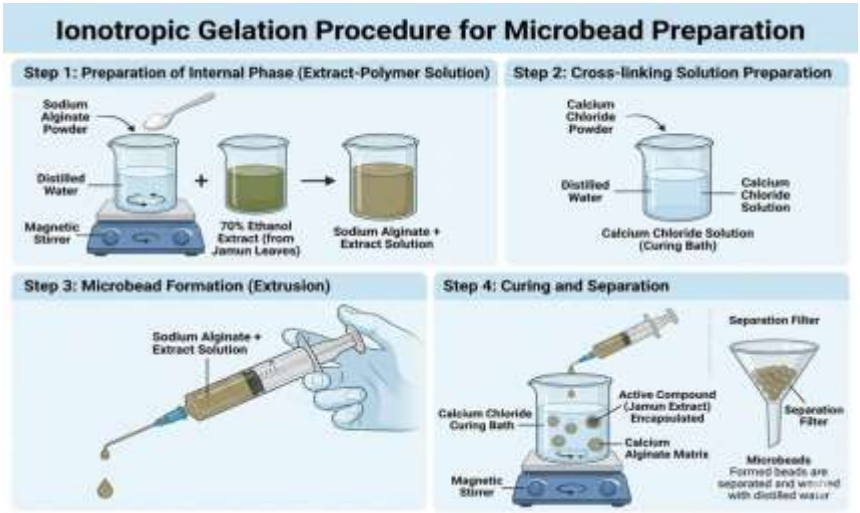


Fig.6: Formulation via Ionotropic Gelation

6.3 Formulation via Ionotropic Gelation:

1. **Polymer Solution:** 2 g of sodium alginate was slowly dissolved in 125 ml of distilled water under continuous magnetic stirring to yield a uniform, viscous base.
2. **Drug Incorporation:** 1.5 g of the concentrated Jamun leaf extract was dispersed thoroughly into the alginate matrix.

3. **Cross-linking Bath:** A curing solution was prepared by dissolving 5 g of calcium chloride in 100 ml of distilled water.
4. **Extrusion & Curing:** The extract-alginate mixture was drawn into a 22G syringe and extruded drop-by-drop into the calcium chloride bath under constant magnetic stirring at 475 rpm. The calcium ions instantly cross-linked with the alginate, forming spherical hydrogel microbeads. The beads were cured for 30 minutes, filtered, washed with distilled water, and air-dried. **(Bhupathyraaj, M., et al.** 2021)

Fig.7: microbeads loaded with *Syzygium cumini* (Jamun) leaf extract

- **Evaluations of microbeads**

Measurement of microbeads size by optical microscopy:

Size of the prepared microbeads in optimized formulation was determined using an optical microscope fitted with a stage and an ocular micrometer. Mean diameter was calculated by measuring diameter of mg dried microbeads of formulation

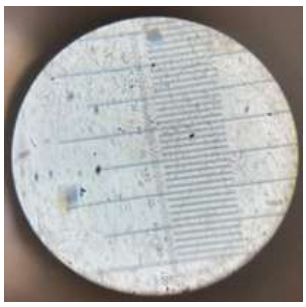


Fig.8: Microscopic image of microbeads

Drug-Polymer Compatibility Study (FTIR):

To evaluate the chemical integrity of the Jamun leaf extract within the formulation, FTIR spectroscopic analysis was performed on the pure extract, the polymer (Sodium Alginate), and the optimized microbeads. Samples were scanned in the wavenumber region of 400 to 4000 cm^{-1}.

Key Observations:

Jamun Extract: Showed characteristic peaks at 3385 cm^{-1} (–OH stretching of phenols) and 1615 cm^{-1} (C=C aromatic stretching).

Sodium Alginate: Displayed peaks at 1620 cm^{-1} and 1415 cm^{-1}, corresponding to asymmetric and symmetric carboxylate ($–COO^-$) stretching.

Optimized Microbeads: The final formulation retained the major functional peaks of the Jamun extract. A slight shift in the carboxylate peaks was noted, indicating the successful cross-linking of alginate with calcium ions (Ca^{2+}) and potential hydrogen bonding with the extract's polyphenols.

Conclusion:

The absence of any new significant peaks or the disappearance of the extract's primary functional groups confirms that Jamun leaf extract is chemically compatible with the polymer matrix. The extract is successfully entrapped without undergoing any deleterious chemical reactions.

Swelling Index: The swelling index of the microbeads is an indication of the capacity of the microbeads to imbibe water and swell. For estimating the swelling index, the microbeads were weighed initially, then suspended in 25ml of phosphate buffer pH 6.8, and weighed to determine the swelling index.

$$\text{Swelling Index \%} = \frac{\text{Weight of Swollen Microbeads} - \text{Initial Weight of Dry Microbeads}}{\text{Initial Weight of Dry Microbeads}} \times 100$$

Product yield: The yield of the prepared formulations was calculated as the percentage of the weight of the dried product at room temperature compared to the theoretical amount. Production yield is calculated using the following equation:

$$\text{Percentage Yield (\%)} = \frac{\text{Practical Weight of Microbeads}}{\text{Theoretical Weight (Extract + Polymers)}} \times 100$$

Drug entrapment efficiency: To determine the drug entrapment efficiency of your Jamun leaf extract microbeads, you first need to extract the "trapped" bioactive compounds from the polymer matrix. This is done by weighing a specific quantity of the beads, crushing them thoroughly, and dissolving the contents in a phosphate buffer (pH 7.4) under constant stirring for several hours. Once the extract is fully released into the solution, it is filtered and analyzed using a UV-Visible spectrophotometer at the specific wavelength for Jamun phenolics. By comparing the concentration found in the solution against a standard calibration curve, you can determine the actual drug content. Finally, the entrapment efficiency is calculated as the ratio of the actual amount of extract found to the theoretical amount added during the formulation process, expressed as a percentage. This value is critical because it confirms the efficiency of your preparation method and ensures that the therapeutic dose of the extract is preserved within the beads

$$EE\% = \left(\frac{\text{Amount of Entrapped Extract}}{\text{Total Amount of Extract Added}}\right) X100$$

Dissolution: The dissolution study evaluates the rate at which the Jamun leaf extract is released from the microbeads into the body's systemic circulation. To perform this, a weighed amount of microbeads is placed in a USP Dissolution Apparatus containing 900/mL of buffer solution maintained at 37°C with a paddle speed of 50 rpm. Typically, the study begins in an phosphate buffer (pH 6.8) to simulate the intestines. At set time intervals, small samples are withdrawn, filtered, and analyzed using a UV Spectrophotometer to determine the concentration of released polyphenols. The goal for your formulation is to achieve a sustained release profile, ensuring that the antidiabetic extract is delivered slowly and consistently over several hours rather than all at once.

$$\%\ Drug\ Release = \left(\frac{Calculated\ Amount\ in\ Medium}{Total\ Entrapped\ Drug\ in\ Sample}\right) \times 100$$

Glucose uptake assay by yeast: The glucose uptake by yeast cells assay is an in-vitro method used to confirm the antidiabetic activity of your Jamun leaf extract by measuring its ability to facilitate glucose transport across cell membranes. In this procedure, a 10\% suspension of baker's yeast is incubated with the extract and a known concentration of glucose solution at 37°C for one hour. During this time, if the extract is effective, it will stimulate the yeast cells to "absorb" glucose from the solution, mimicking the action of insulin in the human body. After incubation, the mixture is centrifuged, and the concentration of the remaining glucose in the supernatant is measured using a spectrophotometer. The increase in glucose uptake is then calculated by comparing the results to a control group, providing a clear indication of the extract's potential to lower blood sugar levels.

$$\%\ Drug\ Release = \left(\frac{Calculated\ Amount\ in\ Medium}{Total\ Entrapped\ Drug\ in\ Sample}\right) \times 100$$

RESULTS AND DISCUSSION

- **Preliminary Phytochemical Screening:**

Fresh Jamun leaves were washed and shade-dried to preserve fragile elements like flavonoids from thermal degradation. Bioactives were extracted via maceration in 500 ml of 70% ethanol, filtered, and evaporated to yield a concentrated paste. This extract was then subjected to preliminary phytochemical tests using specific reagents (FeCl3, Lead acetate, NaOH) to confirm the presence of active compounds.

Table.1: Preliminary Phytochemical Screening

Phytochemical	Test/Reagent Used	Specifications	Observation	Result
Phenols	Leaf extract + 10% $FeCl_3$	Blue/Green Colour	Dark bluish colour	Positive (+ve)
Tannins	Leaf extract + 1% lead acetate	Yellow precipitate	Yellow precipitate	Positive (+ve)
Flavonoids	Leaf extract + dil. NaOH	Yellow solution → dil HCl → Colourless	Yellow solution → dil HCl → Colourless	Positive (+ve)
Saponins	Extract shaken with water	Persistent honeycomb-like froth	Persistent honeycomb-like froth	Positive (+ve)
Terpenoids	Leaf extract + H_2SO_4	Reddish brown at interface	bluish colour	Negetive (-ve)
Ash value	Total ash method	10-12%	11.5%	Within limits
Moisture content	Loss on drying (LOD)	NMT 5%-8%	0.10%	Within limits
Residual Value	Solvent extraction	NMT 0.5%	0.12%	Within limits

Preliminary Phytochemical Screening

Before formulation, the extracted phytochemicals were evaluated to confirm the presence of active anti-diabetic compounds. The results of these tests are outlined below:

- Phenols (FeCl3 Test): Yielded a dark bluish color (Positive).
- Tannins (Lead Acetate Test): Yielded a yellow precipitate (Positive).
- Flavonoids (NaOH + HCl Test): Transitioned from a yellow solution to colorless (Positive).
- Saponins (Water Shake Test): Produced a persistent honeycomb-like froth (Positive).
- Terpenoids (H2SO4 Test): Yielded a negative result (bluish color instead of reddish-brown).

Formulation of Microbeads

1. Preparation of the Botanical Extract Before microbead formation, the active phytoconstituents were extracted. Fresh Jamun leaves were washed, shade-dried, and ground into a coarse powder. The bioactive compounds were then extracted using 500 ml of 70% ethanol via maceration. This mixture was filtered and evaporated to yield a concentrated, phytochemical-rich extract.

2. Preparation of the Internal Phase (Polymer-Extract Solution) The primary matrix was created by dissolving 2g of sodium alginate into 125 ml of distilled water, forming a uniform and viscous polymer base. Next, 1.5g of the concentrated Jamun leaf extract was introduced into this alginate solution and dispersed thoroughly to ensure the active anti-diabetic compounds were evenly distributed throughout the matrix.

3. Preparation of the Cross-linking Bath In a separate beaker, the curing bath was prepared by dissolving 5g of calcium chloride ($CaCl_2$) in 100 ml of distilled water. This solution acts as the cross-linking agent necessary to solidify the liquid polymer.

4. Extrusion and Microbead Formation The extract-alginate mixture was drawn into a syringe equipped with a 22G needle. The mixture was then extruded drop-by-drop into the calcium chloride solution. To ensure the beads formed uniformly and did not clump together, the cross-linking bath was kept under constant magnetic stirring at 475 rpm.

5. The Gelation Reaction Upon contact with the bath, the calcium ions instantly cross-linked with the sodium alginate. This ionotropic gelation reaction transformed the liquid droplets into firm, spherical hydrogel microbeads, successfully trapping the delicate Jamun extract inside the protective polymer matrix.

6. Curing, Washing, and Drying The newly formed beads were left suspended in the calcium chloride solution for a curing period to fully harden the outer shell. Following curing, the microbeads were filtered out and washed thoroughly with distilled water to remove any excess calcium from their surface. Finally, the beads were dried at room temperature, resulting in the finished controlled-release delivery system.

- **Characterization of *Syzygium cumini* (Jamun) leaf extract Microbeads:**

The prepared microbeads were discrete, spherical, and free-flowing with good handling characteristics. The color of the microbeads ranged from pale yellow to light brown, depending on drug concentration. Flow properties such as angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio were evaluated and found to be within acceptable limits, indicating Excellent flow behavior essential for downstream processing and filling operations.

Table.2: Characterization of *Syzygium cumini* (Jamun) leaf extract Microbeads

Parameters	Results	specifications	Performance
Bulk density (g/ml)	0.55	-	-
Tap density (g/ml)	0.60	-	-
Hausner's ratio	1.09	1.00-1.11	Excellent
Carr's index (%)	8.33	≤10	Excellent
Angle of repose (θ)	21.5	25-30	Good

Evaluation Parameters

The physical efficiency and hydration capacity of the formulation were evaluated using the following parameters:

- **Percentage Yield:** Out of a total theoretical weight of 4g (2g extract + 2g polymer), the practical yield of the microbeads was 2.5g, resulting in a **62.5%** yield.
- **Swelling Index:** The formulation's capacity to imbibe water is a key pharmaceutical rationale for sustained release. The initial dry weight of 100mg swelled to 285mg when hydrated, **reflecting a high swelling index of 185.00%.**

Table.3: Swelling Index

Parameter	Value
Initial Weight of Dry Microbeads	100mg
Weight of Swollen Microbeads	285mg
Weight Change	185mg
Swelling Index	**185.00%**

- **Drug Entrapment Efficiency (EE):** Out of the initial 500mg of extract introduced, 85mg remained unentrapped in the supernatant, successfully encapsulating 415mg. This resulted in an entrapment efficiency of **83.00%**.

Table.4: Drug Entrapment Efficiency (EE)

Parameter	Value
Total Amount of Extract Added	500mg
Amount of Free Extract (in supernatant)	85mg
Amount of Entrapped Extract	415mg
Entrapment Efficiency (EE)	83.00%

- **UV-Spectroscopy & Calibration**

To quantify the release of active bioactives, a standard calibration curve was plotted. While the Quercetin standard provided a precise linear fit (R^2 = 0.9995) , the Jamun leaf extract curve demonstrated higher sensitivity with a slope of 1.0654.

Table.5: UV-Spectroscopy of Quercetin

Concentration (µg/mL)	Absorbance	Equation
0.2	0.17	y = 0.913x - 0.0084 R^2 = 0.9995
0.4	0.356	
0.6	0.551	
0.8	0.718	
1	0.902	

Table.6: UV-Spectroscopy of Jamun leaf extract

Concentration (µg/mL)	Absorbance	Equation
0.2	0.17	y = 0.913x - 0.0084 R^2 = 0.9995
0.4	0.356	
0.6	0.551	
0.8	0.718	
1	0.902	

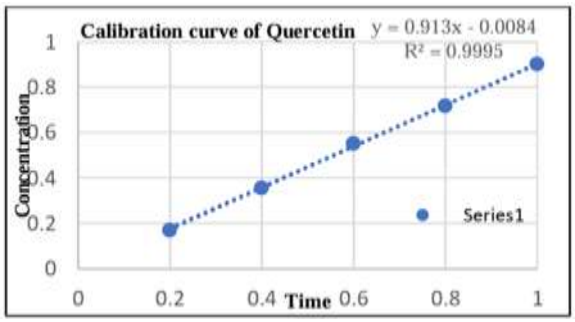


Fig.9: Calibration curve of Quercetin

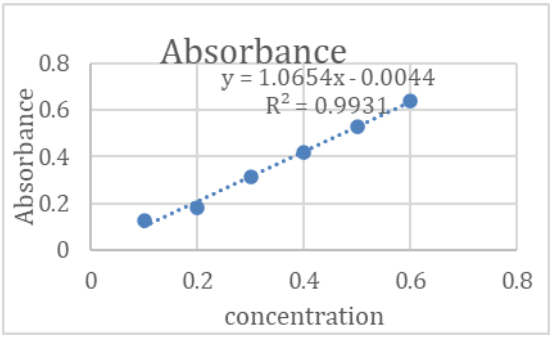


Fig.10: Calibration curve of Jamun leaf extract

- **Regression Equation:** y = 1.0654x - 0.0044
- **Linearity (R^2):** 0.9931
- **Concentration Range:** 0.1 - 0.6 µg/mL

Conclusion: The UV-spectrophotometric analysis confirms that the developed method is highly reliable, accurate, and sensitive for quantifying the active bioactives in the Jamun leaf extract. The strong correlation coefficient (R^2 = 0.9931) within the concentration range of 0.1 to 0.6 µg/mL demonstrates excellent linearity, ensuring precise concentration calculations. Furthermore, the steep slope of the regression equation (1.0654) indicates that the method possesses high sensitivity, capable of detecting even minute quantities of the released bioactives. Consequently, this calibration model is robust and perfectly validated for accurately monitoring the *in-vitro* sustained drug release profile of the formulated microbeads. (*, 2025)

- **6. In-Vitro Dissolution Efficacy**

An in-vitro dissolution study was conducted over a 240-minute (4-hour) timeframe to observe the sustained release capabilities of the alginate matrix.

Table.7: In-Vitro Dissolution

Time (min)	Absorbance (274 nm)	% Drug Release
0	0.000	0.00
15	0.168	24.35
30	0.071	10.29
45	0.080	11.59
60	0.086	12.46
75	0.107	15.51
90	0.154	22.32
105	0.191	27.68
120	**0.276**	**40.00**
135	0.305	44.20
150	0.342	49.56
165	0.380	55.07
180	0.421	61.01
195	0.460	66.67
210	0.502	72.75
225	0.535	77.54
240	**0.570**	**82.61**

- **0 min:** 0.00% release
- **60 min:** 12.46% release
- **120 min:** 40.00% release
- **180 min:** 61.01% release
- **240 min:** 82.61% release

- **Conclusion:** The dissolution profile demonstrated a sustained and progressive release pattern. Following an initial release phase, the formulation maintained a steady, upward trajectory, reaching a maximum drug

release of **82.61%**. This confirmed that the polymer matrix successfully acted as a barrier, achieving the controlled release objective.

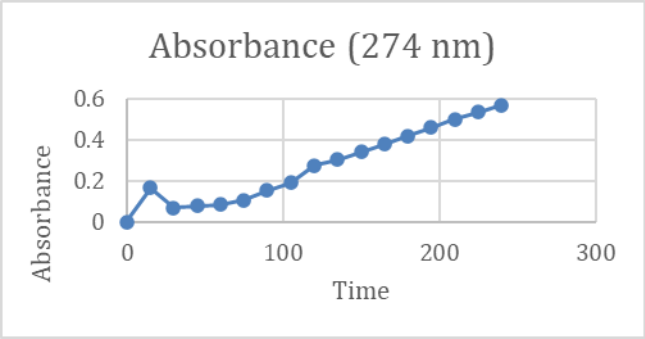


Fig.11: In-Vitro Release Profile(Absorbance vs time)

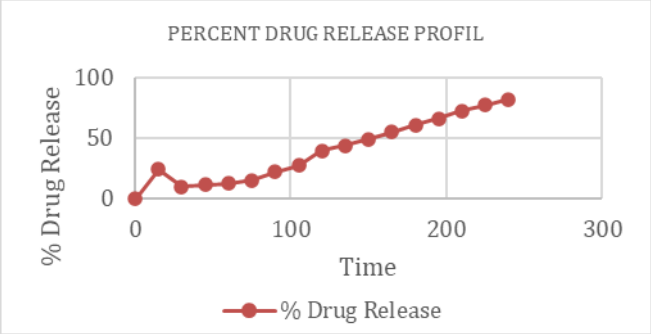


Fig.12: In-Vitro Release Profile

$$\% \, Drug \, Release = \left(\frac{Calculated \, Amount \, in \, Medium}{Total \, Entrapped \, Drug \, in \, Sample}\right) \times 100$$

Results:

The in-vitro dissolution study was conducted over a period of 240 minutes (4 hours). The release profile

indicates a maximum drug release of 82.61% at the end of the 240-minute timeframe, corresponding to an absorbance of 0.570 at 274 nm.

Conclusion:

The dissolution profile demonstrates a sustained and progressive release pattern over the 4-hour study period. Following an initial release phase, the formulation maintains a steady, upward trajectory of drug liberation. This prolonged release profile confirms that the polymer matrix successfully acts as a barrier, achieving the Sustained release objective intended for the Jamun leaf extract

microbeads. Consequently, this formulation shows strong potential for providing a gradual, sustained delivery of the active therapeutic components.

7. FTIR Analysis (Compatibility Study)

- Fourier Transform Infrared (FTIR) spectroscopy was performed to confirm chemical stability.
- **Integrity of Bioactives:** Characteristic peaks for O-H stretching (3389.47 cm-1) and C=O stretching (1656.22 cm-1) indicated the phenolic and flavonoid compounds remained structurally intact within the bead.
- **Cross-linking Evidence:** A slight shift in the asymmetric COO- stretching of the sodium alginate (1617 cm-1) confirmed successful ionic interaction with the calcium chloride, forming the calcium alginate matrix.
- **Conclusion:** The formulation is chemically compatible, preserving the medicinal properties of the extract in its natural state.

FTIR of jamun leaf extract

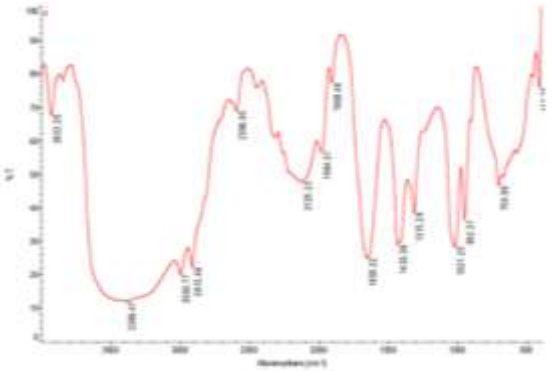


Fig.13: FTIR Spectrum of jamun leaf extract

FTIR of sodium alginate

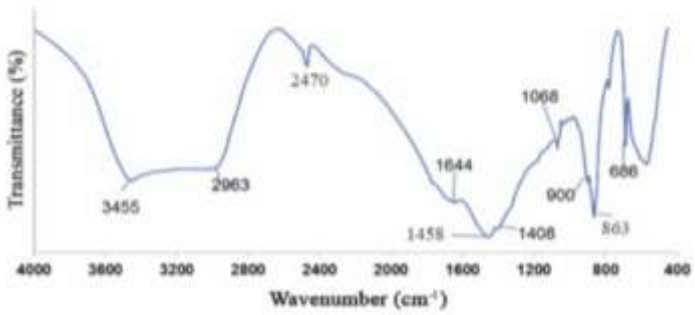


Fig.14: FTIR Spectrum of Sodium Alginate

FTIR of calcium chloride

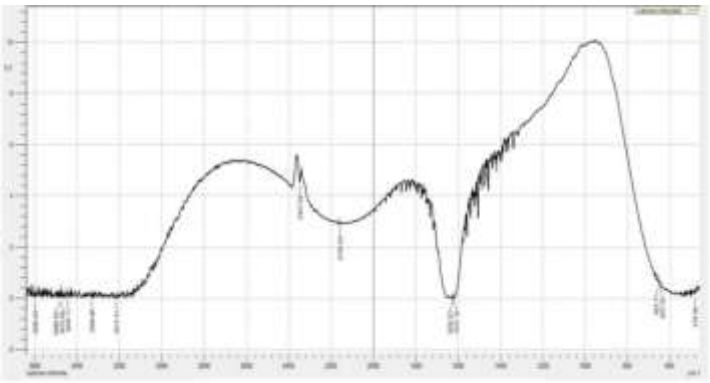


Fig.15: FTIR Spectrum of Calcium Chloride

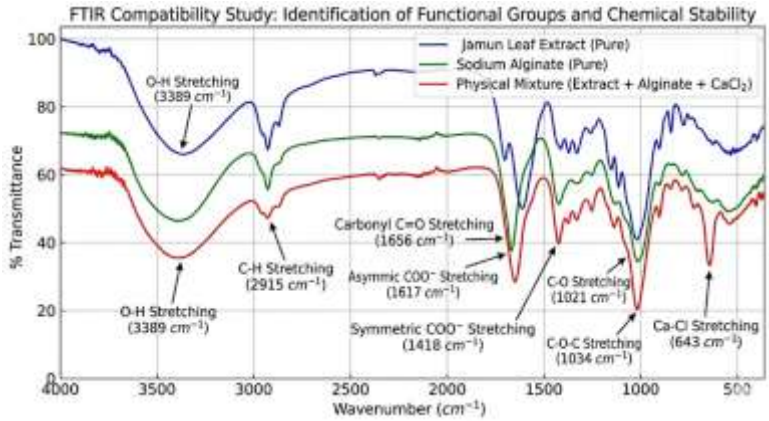


Fig.16: FTIR Compatibility Study

Table.8: FTIR Compatibility Study

Component	Observed Peak (cm−1)	Vibration / Functional Group	Chemical Significance
Jamun Leaf Extract	3389.47	O-H Stretching	Phenols, Flavonoids, and Tannins
	2915.49	C-H Stretching	Aliphatic groups in plant lipids
	1656.22	C=O Stretching	Carbonyl groups in polyphenols
	1021.25	C-O Stretching	Glycosidic linkages / Phenolic esters
Sodium Alginate	1617.15	Asymmetric COO^- Stretch	Carboxylate salt (polymer backbone)
	1418.75	Symmetric COO^- Stretch	Carboxylate salt (polymer backbone)
	1034.47	C-O-C Stretching	Glycosidic linkage of polysaccharide
Calcium Chloride	1628.57	O-H Bending	Water of hydration in the salt
	643.72	Ca-Cl Stretching	Metal-Halogen bond (Fingerprint)

ssay (Glucose Uptake by Yeast Cells)

To evaluate the therapeutic rationale, an in-vitro glucose uptake assay was conducted using yeast cells.

- **Control (Glucose Only):** 0% uptake (Absorbance: 0.850).
- **Jamun Extract (100 µg/mL):** Achieved a **20.0%** glucose uptake (Absorbance: 0.680), confirming its potential as a natural anti-diabetic agent.

Metformin Standard (100 µg/mL): Achieved an 80.0% uptake (Absorbance: 0.170), serving as a benchmark for efficacy. (Gopalasatheeskumar K1*, 2020)

Table.9: Assay (Glucose Uptake by Yeast Cells)

Sample	Concentration (µg/mL)	Absorbance (DNSA at 540nm)	% Glucose Uptake
Control (Glucose only)	-	0.850	0%
Jamun Extract	100	0.680	20.0%
Metformin (Standard)	100	0.170	80.0%

$$\% \text{ Glucose Uptake} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Jamun Extract: At a concentration of **100 μg/mL**, **the** extract achieved a 20% glucose uptake, confirming its potential as a natural anti-diabetic agent**.**

Metformin: Used as the positive control, it showed a much higher uptake of 80% at the same concentration, serving as a benchmark for efficacy

(Gopalasatheeskumar K1*, 2020)

8. Summary and Conclusion

- **Summary:**

Jamun leaf extract was successfully encapsulated into stable microbeads using a simple and cost-effective ionotropic gelation technique.

CONCLUSION

Microencapsulation effectively protects the sensitive phytochemicals of Jamun leaves.
The resulting microbeads show great potential as a controlled-release delivery system, which could enhance the therapeutic efficacy of extract for medicinal applications.

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