EVALUATION OF IPIL-IPIL (Leucaena leucocephala) SEED GUM AS CO-ENCAPSULATING AGENT FOR TARGETED AND CONTROLLED DELIVERY OF POWDERED INSULIN PLANT (Chamaecostus cuspidatus)

Aira B. Dacasin1, Maria Mikaela Isabel H. Liquido1, Ella Denese Anne B. Maglaquit1, Adrian Raymund M. Origenes1, Librado A. Santiago1,2,3, and Mark Kevin P. Devanadera1

Address(es):
1 Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, Manila, Philippines, 1008.
2 Research Center for Natural and Applied Sciences, University of Santo Tomas, Manila, Philippines, 1015.
3 The Graduate School, University of Santo Tomas, Manila, Philippines, 10155.

*Corresponding author: mpgdevanadera@ust.edu.ph

ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is the most common non-communicable disease in the Philippines, characterized by increased blood glucose levels brought by low insulin production or insulin resistance. Chamaecostus cuspidatus is a medicinal plant known for its glucose-lowering property. The controlled release of the C. cuspidatus leaves must be achieved to maximize its antidiabetic property. Leucaena leucocephala is an endemic tree in the Philippines, having its seed as a source of possible co-encapsulating material for drug delivery. Galactomannan, as the main component of the isolated seed gum, can be used as a substitute for an effective moderate drug release to its intended site. Thus, this study evaluates the drug release property and stability of seed gum as a co-encapsulating agent for targeted and controlled delivery of the C. cuspidatus leaves. The encapsulation process of the C. cuspidatus leaves was done through the extrusion method. The stability of the encapsulation was evaluated through in vitro gastrointestinal simulation analysis and was examined using differential scanning calorimetry (DSC), Fourier transform infrared spectrometer (FTIR), and field emission scanning electron microscope (FE-SEM) to verify its surface morphology. The capsules were observed to fully disintegrate at the fed state (pH 5.4) of the simulated gastrointestinal conditions, which is the target site. The fingerprint on the FTIR spectra of the encapsulated drug presented indicates the successful incorporation of the powdered leaves inside the encapsulating material. Morphological micrographs have shown that the resulting capsules were fairly in spherical, having a size of approximately 3.8 mm. Ridges and pores are also present on the surface of the capsules for their immediate disintegration and hydration. Therefore, the L. leucocephala seed gum can be a potential candidate as a co-encapsulating material suitable for effective, targeted, and controlled delivery of C. cuspidatus leaves for maximum antidiabetic benefits.

Keywords: Type 2 diabetes mellitus, co-encapsulation, Chamaecostus cuspidatus, Leucaena leucocephala, seed gum

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder distinguished as chronic hyperglycemia due to insulin resistance or a deficiency in insulin production (Kaneto, 2015). This can be associated with tissue and organ damage, particularly in the heart and kidneys. According to the Department of Health-Philippines, T2DM is the most common type of diabetes and the deadliest non-communicable disease. The tally of the Philippine Statistics Association (PSA) in 2020 shows that T2DM has ranked 4th in the Philippines as the primary cause of death in the country (Cudis, 2021). The number of patients affected by T2DM has increased by 7.8% from the 2019 statistics of T2DM among Filipinos, which medical experts believed became one of the most common comorbidities among adult Filipinos as the subsequent lockdowns and restrictions have affected the disease control and access in the country. Due to the sudden shift of work and school modalities, the subsequent lockdowns and restrictions have affected the disease control and accessibility. The overall increase by 7.8% from the 2019 statistics of T2DM among Filipinos, which medical experts believed became one of the most common comorbidities among adult Filipinos as the subsequent lockdowns and restrictions have affected the disease control and access in the country. Due to the sudden shift of work and school modalities, the subsequent lockdowns and restrictions have affected the disease control and accessibility. The overall increase of Type 2 Diabetes Mellitus, co-encapsulation, Chamaecostus cuspidatus, Leucaena leucocephala, seed gum

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systems, particularly as a thickening agent, binding agent, and disintegrating agent in the pharmaceutical industry, as a microencapsulation coating material (Deodhar et al., 1998; Verma & Razdan, 2003), and helps in the stabilization of foams and emulsion systems (Phillips & Williams, 2009; Mittal et al., 2016), so effective utilization of the bioactive components from C. cuspidatus must be addressed. This includes the drug accumulated in sufficient concentrations in the small intestinal region, which is the intended site of drug release and where most of its absorption happens (Verma & Razdan, 2007). To achieve maximum health benefit of the dried C. cuspidatus, insulin plant leaves are to be homogenized and encapsulated using the seed gums of L. leucocephala and other biopolymers for their effective release in the body.

Even though L. leucocephala is widely abundant in seeds; only limited reports are available on the application of its seed gum in the development of drug delivery. This study provided an avenue to utilize and highlight the L. leucocephala’s seed gum potential as a co-encapsulating agent for drug delivery. Aside from the seed gum of L. leucocephala as encapsulating material, other biopolymers such as alginate will be combined for a successful controlled and targeted delivery of bioactive present in the C. cuspidatus. Thus, the objective of this study focuses on the stability, and drug release property of the isolated seed gum of L. leucocephala as a co-encapsulating material for the effective and targeted delivery of the homogenized C. cuspidatus leaves in the intestinal region by employment of in vitro gastric simulation.

**Material and Methods**

**Materials**

L. leucocephala seeds were collected from a farm in Sta. Elena, Camarines Norte in the Bicol region of the Philippines. The leaves of C. cuspidatus were obtained from the Barangay Milagrosa, Calamba, Laguna, Philippines. Both plants were identified and authenticated by Jose Vera Santos Memorial Herbarium, Institute of Biology, University of the Philippines, Diliman. The chemical and reagents used in this study were of analytical grade. These chemicals and reagents were 70% isopropyl alcohol, sodium alginate, and calcium chloride flakes at 74% concentration; buffer 1 (KCl-HCl buffer at pH 1.4 with 75 mM KCl, 0.01M HCl, 3.0 g/L pepsin, 125 mM NaCl, and 75 mM NaHCO3), buffer 2 (KCl-HCl buffer at pH 2.4 with 75 mM KCl, 0.01M HCl, 3.0 g/L pepsin, 125 mM NaCl, and 75 mM NaHCO3), buffer 3 at pH 8.0 with 1%/w/v pancreatin enzymes, 1.5 g/L bile salt, 6.5 g/L NaCl, 0.835 g/L KCl, 0.22 g/L CaCl2, 0.824 g/L NaH2PO4, 25.20 g/L Na2HPO4, buffer 4 at pH 5.0 with 1%/w/v pancreatin enzymes, 1.5 g/L bile salt, 6.5 g/L NaCl, 0.835 g/L KCl, 0.22 g/L CaCl2, 13.20 g/L NaH2PO4, 1.001 g/L Na2HPO4, and buffer 5 at pH 7.4 with 0.20 g/L KC1, 0.5 g/L NaCl, 2.785 g/L Na2HPO4, 21.37 g/L NaH2PO4.

**Methods**

**Preparation of the Plant Samples**

**Extraction of Seed Gum from L. leucocephala Seeds**

The L. leucocephala seeds were washed, air-dried in 3-5 days, and pulverized with a blender. Then the powdered seeds were weighed and then soaked in distilled water for 24 hours. The filtrate was precipitated with 70% isopropyl alcohol three times in order to complete the extraction process. The isolated crude seed gum was air-dried using Severin Food Dehydrator 0D2940 at 60°C and packaged into a plastic container for further use.

**Homogenization of the C. cuspidatus Leaves**

The leaves of C. cuspidatus were dehydrated first using a Severin Food Dehydrator OD2940. Once the leaves were dehydrated, it underwent homogenization using a blender resulting in a powdered form of the leaves of C. cuspidatus. The resulting powder was stored in an amber bottle wrapped with aluminum foil until further use.

**Physicochemical and Chemical Properties of L. leucocephala Seed Gum**

The isolated crude gum underwent chemical analyses that determined moisture content, total solid content, carbohydrates, total reducing sugar, fat, protein, and total ash content. These physicochemical properties were analyzed in the First Analytical Services and Technical Cooperative (F.A.S.T) Laboratories in Calamba City, Laguna, Philippines. Furthermore, physiochemical properties such as pH determination of the seed gum were also determined.

**pH Determination**

For the physicochemical properties of the solubilized L. leucocephala seed gums, a portable digital pH meter was used. The pH meter was calibrated by preparing the buffer solutions with the two buffer powder packets, pH 4.0 and pH 6.86, included in the pH meter kit. The buffer solution was prepared according to the manual’s instructions, wherein the powder was dissolved in 500 mL of deionized water.

**Moisture Content and Total Solid Content**

To calculate the moisture content of the L. leucocephala seed gum, the mass of the solubilized L. leucocephala seed gum and its mass after drying was determined. Before drying, the mass of the solubilized seed was measured using a digital weighing scale and weighed 239.8g. Then, the solubilized seed gums were dried using the Severin Food Dehydrator OD2940. Afterward, the mass of the dried L. leucocephala seed gum was quantified using the digital weighing scale. The recorded mass of the dried seed gum was 66.3g. With the recorded measurements, the moisture content was calculated using the formula: %moisture (wt/wt) = weight of water in sample/weight of wet sample x 100. As for determining the total solid content, previously recorded masses were used. The total solid content of the L. leucocephala seed gum was computed using the formula: %total solids = weight of dry sample/weight of wet sample x 100.

**Total Carbohydrates Determination**

The carbohydrate content was determined by calculating the remaining percentage after the measurement of the other components was performed. The percentage of carbohydrates was computed by subtracting the percentage of moisture content, protein, lipid, and mineral from 100.

**Reducing Sugar Content Determination: Munson-Walker Method**

Determination of reducing sugars was done based on Jackson & McDonald (1941) with some modifications. A sample from 300g of seed gum was used. The said samples were subjected to heat under controlled temperatures. The formed copper oxide precipitates would be gravimetrically determined by weighing the substance.

**Fat Content Determination: Mojonnier Extraction**

A sample of the seed gum, ammonium hydroxide, and phenolphthalein are placed in a Mojonnier tube. For the first extraction, ethyl alcohol, ethylene ether, and petroleum ether are added, followed by centrifugation. The colorless portion was then transferred to pre-weighed aluminum plates. Subsequently, it was dried on a plate heater at low temperatures to prevent the sample from burning. As for the second extraction, a reduced volume of similar chemicals was added. This is followed by centrifugation and the transfer of the colorless portion. For the final extraction, the procedure was similar to the second extraction. The plates were dried in a 103°C oven for 2–3 hours and were allowed to cool. After cooling, the weights were recorded. The fat percentage was then calculated by dividing the difference between the final and initial plate weight by the sample weight (Momeneb Hamman et al., 2021).

**Protein Content Determination: Kjeldahl Method**

A 300g isolated seed gum was added with a proportional amount of concentrated H2SO4 and mixed in a Kjeldahl flask. The mixture was heated at around 400°C. Once the digestion procedure was done, the mixture was allowed to cool to room temperature, then diluted with deionized H2O. A proportional amount of NaOH was added to the resultant digestion solution. The produced ammonia gas would be distilled into a boric acid solution under alkaline conditions. Here, the solution is titrated with HCl as the standard. Once the endpoint was achieved, the nitrogen content present in the solution was calculated.

**Total Ash Content Determination**

An ignition-gravimetric method was used to determine the percentage of total ash content. The seed gum was accurately weighed and then placed in a silica crucible that had been ignited and weighed. At the bottom of the crucible, the powder was scattered evenly. Then, the crucible was incinerated by slowly increasing the temperature until it was red hot and free of carbon. It was allowed to cool and was weighed after. Through the use of air-dried seed gum as a reference, the percentage of total ash content was calculated (Yadav et al., 2020).

**Encapsulation of Powdered C. cuspidatus Leaves**

**Preparation of metal-ion crosslinker**

The metal-ion crosslinker was prepared based on the method created by Reddy & TAMMISHETTI (2002) with some modifications. Solutions of 1 M CoCl2, 1 M CaCl2, and 1 M CuCl2 will be used as a crosslinking agent in the metal-ion crosslinker. Then, using syringe, 1% w/v of L. leucocephala gum solution will be added dropwise into each solution.
solution. Beads that form a stable and rigid shape will then be used as the metal-ion crosslinker to encapsulate the *C. cuspidatus*.

**Encapsulation using *L. leucocephala* Seed Gum and Sodium Alginate**

Encapsulation of powdered *C. cuspidatus* leaves was done using the manual extrusion method. This procedure was used based on the study of *Gandola et al.* (2020). Varying concentrations (1.9%, 2.1%, and 2.3% w/v) of guar gum solution containing 0.3% w/v of the *L. leucocephala* was prepared in a 5% NaOH solution. This method drops guar solution in a 1M CaCl₂ gelling bath using a 1mL disposable syringe. The capsules produced was double coated with 2% w/v alginate solution. Microparticles were submerged again in a 1M CaCl₂ solution to allow cross-linking with the calcium ions. The resulting capsules underwent hot air circulation drying using a Severin Food Dehydrator OD2940.

**In vitro gastric simulation stability test**

The *in vitro* gastric simulation procedure was performed to assess the bioaccessibility of the bioactive compounds present in *C. cuspidatus*, of which *L. leucocephala* and sodium alginate were used as encapsulating material. This procedure was based on the protocol of Santiago & Devanadera (2016). Several buffer media was utilized for the dissolution tests for the human gastrointestinal environment simulation. Simulated fed and fasted gastric fluids consisted of KCl-HCl buffer at pH 2.4 and 1.4, respectively. These buffers would contain 75 mM KCl, 0.01M HCl, 3.0 g/L pepsin, 125 mM NaCl, and 75 mM NaHCO₃. Furthermore, both simulated intestinal fluids at fed (pH 5.4) and fasted (pH 8.0) states would comprise phosphate buffer containing 1% w/v pancreatic enzymes. 1.5 g/L bile salts, 6.5 g/L NaCl, 0.835 g/L KCl, and 0.22 g/L CaCl₂. In addition, 0.824 g/L Na₃HPO₄ and 25.20 g/L Na₂HPO₄ for fasted intestinal, whereas 13.28 g/L Na₂HPO₄ and 1.001 g/L NaLH₂PO₄ for fed intestinal was also added. Moreover, both fed and fasted colonic fluids contain a phosphate buffer at pH 7.4 with 0.20 g/L KCl, 0.8 g/L NaCl, 2.795 g/L Na₂HPO₄, and 21.37 g/L Na₂HPO₄. The medium was placed in an environment with a specific temperature of 37°C to emulate the physiological temperature, which was kept for 3 hours. A magnetic stirrer was used to simulate the peristaltic movement in the system at a constant rotational speed of 100 rpm. Once the capsules were incubated in a gastric environment for 3 hours, a representative sample was transferred and subjected to the intestinal condition. As for the undissolved capsules, they were transferred to the colonic environment.

**FTIR Analysis**

FTIR analysis was carried out to characterize the isolated seed gum and evaluate the compatibility of the encapsulated powdered leaves of the *C. cuspidatus* using the Perkin Elmer FT-IR Frontier Spectrometer. The solid samples comprised the seed gum of *L. leucocephala* and encapsulated *C. cuspidatus* leaves were grounded to a fine powder form. The IR spectrum used is the mid-IR with a wavenumber from 4000-600 cm⁻¹. Samples were scan 20 times with a measurement time of >30 seconds per scan. Running of samples was done in triplicates. The compound and essential functional groups were determined by identifying the characteristics frequencies as absorption/transmittance bands in the infrared spectrum of the said samples and had a comparison with the characteristics group frequencies of the reference compound.

**Differential Scanning Calorimetry (DSC) for Thermal Stability Analysis**

Thermal analysis using differential scanning calorimetry (DSC) was performed to examine the thermal behavior of the seed gum, and the encapsulated *C. cuspidatus* leaves. The thermal analyzer known as Perkin Elmer DSC 4000 was utilized for the said analysis. The samples passed through a nitrogen atmosphere at a heating range from 30°C to 430°C with a heating rate of 20°C/minute for dried seed gum and 10°C/minute for the encapsulated drug. Runs were performed in triplicates, and the scans were taken for analysis.

**FE-SEM Imaging for Encapsulated *C. cuspidatus* Leaves**

The size and morphological surface of the encapsulated *C. cuspidatus* sample were determined using Dual Beam Helios Nanolab 600. The parameters for imaging were performed at 20.0 kV for FESEM accelerating voltage and at 86 pA for the beam current. Prior to the imaging, the samples were sputter-coated with platinum and were observed at the specified magnifications under a field emission scanning electron microscope (FESEM).

**RESULTS AND DISCUSSION**

**Extraction of *L. leucocephala* Seed Gum and Characterization as Co-Encapsulating Material**

The extraction technique utilized to isolate seed gum from the seeds of *L. leucocephala* resulted in a yield of 19.5% (w/w). The reported yield of the seed gum or the biopolymers known as galactomannan from other literature is 20% (w/w) (*Mittal et al.*, 2016).

A drug's physicochemical properties influence its pharmacokinetic properties, including absorption, distribution, metabolism, excretion (ADME), and pharmacodynamics properties through modulating the interaction of the drug with its target (*Benjamin et al.*, 2010; *Flyn et al.*, 1974). Plant-derived polysaccharides, or the seed gums, possess a beneficial property and biocompatibility, making it suitable for formulating pharmaceutical excipients such as having potential as co-encapsulating material. Similar to the seed gum from *L. leucocephala*, the seed from *Tamarindus indica* (tamarind) also contains gum that has been utilized in developing particular drug delivery systems. However, unlike *L. leucocephala* seed gum, tamarind seed gum has been extensively studied and successfully utilized in the development of drug delivery systems particularly for the oral, intestinal, buccal, and nasal routes (*Nayak & Pal*, 2018). Its physicochemical properties also showed its biocompatibility, providing its suitability as an excipient in ocular drug delivery systems. (*Lynch et al.*, 2020).

The physicochemical properties of the co-encapsulating material, such as its pH, were analyzed to determine the solubility or dissolution rate of a capsule since it greatly depends on the partitioning behavior of the drug from lipid to the aqueous environment (*Pal et al.*, 2018). Furthermore, the chemical properties, which include: the moisture content, carbohydrates, total reducing sugar, fat, and protein content, were analyzed considering that it has a direct influence on the drug absorption and its solubility, and its tendency to resist change or decomposition (e.g., chemical stability) (*Pal et al.*, 2018).

The physicochemical and chemical characterization of the isolated seed gum from *L. leucocephala* are summarized in Table 1. The pH values of the crude seed gum were found to be slightly acidic or near neutral, which is at 6.04. A similar result has been observed in the study of tamarind gum and almond gum, which have a pH value of 6.70 and 5.25, respectively, making them also slightly acidic (*Farooq et al.*, 2014; *Malviya et al.*, 2021). In a drug delivery system, the co-encapsulating material that will be administered must precisely match the physiological needs at the intended site. Because upon entering the gastrointestinal tract with an increasing pH, poly-acidic polymers such as galactomannan will swell, which facilitates its disintegration, and drug release is expected to occur at the intended site. The pH values vary in different gastrointestinal tract segments to maintain cell and tissue homeostasis; for example, the small intestine has a pH value of 5.5–6.8, which is also the intended site for this study (*Balamuralidhara et al.*, 2011). Thus, the pH value of 6.04 of the solubilized *L. leucocephala* seed gums indicates that this co-encapsulating material is non-irritating to the mucous membrane of the gastrointestinal tract and can be utilized for effective targeted delivery of the drug. The percentage of total moisture and solid content of the seed gum was found to be 72.35% (w/w) and 27.65% (w/w), respectively. The total carbohydrate of the seed gum was only 38.7% (w/w). It might be due to other components, including proteins, amino acids, and other non-carbohydrates compounds, which are still bound to the target polysaccharides after the extraction process. The total amount of carbohydrates present in this study is low compared to the other literature that isolated seed gum from the mature seeds of *L. leucocephala* (*Rahim et al.*, 2017). Other compositions from the isolated *L. leucocephala* seed gum were also determined. The total reducing sugars were found to be 0.63% (w/w), the fat content was at 10.6% (w/w), and the protein content of the isolated seed gum was 39.3% (w/w) which is higher than the reported protein content from other literature which is at 2.1% (w/w) (*Mittal et al.*, 2016). Furthermore, the total ash is generally composed of various inorganic mixtures such as carbonates, phosphates, silicates, and silica. This analysis also demonstrates the adulteration levels of a certain natural polymer (*Shiraijuddin et al.*, 2015). The total percentage of the ash content of the seed gum was found to be at 2.44% (w/w), indicating that there is a low level of contamination. The resulting total ash content of the said plant sample is consistent with other literature, which is also low at 5.01% (w/w) (*Mittal et al.*, 2016).

**Table 1 Characterization and compositional analysis of the isolated seed gum from *L. leucocephala* seeds.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observed values for Seed Gum</th>
</tr>
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<tbody>
<tr>
<td><strong>Physicochemical Properties</strong></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.04</td>
</tr>
<tr>
<td><strong>Chemical Properties</strong></td>
<td></td>
</tr>
<tr>
<td>Moisture content (% w/w)</td>
<td>72.35</td>
</tr>
<tr>
<td>Total solid content (% w/w)</td>
<td>27.65</td>
</tr>
<tr>
<td>Carbohydrates (% w/w)</td>
<td>38.7</td>
</tr>
<tr>
<td>Total reducing sugar (% w/w)</td>
<td>0.63</td>
</tr>
<tr>
<td>Fat (% w/w)</td>
<td>10.6</td>
</tr>
<tr>
<td>Protein (% w/w)</td>
<td>39.3</td>
</tr>
<tr>
<td>Total ash content (% w/w)</td>
<td>2.44</td>
</tr>
</tbody>
</table>
Encapsulation of Powdered Leaves of *C. cuspidatus*

Encapsulation, which is commonly adopted in drug delivery systems, is essential for the improved and controlled delivery of active agents to the targeted sites in the body. Applying an ionic crosslinking such as calcium chloride improved the polymer stability, which is necessary for developing an excellent vehicle for oral drug administration (Szekalska et al., 2018). The divalent metal ion crosslinker, which is calcium chloride, was able to help to form a rigid spherical bead, as shown in Figure 1A. These results only indicate that the metal ions could form a bond and crosslink to the seed gum of *L. leucocephala*. This was also evident in the study conducted by Gandola et al. (2020). Their results suggested that the crosslinking effect of calcium chloride exhibited the most stable and compact beads than the other metal ion crosslinker that they utilized in their study (Gandola et al., 2020).

Seed gum (galactomannan) from *L. leucocephala* and sodium alginate served as the encapsulating material for the powdered leaves of *C. cuspidatus*, wherein it successfully enclosed the drug with a membrane forming an insoluble, hydrogel-like bead, as shown in Figure 1B. The resulting encapsulated *C. cuspidatus* leaves were due to the binding interaction formed between the hydroxyl groups of the seed gum of *L. leucocephala* and calcium ions liberated from its solution leading to the formation of an ionic crosslink to the natural biopolymer, as shown in Figure 2.

Ionically crosslinked capsules are known for their excellent biocompatibility and are well-tolerated with a non-permanent network. Moreover, the interaction of calcium ions to the galactomannan is the one that is responsible for the successful entrapment of the powdered leaves of *C. cuspidatus*, encapsulated using the seed gum. The size of the encapsulated *C. cuspidatus* was found to be approximately 3.9 mm (3,900 µm). The said encapsulated material are within the size range of the ‘microcapsules’ which is between 0.2 µm to 5,000 µm (Barbosa-Cánovas et al., 2005).

*In vitro* Gastrointestinal Simulation of Microcapsules for Targeted Drug Release

The capacity of the co-encapsulating material to withstand the acidity of the gastric region and ensure the targeted delivery of the encapsulated *C. cuspidatus* was examined by subjecting the encapsulated *C. cuspidatus* to a simulated gastrointestinal environment. The encapsulated *C. cuspidatus* is composed of *L. leucocephala* seed gum-alginic with homogenized *C. cuspidatus* leaves having a ratio of 17:2, respectively, due to its right consistency, elasticity, and firmness. The capsules were first exposed to the fasted gastric state (pH 1.4). The only observable change after incubation was that the color of the capsules appeared slightly lighter than their original color. (Figure 3A). Exposure of the capsules to the fasted intestinal state (pH 8.0) shows a slight decrease in the number of observed beads (Figure 3B). Moreover, the beads were less bouncy prior to subjecting them to the fasted intestinal condition. No observable change was observed except for a slight change in the color of the beads upon their exposure to the fed gastric environment (pH 2.4) (Figure 3C). However, the beads completely dissolved at the fed intestinal state (pH 5.4), resulting in a murkier solution (Figure 3D).

The pH values in several segments of the gastrointestinal tract are different. For example, the pH values in the stomach are acidic, ranging from pH 1.5 to 3.5. The intestinal pH ranges from slightly acidic to near neutral at pH 5.5 to 6.8. Lastly, the colon pH is near-neutral at 6.4 to 7.4 (Zhu & Chen, 2015). The simultaneous change in pH of the gastrointestinal tract leads to the protonation or deprotonation of the ionizable groups present in the biopolymers used for encapsulation. Moreover, the swelling of the alginate under basic conditions of the simulated intestinal conditions leads to its susceptibility to disintegration and drug release. The seed gum was able to withstand the acidic pH of the gastrointestinal tract due to the presence of alginate. Under basic conditions, the seed gum can quickly disintegrate, thus increasing the release of the drug in the intestinal conditions, which is the intended site of release.

Fourier transform infrared (FTIR) spectroscopy analysis was used to characterize the complex structure present in the seed gum and the encapsulated *C. cuspidatus*. This ensures that the powdered *C. cuspidatus* leaves were incorporated with the seed gum and other biopolymers. On top of that, it is used to determine the chemical alterations that occurred in the co-encapsulating material, galactomannan, caused by the incorporation of the powdered *C. cuspidatus* leaves (Kamaraj et al., 2017). Bands that correspond to functional groups present in galactomannan have been observed in the resulting spectrum. As presented in Figure 4A, the peaks of the dried seed gum (black line) exhibited in the spectrum are similar to pure galactomannan isolated from *L. leucocephala* seeds (orange line) in a study by Rahim et al. (2017). The infrared vibrations spectrum of the sample shows the existence of protein, carbonyl-, ether-, and alkane-containing compounds. The protein and carbohydrate presence of the seed gum was evident based on its chemical analysis. The bands between 1148.21 and 962 cm⁻¹ in the sample represent the C-O and C=O bonds in glycosidic linkages of galactomannan. Moreover, the peaks at around 1314 cm⁻¹ and 869 cm⁻¹ are from the α-D-galactopyranose units and β-D-mannopyranose units of glycosidic linkages (Shirajuddin et al., 2015). The peaks for O-H groups in galactomannan were present in the resulting spectrum at 3279.06 cm⁻¹ and C-H stretch at 2924.49 cm⁻¹. Therefore, this only indicates that galactomannan is the main component of the isolated seed gum from *L. leucocephala*.

The infrared vibration spectrum of the encapsulated *C. cuspidatus* leaves shows the presence of protein, carbonyl-, ether-, and alkane-containing compounds. Figure 4B presents the spectra for the dried seed gum (blue line) and the encapsulated *C. cuspidatus* leaves (black line). The FTIR spectrum of the encapsulated *C. cuspidatus* leaves displayed a strong broadband at 3335.69 cm⁻¹.
corresponding to O-H groups. A similar peak was present at 3279.06 cm\(^{-1}\) for the dried seed gum. Peaks that represent C-H stretch are found at 2919.11 cm\(^{-1}\) and 2850.12 cm\(^{-1}\), which is similar to the C-H stretch at 2924.49 cm\(^{-1}\) and 2854.61 cm\(^{-1}\), respectively, in the dried seed gum spectrum. Four peaks were observed for the C-O-C stretch representing the glycosidic linkages of galactomannan from 1145.73 cm\(^{-1}\) to 1010.81 cm\(^{-1}\). Peaks were present at 822.73 cm\(^{-1}\) and 894.26 cm\(^{-1}\), which can be ascribed to the glycosidic bonds between α-D-galactopyranose units and β-D-mannopyranose units. Similarly, for dried seed gum, peaks were present at 813.29 cm\(^{-1}\) and 869.81 cm\(^{-1}\). The peaks in the encapsulated \textit{C. cuspidatus} leaves spectrum were found to overlap with the FTIR spectrum of the \textit{C. cuspidatus} aqueous plant extract from the study by Saranya et al. (2016). The absorption peaks of the encapsulated \textit{C. cuspidatus} leaves, specifically at 2850.12 (C-H stretch), 1743.18 (C=O stretch), and 1619.04 (N-H bend) cm\(^{-1}\) were almost similar to the spectrum of the \textit{C. cuspidatus} leaves extract from the literature. This implies that the powdered \textit{C. cuspidatus} leaves have been successfully entrapped by the co-encapsulating materials. The peak assignments for dried seed gum and encapsulated \textit{C. cuspidatus} leaves are summarized in Table 2. Overall, the peaks in the spectrum of the encapsulated \textit{C. cuspidatus} leaves indicate that there are no significant changes in the functional groups of the co-encapsulating materials. In addition, based on the overlapping of peaks, it can be inferred that the powdered \textit{C. cuspidatus} leaves were all incorporated inside the seed gum (galactomannan) and alginate.

Table 2 Peak assignments in the Dried Seed Gum and Encapsulated \textit{C. cuspidatus} leaves.

<table>
<thead>
<tr>
<th>Frequencies, cm(^{-1})</th>
<th>Encapsulated \textit{C. cuspidatus} leaves</th>
<th>Bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>3279.06</td>
<td>3335.69</td>
<td>O-H</td>
</tr>
<tr>
<td>2924.49</td>
<td>2919.11</td>
<td>C-H stretch</td>
</tr>
<tr>
<td>2854.61</td>
<td>2850.12</td>
<td>C=O stretch</td>
</tr>
<tr>
<td>-</td>
<td>1743.18</td>
<td>N-H bend</td>
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<tr>
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<td>C-H skeletal vibration</td>
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<td>813.29</td>
<td>822.73</td>
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Figure 4 FTIR spectrum of the (A) dried isolated seed gum (black) with pure galactomannan (orange) from \textit{L. leucocephala} seeds and (B) dried isolated seed gum (blue) with encapsulated \textit{C. cuspidatus} (black).

Stability Analysis of Microcapsules by Analyzing Thermal Behavior of Polymers

Differential scanning calorimetry (DSC) is used to measure the stability of the co-encapsulating material upon subjecting it to heat exposure. The thermal transition temperature (Tm) of the seed gum peaks, along with specific temperatures and enthalpy, are significant in assessing its physical stability and bioavailability.

Based on Figure 5A, the dried seed gum \textit{L. leucocephala} has undergone a glass transition state at 116.66°C with a recorded transition magnitude of 0.610 J/g°C. The first endothermic peak of the co-encapsulating material represents the crystallization of the material. The temperature observed was exactly 189.54°C with an enthalpy of 37.26 J/g. In contrast, the second endothermic peak of the subject sample was seen to have a high endothermic peak, resulting in a temperature of 312.03°C having an enthalpy of ~43.2094 J/g. The two separate endothermic peaks observed are due to the other natural biopolymers, apart from the polymeric carbohydrates (galactomannan), that are bound to the seed gum.

Overall, the isolated, dried seed gum of \textit{L. leucocephala} seeds has high melting temperatures, exhibiting high thermal stability. Likewise, thermal analysis has been used for the determination of the physicochemical interaction between the encapsulate and the encapsulant, as well as the thermal stability of both the \textit{L. leucocephala} seed gum and alginate, which serves as the encapsulating materials for the homogenized \textit{C. cuspidatus} leaves (Gandola et al., 2020). Here, the encapsulated \textit{C. cuspidatus} leaves are further characterized and evaluated via DSC analysis. As shown in Figure 5B, a single peak was observed in the DSC curve with a matching endothermic peak temperature of 167.21°C. Compared to Figure 5A, the endothermic peak temperature exhibited in Figure 5B decreased from 189.54°C to 167.21°C, respectively. This observation can be due to the interaction of the homogenized \textit{C. cuspidatus} leaves and the encapsulant materials (\textit{L. leucocephala} seed gum and alginate). However, the consistency in the degradation of seed gum (galactomannan) for both samples almost remains the same. The observed shift on the endothermic peak between the DSC thermograms only suggests that the encapsulant materials were able to successfully entrap and interact with the \textit{C. cuspidatus} leaves, hence, inhibiting the possible alteration or degradation of the structure of the drug upon exposing it to a high temperature.

Figure 5 DSC thermogram of the (A) dried isolated seed gum from \textit{L. leucocephala} seeds and (B) encapsulated homogenized \textit{C. cuspidatus} leaves with seed gum.

Surface Morphological Characterization of the Encapsulated \textit{C. cuspidatus}

FESEM analysis was performed to determine the surface morphology of the resulting encapsulated \textit{C. cuspidatus}. The efficient delivery of the drug is mainly dependent on the surface morphology of the encapsulated material since this is associated with its drug release mechanism and rate. FESEM analysis reveals information about the shape and surface morphology with direct visualization of the encapsulated material that is necessary for determining its controlled released processes to the targeted site (Yaneva & Georgieva, 2018). The analysis has shown that encapsulated \textit{C. cuspidatus} exhibited a tightly packed external morphology, demonstrating that the biopolymers–seed gum and alginate could entrap the homogenized \textit{C. cuspidatus} properly, as shown in Figure 6. The shape, surface, and size of the microcapsules were also observed. The results revealed that encapsulated \textit{C. cuspidatus} were fairly spherical in shape, their surfaces were bumpy and rough, and the size of the drug was observed to be approximately 3.9 mm. The average size of the encapsulated drug is essential for encapsulation efficiency and drug release behavior. This is because encapsulated size affects the efficiency of drug delivery, the release profile, and drug targeting (Singh et al., 2010). Encapsulated sizes smaller than their usual size tend to aggregate when being stored and transported. Therefore, the size of the encapsulation has to be precisely optimized in order to attain maximum stability leading to a higher drug
release rate to the targeted site. Furthermore, the observed surface of the encapsulated C. cuspidatus was rough due to the presence of ridges and pores, which are necessary to allow the drug to diffuse in or out of the spherical beads and also for its quick hydration.

CONCLUSION

The seed gum was isolated from the L. leucocephala seeds, whose main component is a galactomannan. It was found to be a suitable co-encapsulating agent for targeted and controlled delivery of the powdered C. cuspidatus leaves. The encapsulated C. cuspidatus displayed stability under in vitro gastrointestinal simulation, fully disintegrating at the fed state of the intestine, which is the intended target site. The FTIR spectra of the seed gum and the encapsulated drug have shown that the polymers were able to entrap the drug and form compact beads. The DSC analysis has also proved that the encapsulated C. cuspidatus is thermally stable, ensuring the protection of the components of the C. cuspidatus leaves. Furthermore, based on the SEM analysis, the surface of the spherical beads had ridges and pores, which are essential for the effective release of the drug to the intended site to maximize the anti-diabetic property of the C. cuspidatus. Thus, the seed gum derived from L. leucocephala is an effective co-encapsulating agent and can be utilized as a potential excipient in the formulation of drug dosage forms. The seed gum showed good quality as an encapsulating agent for targeted delivery and controlled release of substances to be encapsulated in the intestinal environment. As the results in vitro gives promising insight on the use of material for drug and nutraceutical delivery system, further study on using animal models for targeted delivery assessment and safety evaluation as per the guidelines set by OECD and FDA for the encapsulating material is recommended for further study.

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