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Research Article

Alginate-Based Microbeads from Encapsulated *Cosmos Caudatus* K. Extracts: *In Vitro* Release Profiles

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Abstract: This study aims to investigate the microencapsulation of *C. caudatus* K. leaves extract under optimal conditions, focusing on its potential as a therapeutic option for type 2 diabetes. In order to determine the inhibitory effect on the α-amylase enzyme, which plays a crucial role in glucose metabolism, biological activity assays were performed. The procedure of effectively encapsulating the substance was achieved at a pH level of 6 using sodium alginate at a concentration of 2% (w/v), resulting in a 77.2% percentage of encapsulation. The experiment involving the inhibition of α-amylase exhibited an IC_{50} value of 69.44 ± 0.924 µg/mL, demonstrating the significant biological activity of the microcapsules. The bioactive substances encapsulated in the microcapsules were released during intervals 30-120 min, at pH conditions of 2.2 and 7.4. The release of bioactive substances was only 2.32 % after 120 minutes at a pH of 2.2, whereas it increased to 64.54 % at a pH of 7.4. This discovery indicates that the microcapsules contain the capability to be utilized for the regulated delivery of drugs. The findings of this research demonstrate the potential to develop herbal medicines with therapeutic properties through microencapsulation, providing an excellent opportunity for managing type 2 diabetes.

Keywords: alginate; microencapsulation; alpha-amylase; realese study; *C. caudatus* K.

Introduction

Botanical bioactive compounds in various food sources have garnered considerable attention from consumers because of their potential to enhance human well-being. The compounds mentioned, including organosulfur compounds, carotenoids, biogenic amines, phytosterols, and biologically active proteins, have been proven to lessen the intensity of various long-lasting ailments. Their therapeutic properties encompass a wide range of health areas, including the management, prevention, and treatment of cancer, diabetes, cardiovascular diseases (CVDs), hypertension, oxidative stress, cognitive health, gastrointestinal health, and lipid profile (1). Indonesia is globally recognized as a prominent producer of medicinal plants due to its rich biodiversity and extensive tradition of utilizing these plants for medicinal purposes. For decades, the country has relied on its abundant plant life as a source of traditional and complementary medicines. Herbal medicines are particularly renowned for their minimal likelihood of causing adverse side effects (2).

Cosmos caudatus Kunth is a tropical vegetable plant native to Indonesia, with origins tracing back to Central America and other tropical regions. The leaves of C. *caudatus* K. are well known for their bioactive components, which include antioxidants and anticancer properties, such as ascorbic acid, quercetin, chlorogenic acid, and polyphenolic compounds (3). Phytochemical testing has revealed that the ethanolic extract of *C. caudatus K.* leaves contains bioactive compounds, including flavonoids, saponins, tannins, and phenolic compounds. These bioactive substances are noteworthy due to their potential health benefits. Quercetin, a potent flavonoid, possesses anti-inflammatory and antioxidant properties. It reduces oxidative stress by neutralizing reactive oxygen species (ROS), thereby protecting cells from damage. Additionally, quercetin can help regulate blood sugar levels by inhibiting the enzyme α-glucosidase, which slows the digestion and absorption of carbohydrates, potentially

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reducing postprandial blood sugar spikes. Its anti-inflammatory effects are particularly beneficial for chronic inflammatory diseases such as diabetes. Furthermore, quercetin has been shown to support the immune system, combat cancer by promoting the apoptosis of cancer cells, and improve endothelial function and blood pressure, all of which may contribute to cardiovascular health (4).

Type 2 diabetes mellitus (T2DM) is a chronic metabolic condition characterized by elevated blood glucose levels and various metabolic abnormalities. The primary contributing factors to T2DM include reduced sensitivity of insulin-sensitive tissues and insufficient insulin production by pancreatic β-cells. Although the pancreas continues to produce insulin, it becomes less effective at facilitating the transport of glucose into cells, leading to inadequate glycemic regulation. This impaired glucose regulation results in a range of complications, including delayed wound healing, peripheral neuropathy, and an increased risk of infection. Therapeutic strategies, such as topical applications and bioactive compound microencapsulation, have shown promise in addressing diabetes-related issues. These approaches aim to improve patient outcomes by expediting wound healing and ensuring the controlled release of therapeutic agents (5,6). Metformin, voglibose, and acarbose are examples of medications used to regulate blood glucose levels by inhibiting the activity of digestive enzymes. However, several studies have indicated that these antidiabetic medications can cause side effects, such as gastrointestinal disturbances, including gas and diarrhea. Therefore, it is essential to develop antidiabetic medications with minimal side effects (7). In 2018, the World Health Organization (WHO) reported that 88% of its 194 member states acknowledged the use of complementary and alternative medicine (CAM), indicating a significant global reliance on these healthcare approaches. This trend is particularly pronounced in developing countries, where traditional medicinal systems, particularly those based on plant sources, are widely embraced. Indonesia exemplifies this trend due to its rich biodiversity and long-standing tradition of utilizing traditional medicine (8). *Cosmos caudatus*, commonly referred to as C. *caudatus* K., is well-known for its therapeutic properties, which numerous studies have highlighted. These benefits include effectiveness against diabetes, high blood pressure, bone deterioration, and microbial infections, among others. The pharmacological properties of the plant are attributed to the presence of flavonoids and polyphenolic compounds (9).

The bioavailability of oral medication needs to be considered when developing candidates for antidiabetic drugs. Therefore, it is necessary to understand the molecular characteristics of the bioactive compounds present in C. *caudatus* K. leaf extract, which can impact its bioavailability (10). Several factors contribute to the widespread use of oral medication administration, including patient preference, the ease of mass production of oral doses, cost-effectiveness, and various advantages of drug delivery. However, the development of oral formulations faces several challenges, primarily due to the pharmacological properties of the drugs, such as limited water solubility and membrane permeability. Additionally, the effectiveness of medication absorption can be hindered by inadequate biological and chemical stability, as well as physiological limitations, including pH levels, metabolic enzymes, and efflux carriers. Moreover, some medications may cause local irritation and induce nausea (11).

Quercetin is known for its very limited oral bioavailability, which restricts its use as a therapeutic medication. This limitation arises from its poor solubility in water, whereas the digestive tract's lining consists of up to 90% water. Additionally, quercetin undergoes rapid metabolism after absorption by enterocytes and its entry into the bloodstream (12). One effective strategy to enhance quercetin's bioavailability is the use of microencapsulation with biodegradable polymers. This process allows quercetin to pass more easily through the stomach and small intestine. Additionally, microencapsulation improves quercetin's bioavailability and maintains its long-term stability. The primary objective of quercetin microencapsulation is to protect the compound during its transit through the gastrointestinal system and to facilitate controlled release in the colon (13).

Microencapsulation is a complex technology for encasing active molecules or substances like vitamins, pigments, antimicrobials, and flavourings in protective coatings, shielding them from environmental influences that could destroy their efficiency. This method is highly effective in several fields, including food science and processing, as it improves the stability, handling, and controlled release of these active substances (14). The primary objectives of microencapsulation are to protect the core material from external influences, mask unpleasant tastes and odors, combine components that are difficult to mix chemically or physically, reduce the likelihood of gastrointestinal irritation from the core material, control its release, and enhance the stability of the core substance (15). The diverse properties of wall materials significantly influence the characteristics of microcapsules, which consist of both the wall and core substances. This selection affects both the chemical and physical properties of the bioactive compounds within. An ideal wall material, such as a polymer, should physically encase the core material without reacting with it. It must also possess desirable attributes, including emulsifiability, stability, and specific solubility characteristics. Therefore, selecting a cost-effective wall material

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that aligns with both the intended application and the properties of the core substance is essential. Common choices for wall materials in microencapsulation include natural, naturally modified, and fully synthetic polymers (16). The selection of the drying technique is crucial during the encapsulation process. Freeze-drying, also known as lyophilization, is one of the most commonly used methods. This technique is ideal for encapsulating highly sensitive bioactive compounds because it avoids exposing the material to high temperatures, unlike spray drying (17). The bioavailability of microcapsules containing the ethanol extract of C. *caudatus* K. leaves can be determined using an in vitro release test. This test is essential for evaluating drug bioavailability, as it correlates with the extent to which the drug is accessible in the body. In vitro release tests play a crucial role in guiding the development of drug formulations and products (18). During the in vitro drug release test, microcapsules are dissolved in two different liquids to evaluate their effects on the stomach and intestines. The first liquid mimics stomach conditions, featuring a low acidity level with a pH of approximately 2.2. In contrast, the second liquid simulates the typical environment of the intestines, with a pH of around 7.4. By using these two fluid types, researchers can assess the medication's behavior in both the stomach and intestines, which is essential for understanding its absorption and release characteristics. Optimizing medication formulations is crucial for ensuring effective drug delivery (15).

One scientific strategy employed in research to evaluate the effectiveness of a natural product with anti-diabetic properties is conducting in vitro investigations to examine its potential to inhibit the alpha-amylase enzyme. Alpha-amylase is responsible for hydrolyzing starch molecules into monosaccharides, which facilitates their absorption into the bloodstream. By suppressing the activity of this enzyme, the breakdown and absorption of glucose can be slowed, potentially leading to a decrease in postprandial hyperglycemia, a condition characterized by elevated blood sugar levels after a meal (19). The alpha-amylase enzyme is crucial for the breakdown of glycogen, starch, and other alpha-linked polysaccharides into glucose and maltose in humans (20). Flavonoids, including kaempferol, apigenin, quercetin, myricetin, luteolin, chrysin, naringenin, and baicalein, have demonstrated significant inhibitory effects on the alpha-amylase enzyme in laboratory tests. This inhibition indicates great potential as a target for treatments aimed at preventing diabetes and obesity. By reducing the digestion and absorption of glucose, these compounds help regulate blood glucose levels and may prevent the development of diabetes and obesity (21).

This study aims to determine the efficacy of microencapsulating C. *caudatus* K. leaf extract under optimal formulation conditions. It involves evaluating the biological activity of the extract, specifically assessing its inhibitory effect on the α-amylase enzyme. Additionally, the study will investigate the effectiveness of microcapsules containing C. *caudatus* K. extract in managing type 2 diabetes. In vitro release experiments will be conducted to measure the amount of active ingredient released from the microcapsules in response to changes in pH and over time.

Materials and Methods

Materials

The research materials used were sourced from Merck and included sodium alginate (at least 99%, grade for molecular biology), glacial acetic acid (primary standard for pharmaceuticals), 3,5-dinitrosalicylic acid (DNS) reagent which contains at least 98% HPLC quality, calcium chloride, aluminum chloride (AlCl3), soluble starch (produced from potatoes, ACS grade), alpha-amylase from *Aspergillus Oryzae* (≥ 150 units/mg protein), acarbose (≥ 95%), D-(+) glucose (analytical standard), and release media (simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)). The powdered leaves of C. *caudatus* K. were purchased from UPT Materia Medica Batu in East Java, along with a species determination letter.

Instrumentations

The following instruments were utilised in this study: a Hitachi's TM 3000 scanning electron microscope (SEM), a particle size analyser (PSA, CILAS 1090), an infrared spectrometer with a Fourier transform (FTIR, Shimadzu Prestige 21 type), and a UV-Vis spectrophotometer (Shimadzu).

Preparation of *C. caudatus* K. Extract

The powder obtained from C. *caudatus* K. leaves was extracted through maceration using ethanol in a volume equal to 4 x the weight of the dried powder, over a total period of 3 x 24 h. The resulting extracts were collected by filtering, and the concentrated extracts were obtained by slowly applying a rotary evaporator vacuum at 110 rpm and 70 °C. Those extracts that are highly concentrated were stored for further examination at 4 °C.

Microencapsulation of *C. caudatus* K Extracts

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A solution of sodium alginate with a weight-to-volume ratio of 2% was prepared in a buffer containing 1% acetate and having a pH value of 6. Then, 1 mL of ethanol (96%) was added to 0.5 g of C. *caudatus* K. extracts. The sodium alginate solution was slowly loaded with the combination. A magnetic stirrer was used for stirring the mixture at 300 rpm till. Following this, the mixture was injected into 40 mL of a 0.1 M calcium chloride solution using a syringe, and continued to be stirred for another half an hour with a magnetic stirrer. Beads of alginate that had formed were rinsed with purified water to eliminate any unused calcium chloride ($CaCl_2$) on their outer layer. Beads were subsequently freeze-dried for 6 h at -55 °C and -60 mmHg of air pressure, or until they turned into powdered microcapsules.

Encapsulation Efficiency

The encapsulation efficiency was used to determine the percentage of chemical compounds that were encapsulated and contained within the polymer solution. This value was an indication of how effective the protective layers were in retaining the extract. Using Equation (1), the encapsulation efficiency was ascertained.

$$Encapsulation\ efficiency\ (\%) = \frac{Total\ flavonoid\ content\ in\ microcapsules}{Total\ flavonoid\ content\ in\ extracts} x\ 100\% \quad (1)$$

Measurement of the total flavonoid content of the samples is conducted using the colorimetric method along with an aluminum chloride solution. A 5 mg of extract and microcapsules of C. *caudatus* K were dissolved in 3 mL of methanol, then incubated at 40 °C for 45 min and centrifuged at 1,000 rpm for 2 min. A 0.6 mL of the solution that was produced and 0.6 mL of 2% $AlCl_3$ were mixed simultaneously.

Alpha-amylase Inhibitory Test. *C. caudatus* K. extracts and microcapsules

Acarbose as a positive control, were prepared at different concentrations (20-100 µg/mL). Alpha-amylase enzyme solution containing 10 U/mL of 250 µL of enzyme was supplemented to each sample. The mixture was left to incubate at 37 °C for a duration of 30 min. Subsequently, the mixture was fused with 250 µL of a solution of soluble starch that is 1% (w/v) and then placed under an incubator set at 25 °C for a 10-min period. In addition, the 500 µL of DNS reagent was incorporate. After heating it to the boiling point, the mixture was left to incubate for 5 min, or until the color turned reddish brown. After cooling the solution under running water, a total of 5 mL of purified water was poured into it. Finally, the absorbance of the solution was determined through measurement with a UV-Vis spectrophotometer set to 480 nm. Three sets of assays were run. Equation (2) was utilised for the purpose of determining the inhibition of alpha-amylase activity.

$$Enzyme\ Inhibition\ (\%) = \frac{Absorbance\ control - Absorbance\ sample}{Absrobance\ control} x\ 100\% \quad (2)$$

The IC_{50} number, which is the amount of inhibition required to stop half of the activity of the alpha-amylase enzyme, was used to illustrate the results. The value of the IC_{50} was determined through the use of a regression equation based on linearity that links sample concentration to enzyme inhibition percentage.

In vitro Release Profiles

Extracts from the microcapsule matrix were released in vitro using simulated gastric fluid (SGF) with a pH of 2.2 and simulated intestinal fluid (SIF) with a pH of 7.4. The microcapsules were added following the preparation of the release media. After that, the mixture was put in the water bath at 37 °C and continually stirred at 100 rpm. 10 mL of the solution were collected at 30, 60, 90, and 120 min intervals. A UV-Vis spectrophotometer with a 420 nm wavelength was then used for analysis. Total flavonoid content, or the amount of substance that was released from the microcapsules was measured using the quercetin standard curve. Equation (3) was utilized to represent the result as percentage of the total amount released (%).

$$Release\ (\%) = \frac{Total\ flavonod\ content\ released\ from\ microcapsules}{Total\ flavonoid\ content\ in\ microcapsules} x\ 100\% \quad (3)$$

Data Analysis

The results were reported in terms of the average value, adjusted for standard deviation. For the purpose of conducting the statistical analysis, the software known as Statistical Package for the Social Science (SPSS) version 26 was utilized. The distinct difference between each variant was further evaluated using Tukey's HSD test and one-way analysis of variance (ANOVA). At the point where the ρ-value was less than 0.05, the differences that were detected were considered to be statistically significant.

Result and Discussion

Microencapsulation of C. *caudatus* K. ethanolic extract was carried out using the freeze-drying method. This method is preferable to spray drying because it prevents the material from being exposed to high temperatures, which can cause its sensitive bioactive components to degrade. Freeze-drying is a method that involves freezing the extract and then drying it under vacuum conditions. This procedure helps maintain the

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bioactive chemicals' effectiveness in the extract. Freeze-drying method enables controlled encapsulation of bioactive substances, protecting them from external factors that may cause degradation. This technique is especially advantageous for encapsulating susceptible bioactive substances, as it shields them from exposure to high temperatures that can modify their properties or stability. The freeze-drying method yields encapsulated powders that can be utilized in solid pharmaceutical firms such as hard capsules or tablets, making them suitable for various usages (17). Microcapsules containing C. *caudatus* K. extract were prepared using a buffer medium with a pH of 6, a 2% (w/v) concentration of Ca-Alginate as a coating, and a stirring time of 30 min, resulting in an encapsulation efficiency of 77.2%. Encapsulation efficiency indeed describes the effectiveness rate of the microencapsulation process, with the percentage of effectiveness increasing with the encapsulation efficiency value. This means the more the active substance is shielded and the coating layer retains its stability, the more efficient the microencapsulation process is. Achieving high encapsulation efficiency is essential for successfully developing microencapsulated products, ensuring the protection and stability of the active substances contained within the microcapsules (14,22).

Alpha amylase inhibition analyses were used to evaluate the microcapsules' biological activity. In vitro biological assay results are shown in Figure 1. The alpha amylase inhibition assay using ascorbic acid and acarbose as positive controls, respectively. A non-encapsulated extract of C. *caudatus* K. was also examined in order to compare it to the microcapsules. The IC_{50} value that the ethanol extract of leaves of C. *caudatus* K. displays is 60.089 ± 0.194 µg/mL. At the concentration mentioned before, this suggests that the extract can block half of the alpha-amylase enzyme's activities. The microcapsules containing the ethanol extract of *C. caudatus* K. had an IC50 value of 69.44 ± 0.924 µg/mL. This value indicates that the microcapsules have the ability to block 50% of the alpha-amylase enzyme activity under the condition where their concentration is 69.44 µg/mL. With an IC_{50} value of 19.18 ± 0.83 µg/mL, acarbose, used as a positive control, can inhibit 50% of the alpha-amylase enzyme's activity at a concentration of 55.41 µg/mL.

Table 1. Acarbose, microcapsules, and ethanolic extracts of C. *caudatus* K. were all tested for their IC_{50} values in relation to their inhibition of alpha-amylase activity.

Sample	IC_{50} value (µg/mL)*
C. caudatus K. ethanolic extract	60.089 ± 0.194^{b}
Microcapsules of C. *caudatus* K	69.44 ± 0.924^{c}
Acarbose	19.18 ± 0.83^{a}

*Various notations, employing the One-Way ANOVA test with a 5% confidence level, show significant variations among conditions.

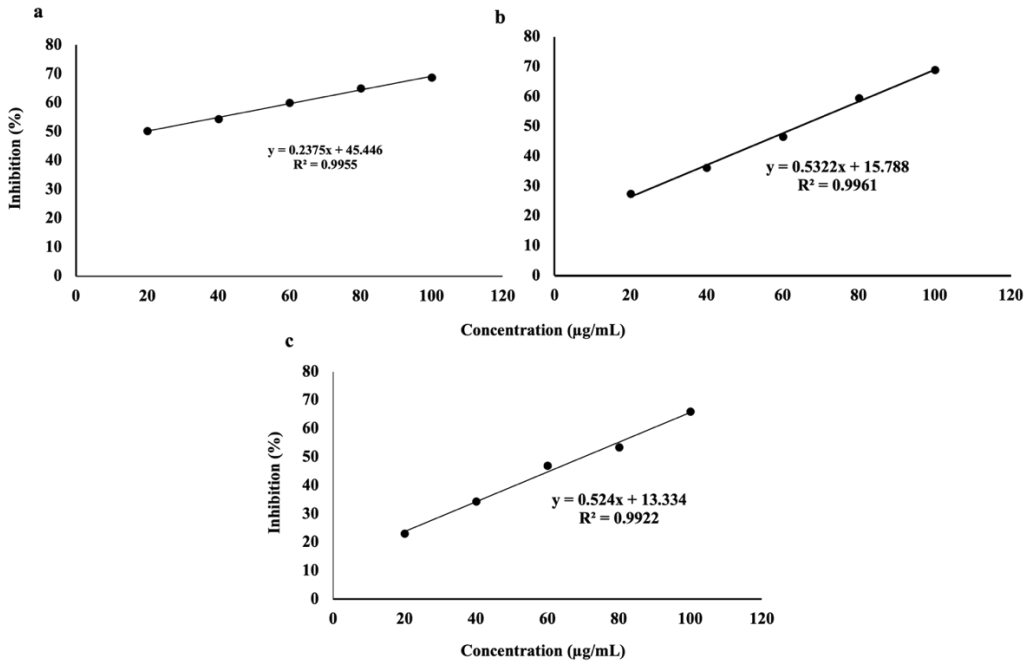

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Figure 1. The IC_{50} values for (a) acarbose, (b) *C. caudatus* K. extract, and (c) microcapsules of *C. caudatus* K. extracts were 19.18 ± 0.83, 60.09 ± 0.19, and 69.44 ± 0.92 µg/mL, respectively, for the inhibition of alpha amylase tests.

Microcapsules, while not as effective as C. *caudatus* K. extract in inhibiting the α-amylase enzyme, still serve a crucial role in maintaining or controlling the biological efficiency of the bioactive compound. This is due to the fact that part of the bioactive compound found inside the microcapsules is maintained inside due to the inability of the compounds to be released entirely. Despite this limitation, C. *caudatus* K. microcapsules continued to function as an alpha-amylase inhibitor. The primary purpose of microcapsules is to provide an ideal controlled release mechanism for the bioactive compound rather than to enhance its biological activity (23,24).

As a positive control, acarbose has the strongest inhibitory effect against alpha-amylase, according to its lowest IC_{50} value. Acarbose is a synthetic medication used to treat type 2 diabetes by inhibiting the enzyme alpha-amylase, which breaks down complex carbohydrates. Its structure resembles oligosaccharides and can be classified as an intermediate between glycosidic linkages. Acarbose competitively inhibits alpha-amylase by binding to its active site, slowing carbohydrate breakdown and reducing postprandial blood glucose levels. Its inhibitory effect is due to mimicking the glucose residue transition state and electrostatic interaction with the enzyme's active site. It acts as a competitive inhibitor of mammalian alpha-amylases by competing with glucose for binding to the enzyme's active site (25).

Flavonoids, one of the active substances found in C. *caudatus* K., are capable of preventing the alpha-amylase enzyme from activating. The alpha-amylase enzyme, which generates complex structures through hydrophobic and hydrogen bond interactions, interacts with flavonoid molecules. There are hydrogen bonds that form between the active enzyme residues and the hydroxyl and carbonyl groups found in flavonoid structure. The enzyme changes form as a result of the interaction between the flavonoid hydroxyl group and the enzyme portion; as a result, one region of the enzyme structure opens, exhibiting a decrease in the alpha helix and an increase in chaos. As a result, this structure covers the enzyme's functional domain. The enzyme's capacity thus attaching substrates is inhibited by this structural alteration, which lowers enzyme activity (26).

Blocking the activity of alpha-amylase is crucial for various reasons, specifically in relation to the control of diabetes and the avoidance of high blood sugar levels. Alpha-amylase is a catalyst that hydrolyzes starch and other polysaccharides into maltose, which is further hydrolyzed by alpha-glucosidase into glucose. This mechanism is essential for the breakdown and assimilation of carbohydrates in the human body. However, inadequate regulation of carbohydrate digestion can result in increased levels of blood glucose after meals, which can contribute to the onset of diabetes and other metabolic problems. Acarbose and other alpha-amylase inhibitors can function as carbohydrate blockers, reducing the ability of carbohydrates in the GI diet to be digested and absorbed. This method is very beneficial in the control of diabetes, high blood sugar, high blood lipid levels, and obesity. Alpha-amylase inhibitors can limit the digestion and absorption of carbohydrates, resulting in a decrease in blood glucose levels. This can be advantageous for preventing or managing diabetes. Furthermore, the inhibitory action of protein on alpha-amylase typically entails physically obstructing the active sites of many subsites of the enzyme, which is a crucial aspect of its efficacy (27).

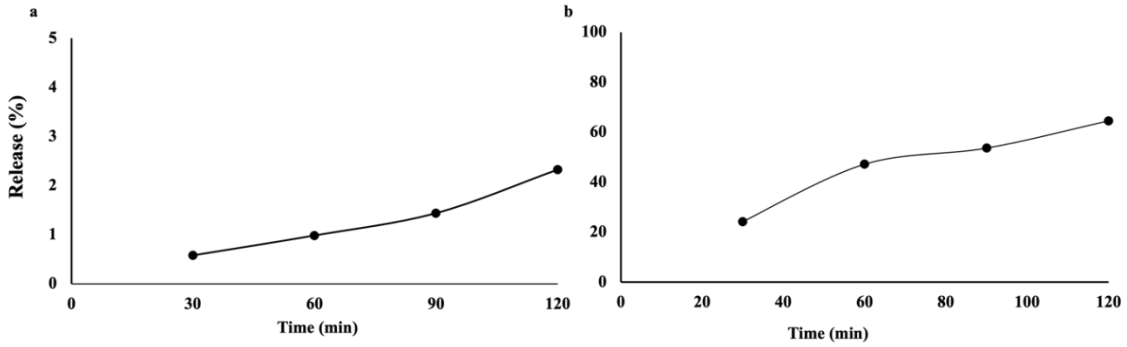

Figure 2. Bioactive chemical release from C. *caudatus* K. extract microcapsules at (a) pH of 2.2 and (b) pH of 7.4.

Conducting release tests, which determine the microcapsules' ability to hold onto their core content until it can reach the target site, is one method to evaluate the quality of the microcapsules. The entire drug release

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mechanism was affected by the materials features of the protective gel that encapsulated the microcapsules (28). The current study involved the placement of C. *caudatus* K. extract microcapsules in pH 2.2 and 7.4 media, followed by an analysis of the extract release amounts at various time intervals. The release behavior of the microcapsule extract showed a significant effect on the pH value at 37 °C. At a pH of 2.2, the release of microcapsules was lower than the release caused by a pH of 7.4, according to the release profile shown in Figure 2. The amount of extract released at pH 2.2 is defined as the initial burst (approximately 2%); after that, practically no further extract was released from the microcapsules. Sodium alginate's pKa influences the low percentage of active ingredient release in the pH 2.2 buffer medium. In an acidic environment, such as the stomach, sodium alginate has a comparatively low ability to swell due to its low swelling ratio. The reduced swelling ratio of sodium alginate in the gastric is due to its low swelling ability, indicating that the polymer matrix chains do not stretch easily. The alginate structure becomes denser and less porous, resulting in a lower swelling ratio. As a result, the drug is released from the microcapsules more slowly in the gastric environment (12,29,30).

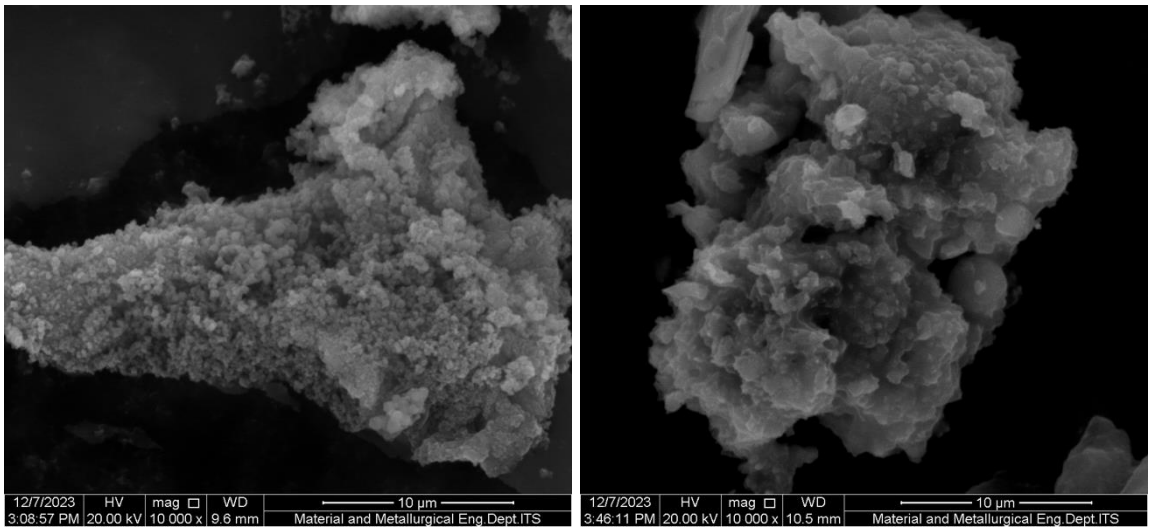

Figure 3. The scanning electron micrographs of (a) microcapsules released at a pH of 2.2 and (b) samples released at a pH of 7.4. The magnified versions approximated 10,000 times.

The SEM picture of alginate-based microcapsules at pH 2.2 in figure 3(a) reveals that these microcapsules exhibit a slow-release profile, with their morphology remaining intact and showing no signs of lysis. This indicates that the microcapsules are resistant to the acidic environment of the stomach, which is characterized by a pH of approximately 2.2. The smooth surfaces and slightly uniform forms of the microcapsules, as observed in the SEM image, suggest that the encapsulation process has been successful, with the active component being well encapsulated within the microcapsule walls (31). This encapsulation is crucial for maintaining the stability of the drug during its passage through the digestive system, ensuring that the active component is released at the desired site and time (32). The cohesiveness of the microcapsule walls, as indicated by the SEM data, further supports the resistance of these microcapsules to the acidic pH 2.2 medium. This cohesiveness is likely due to the interaction between the alginate and the active component, which forms a stable matrix that can withstand the acidic conditions of the stomach. The lack of significant microcapsule breakage and the absence of active component release in the SEM image at pH 2.2 suggest that the microcapsules are designed to protect the active component from degradation and to control its release in a manner that is beneficial for therapeutic purposes (33). In summary, the SEM analysis of alginate-based microcapsules at pH 2.2 demonstrates their ability to resist the acidic environment of the stomach, maintaining their integrity and protecting the encapsulated active component. This characteristic is crucial for the development of oral drug delivery systems, as it ensures that the drug is released at the desired site and time, enhancing its therapeutic efficacy and minimizing potential side effects (34).

In contrast, the release value increased significantly at pH 7.4 and reached at 64.54% after 120 minutes. When carboxylate ions undergo ionization at a pH higher than the pKa value, electrostatic repulsion occurs, expanding the hydrogel's network structure. This expansion enhances the hydrogel's ability to absorb and release drugs loaded within it. With the increase in the number of carboxylate groups in the hydrogel, the network structure of the hydrogel is expanded due to electrostatic repulsion also increasing—consequently, the hydrogel's capacity for swelling rises. The increased enlargement of the hydrogel as a result of ionization of carboxylate

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groups is then expected to increase the percentage of drug release, as the hydrogel's capacity to absorb and release drugs also increases (35,36).

The SEM picture in Figure 3(b) shows that the microcapsules have a rough surface and a deformed shape, which suggests that active substances are leaking out of them. The release is assisted through several pores, causing the core component to spread and distribute unevenly, resulting in an irregular structure. The study found that the active components of the microcapsules are released to a large extent at a pH of 7.4, leading to the formation of a more porous and permeable structure. This observation is consistent with the established behavior of bioactive components, which are often released more efficiently at a pH of 7.4 instead of at a pH of 2.2 when the environment is acidic (37). The pH 7.4 environment, which is closer to the physiological pH of the intestines, is more conducive to the release of encapsulated active substances. This is because the alginate matrix, which is the encapsulating material, degrades more rapidly in an alkaline environment compared to an acidic one. The degradation process of the alginate matrix at pH 7.4 leads to the release of the encapsulated active substances, which can then be absorbed into the bloodstream. The alginate matrix undergoes dissolution at a pH of 7.4, resulting in the release of the encapsulated active substances. These substances are subsequently able to be absorbed into the circulation (18). The SEM analysis and the study's findings highlight the importance of the pH environment in determining the release profile of encapsulated active substances. The ability of microcapsules to bear up against the stomach's acidity (pH 2.2) and maintain their integrity, as well as their ability to release the encapsulated active substances more effectively in a more alkaline environment (pH 7.4), is crucial for the development of effective oral drug delivery systems. This ensures that the drug is released at the desired site and time, enhancing its therapeutic efficacy and minimizing potential side effects (38). In summary, the SEM analysis and the study's observations underscore the significance of the pH environment in influencing the release profile of encapsulated active substances. The ability of in order to bear up against the stomach's acidity and release their contents more effectively in an alkaline environment is a key factor in the advancement of effective tablet medication delivery method, ensuring optimal delivery of medication and therapeutic efficacy (39).

Conclusion

The study involved using the freeze-drying method to encapsulate *C. caudatus* K. extracts, using sodium alginate and calcium chloride as the coating agents. The encapsulation efficiency of the microcapsules, which were made using 2% (weight/volume) sodium alginate at pH 6 and stirred for 30 min, was found to be 77.2%. The alpha-amylase inhibition experiment using microcapsules demonstrated substantial biological activity with an IC_{50} value of 69.44 ± 0.924 µg/mL. Results from the in vitro release study demonstrated that the microcapsule containing *C. caudatus* K. extract exhibited a lower rate of release at pH 2.2 as opposed to pH 7.4. The SEM image at pH 2.2 reveals alginate-based microcapsules with morphology that remains intact, showing no signs of lysis. The active components of the microcapsules are released significantly at pH 7.4, resulting in a more porous and permeable structure. Plant extracts microencapsulated can serve as a natural product enhancement method while maintaining biological functions.

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