

In Vitro Alpha-Amylase Inhibitory Activity of Microencapsulated *Cosmos caudatus* Kunth Extracts

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Abstract: The existence of phytochemicals in *Cosmos caudatus* Kunth, predominantly phenolic compounds, offers several health benefits. Nevertheless, the bioactive compounds are usually susceptible to degradation, and therefore may reduce their biological activity. This work aims to carry out microencapsulation of *C. caudatus* K. extracts by spray drying technique. The in vitro alpha-amylase inhibitory activity of the microencapsulated product is also investigated. The effect of manufacturing conditions, including pH, the concentration of wall materials, and stirring time, was evaluated. The optimal conditions for microcapsules formation were selected based on the activity of microcapsules as inhibitors for the alpha-amylase enzyme, pointing out by the lowest number of IC_{50} . Results showed that microcapsules prepared in pH 4, 0.05% of chitosan, and 90 min stirring time had optimum efficiency, with the IC_{50} value of $92.85 \pm 1.21 \mu\text{g/mL}$. The FTIR (Fourier-Transform infrared) analysis showed that the $-C-N$ stretching amine functional group appeared at wavenumber 1285 cm^{-1} , and the $-P=O$ phosphate bending appeared at 1206 cm^{-1} . Characterization with PSA (particle size analyzer) and SEM (scanning electron microscope) indicated that microcapsules had predominantly spherical forms with a mean diameter of $38.92 \mu\text{m}$. This work confirms the important role of microencapsulation in developing plant extracts with retained biological functionalities.

Keywords: chitosan; *Cosmos caudatus* Kunth; microencapsulation; spray-drying

■ INTRODUCTION

Diabetes mellitus (DM) is a metabolism disorder caused by increased blood sugar levels due to a lack of insulin or insulin resistance [1]. Diabetes can cause various complications, both acute and chronic, if not adequately controlled. Data from the World Health Organization (WHO) shows that 8.5% of adults aged 18 years and over were suffering from diabetes in 2014. In 2016, diabetes was the direct cause of 1.6 million deaths; and in 2012, high blood glucose was the cause of 2.2 million other deaths [2]. Diabetes mellitus generally can be classified into DM type 1 and DM type 2 [3].

The present treatment of diabetes mellitus is focused on controlling and lowering the blood glucose levels to a normal level. However, most modern drugs have many side effects causing some serious medical problems

during treatment. In addition, mild to severe adverse effects have been reported for some chemical-based drugs [4-5]. For example, metformin has been reported to have a few side effects, including gastrointestinal symptoms, nausea, and vomiting [6]. In addition to modern therapies, traditional medicines have been used for a long time and play an important role as alternative medicines [7-8]. According to the WHO (world health organization), a plant-based traditional system of medicine is still the dominant support of about 75–80% of the world's population, mainly in developing countries having a variety of plants [9].

Cosmos caudatus Kunth is an edible plant that is commonly used as an additive and flavor enhancer [10-11]. This plant contains bioactive compounds, including phenolic compounds, flavonoids, flavanones,

polyphenols, saponins, tannins, alkaloids, and essential oils [12]. The flavonoids contained in *C. caudatus* K. leaves, such as myricetin, quercetin, kaempferol, luteolin, and apigenin, have been proposed to have anti-diabetic activity [10].

The stability of natural bioactive compounds, *i.e.*, preservation of their functional properties, could be improved using encapsulation techniques, such as spray drying, spray cooling, coacervation, extrusion, and polymerization [13]. Microencapsulation is a technique that can be used to control a drug or bioactive compound release [14]. In addition, this technique also increases the absorption of active substances when they enter the human body, maximizing the functions of active substances [14]. A microencapsulation delivery system is described as the formulation of a particle dispersed at diameters ranging from 1 to 1000 μm [13,15]. Microencapsulation is increasingly in demand in controlled release because it is relatively at ease in the delivery system. The use of this technique, among others, is to control the release of active compounds from medicinal ingredients; thus, the active compounds are protected from their environment or unwanted effects such as the influence of light, humidity, and oxygen [14-15].

In the food industry, spray drying is one of the oldest and most widespread drying technologies used for microencapsulation of natural products because of its flexibility, good quality powder particles, rapid solubility of the capsules, and affordability [15-16]. In a spray drying process, the encapsulation structures are comprised of two components, the core (bioactive compounds) and the wall materials. The bioactive materials are dissolved in a polymer solution and subsequently atomized into a hot chamber, resulting in the rapid removal of the solvent [9]. The wall material used in this study is chitosan.

Chitosan is one of the natural biodegradable groups of polymers that have been extensively used for microencapsulation [17]. This natural polysaccharide has many pharmaceutical applications, such as oral and parenteral delivery of drugs. As a natural product, chitosan is a renewable pharmaceutical adjunct with good

biocompatibility [18-19]. One of the methods used in the preparation of spray-drying microencapsulation is ionic gelation. The benefits of ionic gelation when using chitosan as wall materials include simple procedures, flexibility to produce particles in a wide range of sizes, and more stable particles in suspension [20-21]. The principle of ionic gelation is the electrostatic interaction between the amine groups on the positively charged biopolymer, *i.e.*, chitosan that binds to the negatively charged polyanions cross-linker [22].

In the manufacture of chitosan-*C. caudatus* K. microcapsules, polyanions cross-linker are needed. The addition of sodium tripolyphosphate (Na-TPP) in the manufacture of chitosan microcapsules strengthens chitosan matrices [17]. Chitosan mechanical properties were improved by chitosan cross-linking with Na-TPP [22]. The preparation of microcapsules is influenced by several factors, including pH, chitosan concentration, and stirring time [23-25]. Chitosan dissolves easily in weak acid solution; hence, selecting the correct pH will produce the finest microcapsules. The concentration of coating material dramatically affects the particle size and the efficiency of the microencapsulation. The stirring time also influences the shapes and sizes of the microcapsules produced [26].

One method to determine the anti-diabetic activity of the natural product is by *in vitro* analysis, by investigating the inhibitory activity against alpha-amylase enzyme [27]. The inhibition of the enzyme involved in the hydrolyzing carbohydrates such as alpha-amylase is significant for lessening hyperglycemia [28]. In humans, alpha-amylase is an important enzyme that hydrolyzes the alpha glycosidic bonds, alpha-linked polysaccharides, like starch and glycogen, producing glucose and maltose [29]. The inhibition of alpha-amylase has reduced glucose absorption into the blood by postponing the digestion of carbohydrates. Therefore, this work aimed to study the encapsulation process of *C. caudatus* K. extracts by spray drying, using chitosan-Na-TPP as wall materials, proposing to assess concurrently the effect of pH, the concentration of wall materials, and stirring time. The determination of the alpha-amylase activity in each condition on

microcapsules preparations is designed to examine that the bioactive compounds can be released from the microcapsules. As a result, have biological activity as an alpha-amylase inhibitor.

■ EXPERIMENTAL SECTION

Materials

The research materials used were purchased from Merck: soluble starch (from potato, ACS grade), 3,5-dinitrosalicylic acid (DNS) reagent ($\geq 98\%$, HPLC grade), acarbose ($\geq 95\%$), glacial acetic acid (pharmaceutical primary standard), alpha-amylase from *Aspergillus oryzae* (≥ 150 units/mg protein), chitosan (low molecular weight, 50,000–190,000 Da), sodium tripolyphosphate (Na-TPP, technical grade, 85%), D-(+) glucose (analytical standard). The *C. caudatus* K. leaves powder was obtained from UPT (*Unit Pelaksana Teknis*) Materia Medica Batu, East Java, accompanied with a determination letter of species.

Instrumentation

Instruments used in this study were an FTIR spectrometer from Shimadzu Prestige 21 and a Shimadzu UV-Vis spectrophotometer. The size and distribution of the microcapsules particles were determined using a CILAS 1090 PSA. The shape and morphology of microcapsules were observed using an SEM TM 3000 Hitachi, with 5000 \times magnification.

Procedure

Extract preparation

Cosmos caudatus K. leaves powder was extracted using the maceration technique, with ethyl alcohol, in the volume of 4 \times dried weight, for 3 \times 24 h. The resulted extracts were collected through filtration, and a rotary evaporator vacuum was used with a slow speed at 110 rpm, at 70 °C to obtain the concentrated extracts. The concentrated extracts were kept at 4 °C for subsequent analysis.

Microencapsulation procedures

The *C. caudatus* K. extract (0.5 g) was dissolved with 17.5 mL of distilled water. Then, 50 mL of 1% chitosan solution (w/v) in 2% acetic acid (v/v, pH variations of 4,

5, and 6) were added slowly to the extracts solution and stirred with a magnetic stirrer for 60 min at a speed of 500 rpm. After that, 175 mL of 0.3% Na-TPP solution (w/v) was added slowly and stirred again with a magnetic stirrer for 60 min. The colloid microcapsules in chitosan and Na-TPP were then dried using a spray dryer with an inlet temperature of 105 °C, an outlet temperature of 85 °C, and an air pressure of 1 bar. The process was repeated under the influence of different concentration of chitosan solution at: 0.05%; 0.1%, and 0.2% (w/v). The initial pH contributing to the optimum inhibition of alpha-amylase activity was used, whereas other conditions were the same. Finally, the effect of stirring time was determined by repeating the process using different stirring times at 30, 60, and 90 min. The optimum conditions were determined based on the activity of microcapsules as inhibitors for the alpha-amylase enzyme, indicated by the lowest value of IC₅₀.

Alpha-amylase inhibition assay

All samples (extracts, microcapsules, or acarbose) were prepared in various concentrations (10 to 100 $\mu\text{g/mL}$ for *C. caudatus* K. extracts, 10 to 200 $\mu\text{g/mL}$ for microcapsules, and 1–10 $\mu\text{g/mL}$ for acarbose). Next, 250 μL of samples were added to 250 μL of the alpha-amylase enzyme solution (10 U/mL). The mixture was incubated for 10 min at 25 °C. Then, 250 μL of 1% soluble starch solution (w/v) was added to the mixture and incubated for 25 °C for another 10 min. Finally, 500 μL of DNS reagent was added, and all the mixture was incubated in boiling water for 5 min until the color of the solution changed to brownish red. The solution was cooled under running water. The solution mixture was diluted with 5 mL of distilled water, and the absorbance was measured at 490 nm using a UV-Vis spectrophotometer. Experiments were conducted in triplicates. Percentage of inhibition activity of the alpha-amylase enzyme was calculated using the following equation:

$$\text{Percentage of enzyme inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100\%$$

The IC₅₀ value was calculated to determine the 50% inhibitory capacity of the reaction at a certain concentration.

Data analysis

Results were expressed as mean \pm standard error of the mean. Statistical analyses were conducted using Statistical Package for The Social Science (SPSS) v.16 software. The one-way analysis of variance (ANOVA) followed by Tukey's HSD test was used to determine the real difference from each variation. The differences at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In this study, the spray drying process was used to produce microcapsules from *C. caudatus* K. extract. This process is the most common drying method used to prepare microparticles based on chitosan. The addition of cross-linking agents like Na-TPP improves the biocompatibility and performance of drugs [22]. In the microcapsules' formulation, various aspects, including pH, the concentration of the core material, or stirring time, play a role in the microcapsules' properties. The optimal conditions were chosen based on the biological activity of microcapsules acting as alpha-amylase inhibitors, indicated by the smallest number of IC_{50} .

The inhibitory activity test of the alpha-amylase enzyme was conducted first to the *C. caudatus* K. extract and acarbose. Results are shown in Fig. 1 and Table 1.

The *C. caudatus* K. extract had an IC_{50} value of $73.07 \pm 0.39 \mu\text{g/mL}$ against alpha-amylase, whereas acarbose as a reference had a lower IC_{50} value of $4.83 \pm 0.08 \mu\text{g/mL}$. These results are understandable since acarbose is a known oral anti-hyperglycemic drug acting as alpha-amylase competitive inhibitor [30]. Furthermore, the *C. caudatus* K. extracts contain a mixture of various secondary metabolites [12], and not all these compounds

have activity as an inhibitor for alpha-amylase. Compounds that are active as alpha-amylase inhibitors are phenolic compounds. They can bind covalently to alpha-amylase and alter their activity due to the ability to form quinones or lactones that react with nucleophilic groups on the active sites in the enzyme [27,31].

The inhibitory activity assay of the alpha-amylase enzyme against the microcapsules of *C. caudatus* K. extract in different pH variations, chitosan concentrations, and stirring times are presented in Fig. 2 and Table 2. The IC_{50} values obtained in the microcapsules with pH variations of 4, 5, and 6 were 132.22 ± 0.30 , 150.40 ± 0.77 , and $169.84 \pm 0.77 \mu\text{g/mL}$, respectively (Table 2). From these, the increase in pH will increase the IC_{50} value, which means that the inhibitory activity for the microcapsules against alpha-amylase enzyme was lower than those in the non-encapsulated *C. caudatus* K. extracts. These may be caused by the bioactive compounds contained in the microcapsules that cannot be released thoroughly and some retained in the microcapsules.

Nevertheless, microcapsules of the *C. caudatus* K are still active as an inhibitor for alpha-amylase. Microencapsulation's main purpose is not to increase biological activity but to protect and control the release of the active compounds [14-15]. Therefore, microcapsules

Table 1. The IC_{50} of the *C. caudatus* K. extract and acarbose on alpha-amylase inhibition

Sample	IC_{50} ($\mu\text{g/mL}$)*
<i>C. caudatus</i> Kunth extracts	73.07 ± 0.39^b
Acarbose	4.83 ± 0.08^a

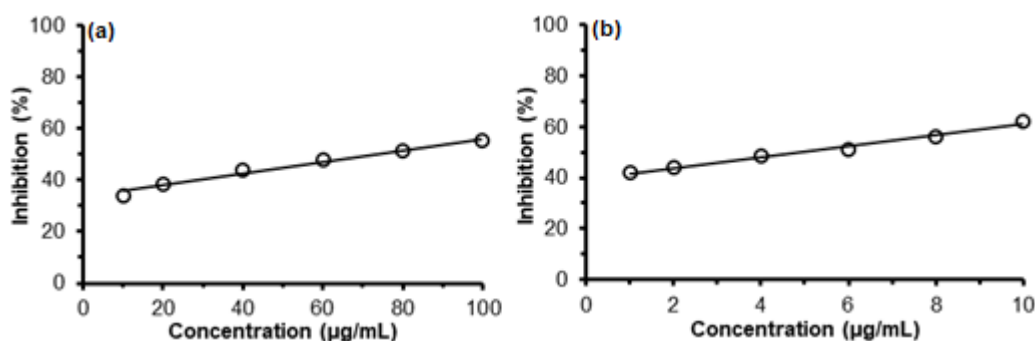


Fig 1. The inhibitory potency of (a) *C. caudatus* K. extracts; (b) acarbose, against alpha-amylase activity

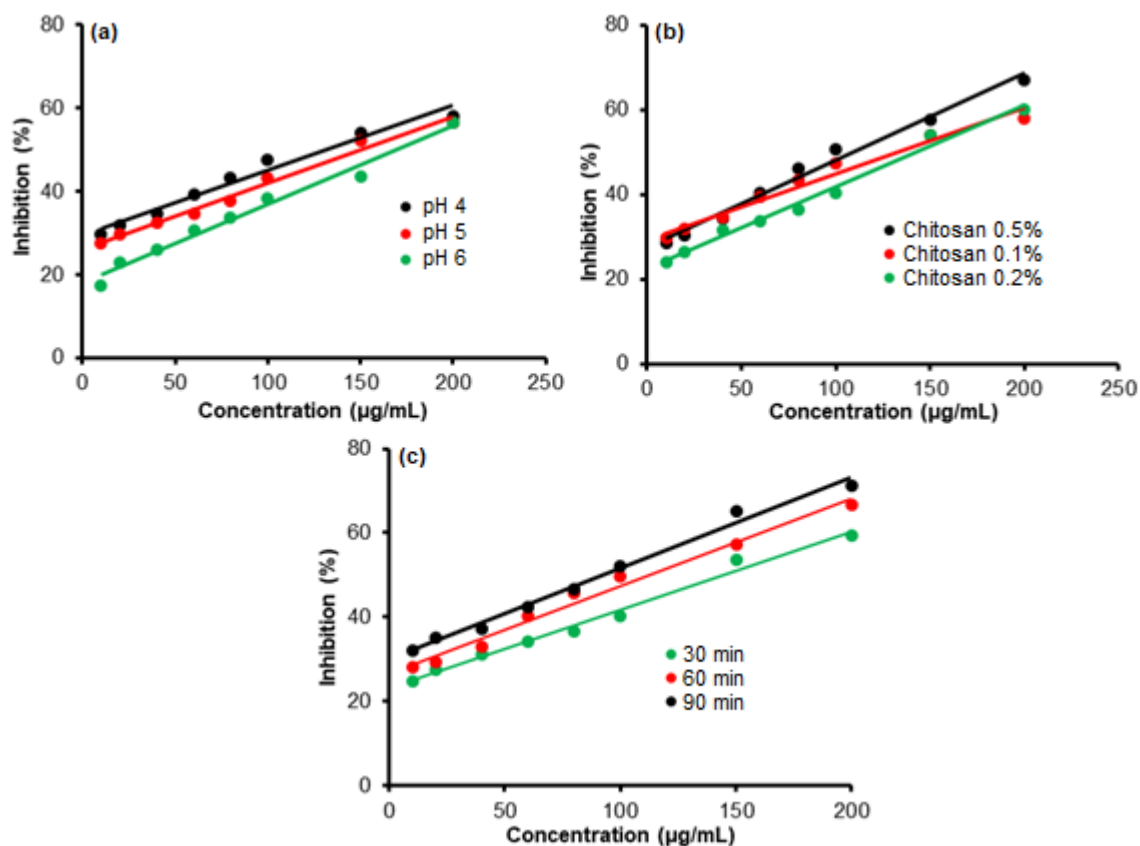


Fig 2. The inhibitory potency of microcapsules of *C. caudatus* K. prepared in (a) different pHs; (b) different chitosan concentrations (w/v); and (c) different stirring times against alpha-amylase activity

Table 2. The IC_{50} values of microcapsules of *C. caudatus* K. with pH, chitosan concentration, and stirring time variations

Sample	IC_{50} ($\mu\text{g/mL}$)*	Sample	IC_{50} ($\mu\text{g/mL}$)*	Sample	IC_{50} ($\mu\text{g/mL}$)*
Microcapsules pH 4	132.22 ± 0.30^a	Microcapsules with 0.05% (w/v) chitosan	109.88 ± 0.46^a	Microcapsules with 30 min stirring time	144.70 ± 2.15^c
Microcapsules pH 5	150.40 ± 0.77^b	Microcapsules with 0.1% (w/v) chitosan	132.22 ± 0.30^b	Microcapsules with 60 min stirring time	112.37 ± 2.09^b
Microcapsules pH 6	169.84 ± 0.48^c	Microcapsules with 0.2% (w/v) chitosan	142.96 ± 0.86^c	Microcapsules with 90 min stirring time	92.85 ± 1.2^a

prepared in pH 4 were the optimum conditions with the lowest IC_{50} toward alpha-amylase enzyme.

The increase in pH increases the IC_{50} value. It means that the inhibitory activity of the alpha-amylase decreased. Microcapsules prepared in pH 4 resulted in the highest inhibitory activity for the enzyme. The protonation process in an acid environment to neutral solution occurs due to the pK_a value of the amino group in chitosan (~ 6.5) [32]. An increase in solubility of chitosan can be made despite the protonation of the

amino group in an acidic solution. The practice of this property has great importance on biomedical applications when chitosan is used to deliver drugs to acidic environment targets. In pH 4, chitosan received more proton donors; as a result, more amine groups (NH_2) will be protonated to NH_3^+ ions which will ionically cross-link with $\text{P}_3\text{O}_{10}^{5-}$ ions from Na-TPP, forming microcapsules. A large number of protonated chitosan will further increase chitosan's ability to absorb the bioactive compounds from the extracts; and, as a

result, increase the inhibitory activity for the alpha-amylase enzyme. A large number of protonated chitosan will further increase chitosan's ability to absorb the bioactive compounds from the extracts and increase the alpha-amylase enzyme's inhibitory activity.

This observation was similar to a previous report that demonstrated pH 4 was the most effective pH for designing chitosan-containing microparticles [32]. At pH 4, biopolymers' charge densities of opposing signs were stoichiometrically balanced. If the pH was too low, the ionization degree of amine groups on the chitosan molecular chain did not assist the formation of a homogeneous particle in the system. When pH values were higher than 4, both ionization degree and solubility of chitosan reduced, which perhaps stimulated the discrepancy in size distribution [33].

The resulting IC_{50} values on the microcapsules with a variation of the chitosan concentration of 0.05%, 0.1%, and 0.2% (w/v) were 109.88 ± 0.46 , 132.22 ± 0.30 , and 142.96 ± 0.86 $\mu\text{g/mL}$, respectively. Increasing the chitosan concentration will increase the IC_{50} value, which means that the alpha-amylase inhibitory activity decreases. Microcapsules with the lowest chitosan concentration of 0.05% (w/v) resulted in the optimal inhibitory activity since this concentration had the lowest IC_{50} value.

The higher the concentration of chitosan, the inhibitory activity of the microcapsules about the alpha-amylase enzyme decreases. The optimum microcapsule condition was obtained at the lowest chitosan concentration of 0.05% (w/v), resulting in the best inhibitory activity seen from the lowest IC_{50} number. The greater the amount of chitosan, the more ammonium ions from chitosan can bind to the extract compounds; therefore, microcapsules provide the best inhibitory activity against the alpha-amylase. The higher the chitosan concentration, the smaller the space between the pores, preventing the active ingredients from diffusing from the microcapsules [34-35]. Previous research showed similar results [34]; optimal chitosan concentration in limonene essential oil microencapsulated in chitosan was not the highest concentration used.

The longer the stirring time to prepare microcapsules, the lower the IC_{50} values obtained. Microcapsules prepared in 30, 60, and 90 min stirring time had IC_{50} of 144.70 ± 2.15 , 112.37 ± 2.09 , and 92.85 ± 1.2 $\mu\text{g/mL}$, respectively. The longer the stirring time, the more homogeneous and the coating process on the microcapsules increases. The longer the stirring process, the longer it takes to break the particles into smaller pieces and have a longer dispersion of particles aggregates [26]. In this study, microcapsules with a stirring time of 90 min resulted in the highest inhibitory activity toward the alpha-amylase enzyme. This result agreed with an earlier study that increasing stirring time and stirring speed had better distribution and better structures of microparticles [23].

Fig. 3 displays the FTIR results of the Na-TPP, chitosan, *C. caudatus* K. extracts, and the microcapsules of the *C. caudatus* K extracts, and the assignment of the peaks of interest is tabulated in Table 3. All FTIR spectra, except for Na-TPP, exhibit a strong and broad antisymmetric band at about $3500\text{--}3400$ cm^{-1} that results from overlapping of the O-H and N-H stretching vibrations of functional groups engaged in hydrogen bonds (peak 1). The Na-TPP FTIR spectrum showed their characteristic bands at the region of $1250\text{--}1150$ cm^{-1} (peak 5) related to the phosphate group (P=O) and $980\text{--}960$ cm^{-1} from symmetric and antisymmetric stretching vibrations in the PO_3 group (peak 6). The spectrum of chitosan exhibits characteristic absorption bands at $1650\text{--}1600$ cm^{-1} (peak 2, C=O stretching in amide group, amide I vibration) and $1360\text{--}1150$ cm^{-1} (peak 5, N-H bending in amide group, amide II vibration). The FTIR spectrum of *C. caudatus* K extracts shows that the extracts' secondary metabolites compounds were mostly flavonoid compounds [38-39]. These were deduced from the characteristic peaks seen in the spectra numbered 1 to 5. Number 1 to 5 peaks were as follows: $3500\text{--}3400$ cm^{-1} ; $3000\text{--}2850$ cm^{-1} ; $1650\text{--}1600$ cm^{-1} ; $1440\text{--}1430$ cm^{-1} ; and $1360\text{--}1250$ cm^{-1} . Those peaks were identified as alcohol O-H stretching (peak 1), C-H alkanes (peak 2), C-H alkanes and C=C aromatics (peaks 3 and 4), and C-H alkanes from the

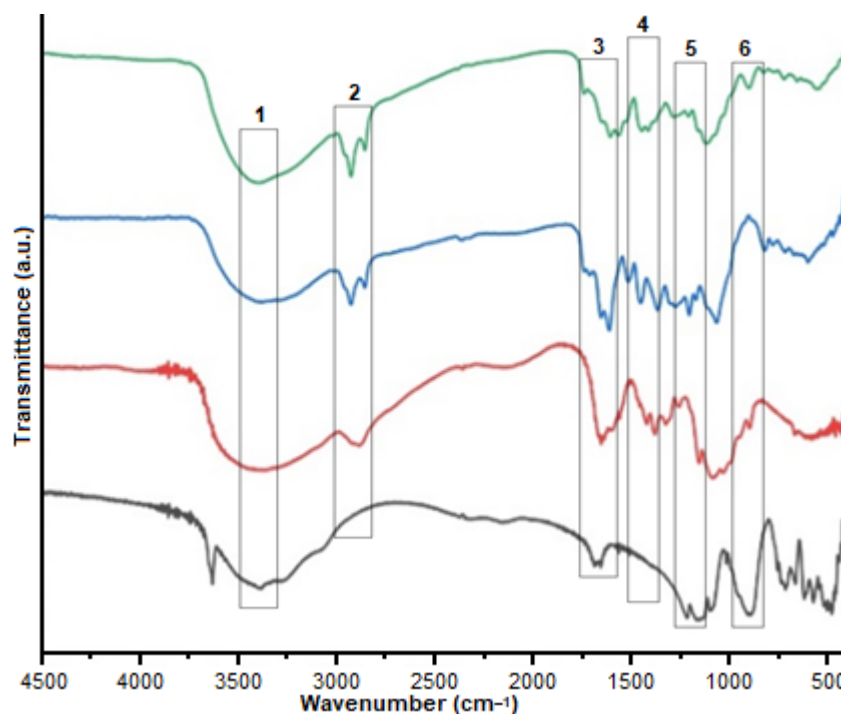


Fig 3. FTIR spectra of: (a) Na-TPP; (b) chitosan; (c) *C. caudatus* K. extract; and (d) microcapsules of *C. caudatus* K. extract

Table 3. Assignment of the FTIR spectra from Fig. 3

Peak number	Na-TPP [36-37]	Chitosan [36-37]	<i>C. caudatus</i> K. extract [38-39]	Microcapsules of <i>C. caudatus</i> K. extract [35-39]
1	3500–3400 cm ⁻¹ for O–H alcohol	3500–3400 cm ⁻¹ for O–H alcohol	3500–3400 cm ⁻¹ for O–H alcohol	3500–3400 cm ⁻¹ for O–H alcohol
2	(undetected)	3000–2900 cm ⁻¹ for C–H alkanes	3000–2850 cm ⁻¹ for C–H alkanes	2970–2850 cm ⁻¹ for C–C alkanes
3	(undetected)	1650–1600 cm ⁻¹ for C–H alkanes and C=C aromatics	1650–1600 cm ⁻¹ for C–H alkanes and C=C aromatics	1600–1650 cm ⁻¹ for C–H alkanes and C=C aromatics
4	(undetected)	1440–1430 cm ⁻¹ for C–H alkanes and C=C aromatics	1440–1430 cm ⁻¹ for C–H alkanes and C=C aromatics	1440–1430 cm ⁻¹ for C–H alkanes and C=C aromatics
5	1250–1150 cm ⁻¹ for P=O stretching	1360–1150 cm ⁻¹ for C–N stretching	1360–1250 cm ⁻¹ for C–H alkanes	1360–1150 cm ⁻¹ for C–N stretching
6	980–960 cm ⁻¹ for P–O stretching in PO ₃ group	(undetected)	(undetected)	980–960 cm ⁻¹ for P–O stretching in PO ₃ group

aromatics nucleus (peak 5).

The FTIR spectra of the extracts and microcapsules of *C. caudatus* K. exhibit some similarities with some distinct features in the spectrum of microcapsules. The similarities indicate that microcapsules were loaded with the bioactive compounds from the *C. caudatus* K. extracts, whereas the differences propose that the microencapsulation is conducted successfully. As shown

in Fig. 3, a new absorption peak in the microcapsules spectrum at 1360–1150 cm⁻¹ indicates a C–N functional group from amines, which may derive from chitosan as coating materials microcapsules. In addition, in the microcapsules spectrum, new absorption also appeared at a wavenumber of 980–960 cm⁻¹, suggesting a P=O functional group that possibly originated from Na-TPP used as a cross-linker agent in microencapsulation.

The obtained microparticles were then characterized with their size and surface structure. Particle size distribution analysis can be useful to analyze the microparticles' properties and deduce their stability. The mean average size of the particles was 38.92 μm , as shown in Fig. 4. Considering the SEM images (Fig. 5), the microcapsules formulated in optimum conditions, pH 4, 0.05% (w/v) chitosan concentration, and 90 min stirring time present more spherical and regular shapes than the morphological shapes of the *C. caudatus* K. extract, even though the surface of the microcapsules was partially rough. In this work, the sizes and the microcapsules surfaces containing *C. caudatus* K. extracts appear similar to those of the common microparticles. Other authors obtained similar results that displayed small and spherical microparticles with some variabilities and roughness from the spray drying process [16-17,19]. Nonetheless, the microcapsules product was already in the range of microparticles sizes, with a mean diameter of 38.92 μm .

The optimal IC_{50} value from all optimal pH, chitosan concentration, and stirring time was $92.85 \pm 1.2 \mu\text{m}$. This number is comparable with the IC_{50} value of *C. caudatus* K. extract with the IC_{50} of $73.07 \pm 0.39 \mu\text{m}$. Therefore, *C. caudatus* K. extract's microcapsules also have a high capacity for alpha-amylase enzyme inhibitors.

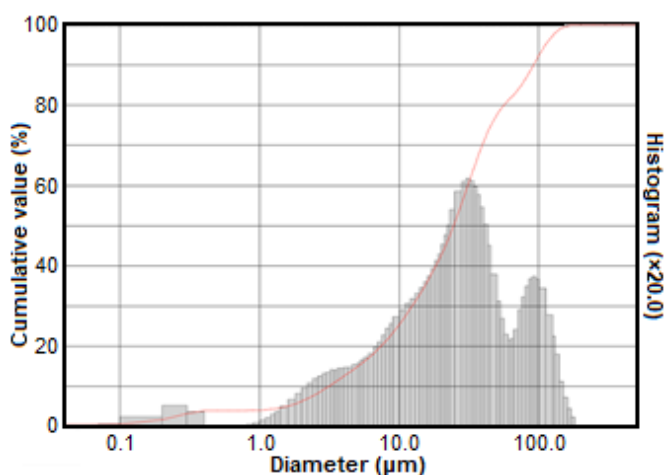


Fig 4. Particle size distribution from microcapsules of *C. caudatus* K. extracts prepared in pH 4, 0.05% (w/v) chitosan concentration, and 90 min stirring time. The mean diameter was 38.92 μm

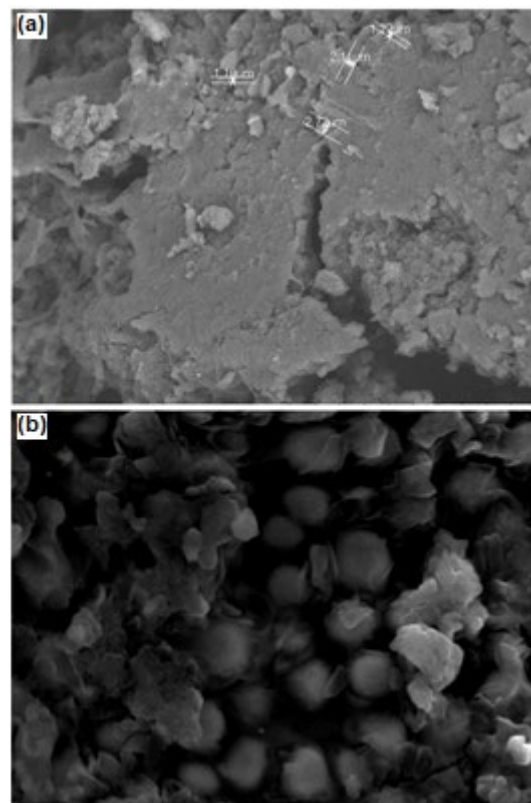


Fig 5. SEM images of the (a) *C. caudatus* K. extracts; microcapsules of *C. caudatus* K. extracts prepared in pH 4, 0.05% (w/v) chitosan, and 90 min stirring time. The magnification was 5000 \times

CONCLUSION

This study has successfully developed microencapsulation of *C. caudatus* K. extracts under variation of pH, chitosan concentration, and stirring time; and determined their in vitro biological activity. The activity of microencapsulated *C. caudatus* K. extracts increased by increasing stirring time and chitosan concentration and decreasing pH. The microencapsulated products resulted in high in vitro alpha-amylase inhibition with the IC_{50} value of $92.85 \pm 1.2 \mu\text{g/mL}$. The FTIR analysis showed that cross-linking was developed between chitosan and Na-TPP. The morphological analysis by SEM indicated that the surface of encapsulated extracts was rough and mostly spherical; nevertheless, PSA analysis showed that the micro-sized particles were achieved at a mean diameter of 38.92 μm .

The obtained results suggest that microencapsulation of *C. caudatus* K. extracts allow a new approach in utilizing bioactive compounds from plant nutraceuticals.

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■ AUTHOR CONTRIBUTIONS

EH and SAD conducted the experiment, AS and AR conducted the analysis, wrote, and revised the manuscript. All authors agreed to the final version of this manuscript.

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