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Synthesis and Characterization of Cinnamon Loaded BSA Microparticles with Antidiabetic Properties

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Abstract

Conventional medicine that is being used to treat diabetes exert adverse side effects and therefore scientists have focused on natural hypoglycemic agents. “Sri Wijaya” (CCSW) is an accession of *Cinnamomum zeylanicum*, which shows higher hypoglycemic activity. Pressured water extract of its dried quills can be used as an antidiabetic nutraceutical. Higher stability, ease of storage and transportation, make powder form nutraceuticals more preferred. The objective of this study was to develop cinnamon encapsulated microparticles as a powder form nutraceutical with higher hypoglycemic activity. Four different products were synthesized. Two of them were synthesized using bovine serum albumin (BSA) (8.8 % (w/v), pH=5) in the presence of citric acid and ascorbic acid as cross-linking agents separately. The other products were synthesized using BSA (20 mg/ mL, pH=9) in the presence of same cross-linking agents. Antidiabetic activity of the products was determined using alpha-amylase and alpha glucosidase inhibition assays and compared with that of crude cinnamon extract and positive control acarbose. Since the product synthesized using BSA (20 mg/ mL, pH=9) and citric acid showed the highest alpha amylase inhibition activity, solubility, cinnamon loading percentage and cinnamon entrapment efficiency those conditions were concluded as the optimum conditions required to synthesize microparticles with higher hypoglycemic activity. Particle size, polydispersity index, and zeta potential of that product were 1.281 ± (0.004) µm, 0.460 ± (0.018) and -1.09 ± (0.03) mV respectively. According to the SEM image, microparticles have a spherical morphology. The UV-visible spectrum and the FT-IR spectrum confirm the entrapment of cinnamon compounds.

Keywords: Cinnamon; Cross-linking agent; Diabetes; Microparticles; Nutraceutical.

Abbreviations: CCSW: *Cinnamomum zeylanicum* “Sri Wijaya”; BSA: Bovine serum albumin; CLMP: Cinnamon Loaded Microparticles.

INTRODUCTION

Diabetes mellitus, simply referred to as diabetes is the most common endocrine illness (Hudaib *et al.,* 2018). In 2008, World Health Organization has estimated that approximately 2.9 million deaths occur due to diabetes mellitus every year (Desoky *et al.,* 2012). The major types of diabetes mellitus are type 1 diabetes and type 2 diabetes (Njagi *et al.,* 2014). Type 1 diabetes is an autoimmune disease, and it typically develops in children and young adults. The immune system of the individuals who suffer from this disease, mistakenly destroys the pancreatic beta cells which make insulin. Type 1 diabetes patients should regularly administer insulin medication. The most common kind of diabetes mellitus is the type 2 diabetes (Riaz, 2009). This is a disorder which can be characterized by insulin resistance and beta cell dysfunction (Desoky *et al.,* 2012).

Since current conventional diabetes medicines exert adverse side effects, scientists have focused more on hypoglycemic agents of natural sources, which have been used in traditional medicine systems such as Ayurveda (Desoky *et al.,* 2012). Nearly 72.8 % of the world diabetic population rely on herbal medicine for the treatment of their diabetes mellitus (Hudaib *et al.,* 2018). In traditional medicine systems of China, Korea and Russia, cinnamon has been used as a medicine for diabetes mellitus (Hudaib *et al.,* 2018). Various chemical compounds in cinnamon can contribute for the anti-diabetic property in different ways. Methyl hydroxychalcone polymer of cinnamon has an ability to stimulate glucose oxidation, thus helps to decrease the glucose content. Some compounds in cinnamon such as polyphenol type-A polymers act as insulin (Gan and Rao, 2014). Cinnamon can reduce the intestinal glucose absorption by inhibiting the enzymes like α-amylase and α-glucosidase which involve for the carbohydrate metabolism. A proanthocyanidin called cinnamtannin B1 is responsible for this activity. The same compound can further contribute to decrease the blood glucose level by

stimulating cellular glucose uptake by membrane translocation of GLUT-4, stimulating glycogen synthesis, inhibiting gluconeogenesis, and stimulating insulin release. Cinnamon can also act against diabetic retinopathy and neuropathy (Constantine *et al.,* 2013).

There are approximately 250 species of cinnamon all over the world (Gan and Rao, 2014). Among them Ceylon cinnamon (*Cinnamomum zeylanicum*) can be consumed in higher doses for longer durations without toxic effects due to the very low coumarin content. CCSW is one of the accessions of *Cinnamomum zeylanicum* that is a newly developed accession of cinnamon, and it shows higher anti-diabetic activity (Wariyapperuma *et al.,* 2020). Therefore, CCSW accession of Ceylon cinnamon can serve as a promising candidate to develop antidiabetic nutraceuticals.

In developing effective antidiabetic nutraceuticals from Ceylon cinnamon, the active compounds should be extracted. According to the previous studies, pressured water extraction is the most effective method to prepare an aqueous extract of cinnamon with antidiabetic activity. It is desirable to have aqueous plant extracts of plants in the powder form to enhance the durability, stability and the ease of storage and transportation. Plant extracts can be encapsulated in microparticles to convert them into more stable form (Amritham *et al.,* 2016). Microencapsulation is an advanced technology using which one or more active compounds can be encapsulated within an inert material, making a tiny sphere of diameter ranging from 1 µm to several 100 µm. Microencapsulation is performed for protecting the sensitive bioactive compounds as well as for their safe delivery. Nowadays BSA is used as a common encapsulant since the microparticles produced using this protein are less toxic, nonantigenic, biocompatible and biodegradable (Choudhury *et al.,* 2021). Microparticulate drug delivery systems proffer a number of significant advantages including effective protection of the encapsulated active agent against enzymatic degradation, enhancement of peptide stability, site-specific and controlled drug release (Wong *et al.,* 2018; Singh *et al.,* 2010)

The objective of the study was to synthesize and characterize cinnamon encapsulated BSA microparticles with antidiabetic properties.

MATERIALS AND METHODS

Raw Materials

Cinnamomum zeylanicum quills (Accession: Sri Wijaya)

Chemicals

Bovine Serum Albumin (BSA), Ethanol, Citric acid, Ascorbic acid, Sodium hydroxide, Hydrochloric acid, Acarbose, Alpha amylase, Starch, Dinitrosallicylic acid reagent (DNS), Sodium hydrogen phosphate, Sodium dihydrogen phosphate, Alpha glucosidase, para-nitrophenyl-d-glucopyranoside (pNPG), Dimethyl sulfoxide (DMSO) and Anhydrous KBr

Instruments

Electric grinder (Sumeet, India), Analytical balance (Kern ALJ 120-4 Germany), Pressure cooker (Prestige India), Centrifuge machine, Magnetic stirrer, pH meter, Thermometer, Electric oven, Microplate reader (Spectra Max M5, Molecular Devices, CA, USA), Malvern Zetasizer Nano ZS apparatus (Malvern Instruments Ltd., Malvern, UK), Field emission scanning electron microscope (Hitachi SU6600 FE-SEM), UV-VIS spectrophotometer (Agilent Technologies, Germany. Cary 60), FT-IR (Fourier transform infrared) spectrometer (PerkinElmer, L 1600300 Spectrum TWO LITA, Liantrisant, UK).

Preparation of cinnamon quills

Dried quills of *Cinnamomum zeylanicum* (which were obtained from Cinnamon Research Institute, Thihagoda, Matara) were ground into fine powder and stored at -10°C.

Preparation of the extract

Cinnamon powder (10.00 g) was mixed with distilled water (200.0 mL) and digested under pressure for 10 minutes using a pressure cooker. The extract was filtered through a muslin cloth. The filtrate was centrifuged at 3000 rpm for 15 minutes and then the supernatant was used.

Preparation of cinnamon loaded microparticles (CLMP)

Based on previous research studies, two different BSA solutions and two different crosslinking agents were used to prepare four products as mentioned in the table 01. pH values of BSA solutions were set to the required value by using NaOH (1 M) and HCl (1 M) solutions.

Table 1. BSA solutions and crosslinking agents which were used to prepare microparticles.

Product	BSA solution		Crosslinking agent
	Concentration	pH	
A	20.00 mg/ mL	9	Citric acid
B	20.00 mg/ mL	9	Ascorbic acid
C	8.85 % w/v	5	Citric acid
D	8.85 % w/v	5	Ascorbic acid

For the synthesis of cinnamon loaded BSA microparticles, the aqueous cinnamon extract (4.00 mL) was mixed with ethanol (16.00 mL). The mixture was added to a BSA solution (4.00 mL) at 1.00 mL/ minute addition rate while constantly stirring the BSA solution at 600 rpm. During the addition process the temperature of the system was maintained at around 4 °C. Then the crosslinking agent (8 %w/v, 230 µL) was added. The

reaction mixture was stored for twenty-four hours at 4 ℃. The microparticle bearing solution was centrifuged at 3000 rpm for 30 minutes and the microparticles were collected. The particles were washed with distilled water and dried at 50 ℃ until a constant weight was observed. The particles were stored at 4 ℃ for further analyses.

Determining the yield of CLMP

Weight of the product was measured. Total concentration of all compounds in cinnamon extract was determined by evaporating cinnamon extract (1.00 mL) and weighing the remainder. The yield was calculated according to the following equation.

$$\text{Yield} = \frac{\text{Weight of the product (g)}}{\text{Weight of used BSA + Weight of cinnamon extract}} \times 100$$

Determining antidiabetic activity of CLMP

Antidiabetic activity of the pressured water extract of cinnamon quills, positive control acarbose and the CLMP were determined by carrying out *in-vitro* alpha amylase inhibition assay and alpha glucosidase inhibition assay. The antidiabetic activity of the products was compared with the antidiabetic activity of the acarbose and the crude extract.

Alpha amylase inhibition assay

Alpha amylase inhibition activity of CLMP, pressured water extract of cinnamon and acarbose were determined by following the method described by Aiyegoro *et al.* (2017) with slight modifications. (Aiyegoro *et al.,* 2017)

Alpha amylase enzyme (0.05 mg mL^{-1}, 250 µL) in sodium phosphate buffer (0.02 M, pH 6.9) was added to the sample (500 µL). The mixtures were incubated at room temperature for 15 minutes. Then starch solution (1 %, 250 µL) in sodium phosphate buffer (0.02 M, pH 6.9) was added. The reaction mixtures were incubated at room temperature for another 15 minutes. DNS reagent (250 µL) was added and boiled for 5 minutes, and absorbance was measured at 540 nm by microplate reader.

The same procedure was repeated by adding sodium phosphate buffer (0.02 M, pH 6.9, 250 µL) instead of alpha amylase enzyme. In addition to that, the above procedure was repeated for two other reaction mixtures. One amongst them was prepared by adding sodium phosphate buffer (0.02 M, pH 6.9, 500 µL) instead of plant extract and the other was prepared by adding sodium phosphate buffer (0.02 M, pH 6.9, 750 µL) instead of both plant extract and the enzyme. The percentage inhibition of alpha amylase enzyme was calculated using the following formula. IC_{50} values on alpha amylase enzyme were determined by using GraphPad Prism 8 software, based on inhibitory percentage values.

$$\text{Inhibition (\%)} = \frac{\text{(P-Q) - (R-S)}}{\text{(P-Q)}} \times 100$$

P : Absorbance at 540 nm without inhibitor and with enzyme
Q : Absorbance at 540 nm without inhibitor and enzyme
R : Absorbance at 540 nm with inhibitor and enzyme
S : Absorbance at 540 nm with inhibitor and without enzyme

Alpha glucosidase inhibition assay

Alpha glucosidase inhibition activity of the products, pressured water extract of cinnamon and acarbose were determined by following the method described by Jayawardena *et al.* (2018) with slight modifications. [14]

Alpha glucosidase enzyme (0.5 U mL^{-1}, 25 µL) in sodium phosphate buffer (0.10 M, pH 6.8) was added to the sample (50 µL) and incubated at 37 ℃ for 10 minutes. p-NPG (1.25 mM, 25 µL) in sodium phosphate buffer (0.10 M, pH 6.8) was added and incubated at 37 ℃ for 10 minutes and the absorbance was measured at 405 nm by a microplate reader. The same procedure was repeated by adding sodium phosphate buffer (0.10 M, pH 6.8, 25 µL) instead of the enzyme, sodium phosphate buffer (0.10 M, pH 6.8, 50 µL) instead of cinnamon extract and sodium phosphate buffer (0.10 M, pH 6.8, 75 µL) instead of both cinnamon extract and enzyme. The percentage inhibition of the alpha glucosidase enzyme was calculated using the following formula. IC_{50} values on alpha glucosidase enzyme were determined by using GraphPad Prism 8 software, based on inhibitory percentage values.

$$\text{Inhibition (\%)} = \frac{\text{(P-Q) - (R-S)}}{\text{(P-Q)}} \times 100$$

P : Absorbance at 405 nm without inhibitor and with enzyme
Q : Absorbance at 405 nm without inhibitor and enzyme
R : Absorbance at 405 nm with inhibitor and enzyme
S : Absorbance at 405 nm with inhibitor and without enzyme

Characterization of microparticles

Solubility

CLMP (5.0 mg) was added to 5.00 mL of distilled water and stirred for 30 minutes at 600 rpm at room temperature. After that the mixture was centrifuged at 10 000 rpm for 15 minutes. Pellet was dried at 100 ℃ in an oven until a constant weight was observed. The procedure was triplicated. The solubility was calculated according to the following formula.

$$\text{Solubility (\%)} = \frac{\text{Weight of the product added (g) - Weight of the pellet (g)}}{\text{Weight of the product added (g)}} \times 100$$

Cinnamon loading and entrapment efficiency
A concentration series from the pressured water extract of cinnamon quills was prepared. Absorbance of the concentration series was measured at 310 nm. A graph of absorbance at 310 nm vs concentration of cinnamon extract was plotted (Akkawi *et al.,* 2015). Absorbance of the supernatants which remained after the separation of the product containing pellets, were measured at 310 nm. By comparing the absorbance value of each supernatant with the standard curve, the concentration of remaining cinnamon compounds in the supernatants was found. The procedure was triplicated. Cinnamon loading and entrapment efficiencies were determined by using following formulae.

Weight of cinnamon loaded = Weight of cinnamon added - Weight of cinnamon in supernatant

$$\text{Cinnamon loading (\%)} = \frac{\text{Weight of cinnamon loaded}}{\text{Total weight of the product}} \times 100$$

$$\text{Cinnamon entrapment efficiency (\%)} = \frac{\text{Weight of cinnamon loaded}}{\text{Weight of cinnamon added}} \times 100$$

Determination of particle size and zeta potential
The particle size, polydispersity index (PDI) and zeta potential of the product were determined with the Malvern Zetasizer Nano ZS apparatus. An aqueous suspension of CLMP was diluted 1:100 with ultrapure water and the solution was placed in a disposable polystyrene cuvette and the particle size measurement was obtained. The solution was also placed in a folded capillary zeta cell and the zeta potential measurement was obtained. Both procedures were triplicated.

Morphological observations
Field emission scanning electron microscope was used to visualize the morphology and shape of the oven-dried product. Sample was mounted onto the sample stub using carbon tapes and the images were taken after gold sputter coating for 15 seconds.

UV-visible absorbance spectra
The UV-visible absorption spectra were analyzed using a UV-visible spectrophotometer from 200 to 500 nm within a 1 cm quartz cell. A 0.8 mg/mL synthesized nanoparticle sample was prepared after dissolving it in DMSO and its absorbance spectrum was obtained. It was then compared with the absorbance spectra of 1 mg/mL pure BSA, 0.07 mg/mL aqueous cinnamon extract and 0.8% w/v citric acid. The absorbance spectrum of distilled water was subtracted from all sample spectra.

FT-IR spectroscopy
The molecular characteristics of the product were examined and compared with that of pure BSA, oven dried aqueous cinnamon extract and pure cross-linking agent, citric acid using an FT-IR spectrometer. Each sample was mixed with anhydrous KBr in a 1:10 ratio and ground using a motor and pestle until a fine powder was obtained. A small portion of the powder was placed in the pellet forming mold and pressed under pressure. Then the pellet was placed in the FT-IR spectrometer and scanned in the wavenumber range of 750-4000 cm^{-1}.

Statistical Analysis
The obtained data were statistically analysed by one-way analysis of variance (ANOVA) using Minitab software package. The results were expressed in the form of mean ± standard deviation of triplicate determinants. The level of significance was taken at 5% confidence interval ($p < 0.05$).

RESULTS AND DISCUSSION

Physical appearance of the products

Table 2. Yield and morphology of the products.

Product	Yield (%)	Morphology
A	71.50	Brown colour powder
B	75.36	Brown colour powder
C	69.62	Brown colour gel
D	67.99	Brown colour gel

Antidiabetic activity

Table 3. IC_{50} values on alpha amylase and alpha glucosidase enzymes.

Sample	IC_{50} value on alpha amylase enzyme (µg/mL)	IC_{50} value on alpha glucosidase enzyme (µg/mL)
Pressured water extract	131.27 (±1.64) [e]	141.25 (±0.21) [b]
Acarbose	140.37 (±1.17) [c]	224.45 (±0.21) [a]
A	117.60 (±1.73) [f]	119.25 (±0.07) [e]
B	134.97 (±0.32) [d]	112.40 (±0.57) [f]
C	151.00 (±0.76) [a]	137.75 (±0.21) [c]
D	146.23 (±0.56) [b]	122.70 (±0.28) [d]

means that do not share a letter are significantly different from each other. ($p < 0.05$)

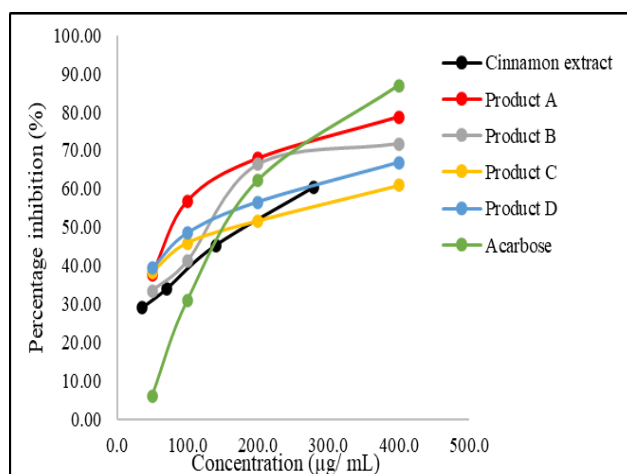

Figure 1. The graph of percentage inhibition of α amylase enzyme (%) vs concentration (µg/ mL).

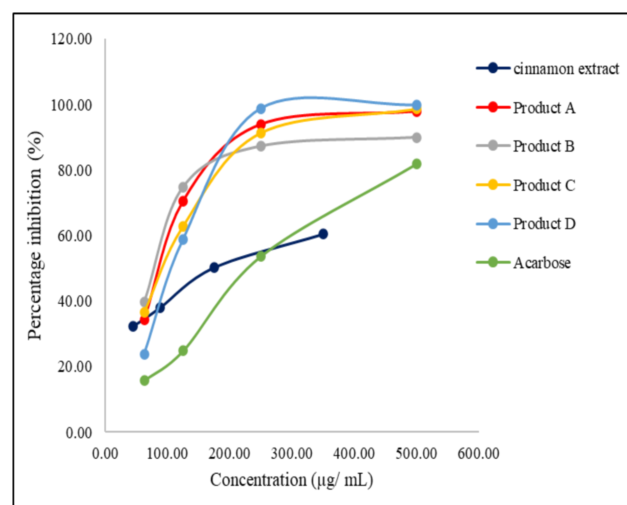

Figure 2. The graph of percentage inhibition of α glucosidase enzyme (%) vs concentration (µg/ mL).

Characterization of the products

Table 4. Solubility, cinnamon loading percentage and cinnamon entrapment percentage of the products.

Product	Solubility in water (%)	Cinnamon loading percentage (%)	Cinnamon entrapment efficiency (%)
A	53.00 (±1.00) [a]	3.69 (±0.01) [a]	77.97 (±0.03) [a]
B	49.00 (±1.00) [a]	3.33 (±0.10) [b]	74.11 (±0.02) [b]
C	30.33 (±1.53) [b]	0.76 (±0.00) [c]	67.51 (±0.02) [c]
D	22.33 (±2.52) [c]	0.72 (±0.01) [c]	62.03 (±0.05) [d]

means that do not share a letter are significantly different from each other. ($p < 0.05$)

Table 5. Particle size, PDI and zeta potential of the product.

Parameter	Mean ± Standard deviation
Particle size	1.281 ± (0.004) µm
PDI	0.460 (± 0.018)
Zeta potential	-1.09 (±0.03) mV

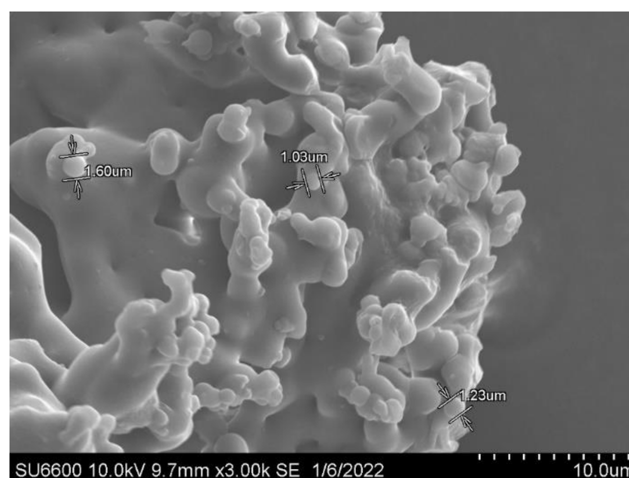

Figure 3. SEM image of CLMP.

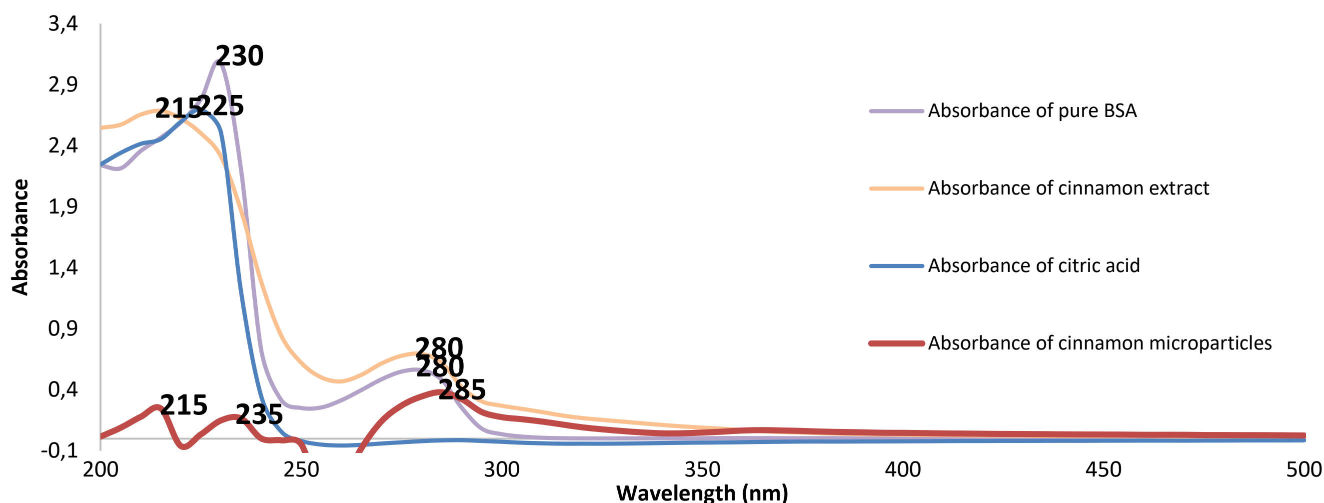

Figure 4. UV-visible absorbance spectra of pure BSA, aqueous cinnamon extract, citric acid, and CLMP.

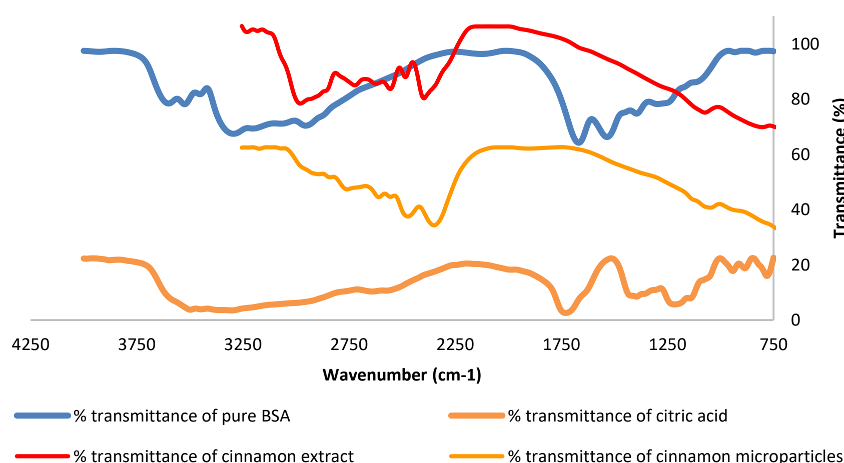

Figure 5. FT-IR spectra of pure BSA, aqueous cinnamon extract, pure citric acid, and CLMP.

Discussion

Micro formulations are an exciting novel method that could be used to improve the stability, bioavailability, poor solubility and absorption of natural products (Singh *et al.*, 2010). Even though cinnamon has many therapeutic properties, the desired effect could be lost in processing cinnamon products. Encapsulating natural products into various matrices is a common technique used to overcome the loss of the active ingredients in natural products during processing.

In the current study, 4 different products (product A, B, C and D) were formulated by encapsulating cinnamon into BSA, as mentioned in table 1.

Products A and B were in powder form while products C and D were gel like. For the preparation of products C and D, the pH value of the BSA solution was set to 5. The isoelectric point of BSA is around 4.7 (Amritham *et al.,* 2016). Hence pH value of the used BSA solution was very close to the isoelectric point of BSA. Therefore, at pH 5, the BSA molecules show less tendency to become ionized. Hence the aggregation of BSA molecules with each other occur rapidly in order to minimize the contact with highly polar aqueous medium. This may be the reason for the formation of gel like product instead of a powder form product.

For the preparation of products A and B, the pH of the BSA solution was set to 9. At that pH value the protein molecules are ionized. Therefore, they tend to repulse each other. This results the separation of the protein particles from each other as much as possible. Hence the resulted particles become smaller and smaller (Constantine *et al.,* 2013; Amritham *et al.,* 2016). This can result in a powder like fine particle in microscale.

According to table 2, the highest yield has been obtained for the product B which was followed by products A, C and D. This suggests that the highest yield

for CLMP can be obtained by using ascorbic acid as the cross-linking agent if the pH value of the used BSA solution is around pH 9.

The antidiabetic activity of natural products can be measured using the inhibitory potential of alpha-amylase and alpha-glucosidase enzymes.

Product A has shown the lowest IC50 value [117.60 (±1.73) µg/mL] (Table 3). That means the product A has the highest inhibitory activity on alpha amylase enzyme. Both products A and B have shown higher inhibitory activity than the crude cinnamon extract and the positive control acarbose. This confirms that the activity of cinnamon extract become enhanced when encapsulated. Since products C and D are not powder form products, they may not be in microscale. That may be a reason for their low alpha amylase inhibitory activity. The graph of percentage inhibition on alpha amylase enzyme vs. concentration is also shown in figure 1.

Cinnamon loading percentage and the cinnamon entrapment efficiencies of the products also support the obtained results for the alpha amylase inhibitory assay (Table 4). Product A has shown the highest cinnamon loading percentage and cinnamon entrapment efficiency. That may be the reason for its higher activity than the other products.

According to table 3, all the products have shown higher inhibitory activity on the alpha glucosidase enzyme in comparison with the crude cinnamon extract and acarbose. However, the inhibitory activity of products C and D is lower than that of products A and B. The higher activity of products A and B may be due to their smaller size and higher surface area.

The lowest IC50 value [112.40 (±0.57) µg/mL] has been recorded for product B. Therefore, the highest inhibitory activity on alpha glucosidase enzyme has been shown by product B even though the cinnamon loading percentage and cinnamon entrapment efficiency of product A is greater than that of product B. But the IC50 values for products A and B [119.25 (±0.07) µg/mL and 112.40 (±0.57) µg/mL respectively] are somewhat closer to each other. The graph of percentage inhibition on alpha glucosidase enzyme vs. concentration is shown in figure 2.

The solubility of the products in water is also given in table 4. The water solubility of products A and B is greater than that of products C and D. As explained by Amirtham *et al.* (2016), higher solubility of the product A and B may be due to their smaller size. Smaller the size greater is the surface area. Therefore, the solubility becomes greater (Amritham *et al.,* 2016; Amighi *et al.,* 2020).

Table 4 shows the cinnamon loading percentage of the products. The percentage values obtained for products C and D do not show statistically significant difference, but they show statistically significant difference to the results obtained for other products. The cinnamon loading percentage of products A and B is significantly greater than that of products C and D. The concentration of the BSA solutions which were used for the synthesis of products C and D were 8.8 % (w/v) while the concentration of the BSA solutions which were used for the synthesis of products A and B were 20 mg/mL. When the concentration of the initially used BSA solution becomes higher, the ratio between the loaded cinnamon compounds and BSA decrease. That is the reason for the observed results.

The cinnamon loaded percentage of product A [3.69 (±0.01) %] is greater than that of product B [3.33 (±0.10) %]. According to Amighi *et al*. (2020) the particles synthesized using citric acid as the cross-linking agent is greater in size compared to the particles synthesized using ascorbic acid as the cross-linking agent. When the size of the particle is greater, the particle can accommodate more bioactive compounds (Amritham *et al.,* 2016). That may be the reason for the slight increment of the cinnamon loading percentage of product A than product B.

Table 4 shows the cinnamon entrapment efficiencies of the synthesized products. The data obtained for all the products have shown statistically significant difference from each other. The highest cinnamon entrapment efficiency has been reported for product A, followed by product B, C and D.

Since products C and D were synthesized at pH 5, BSA molecules tend to aggregate with each other due to their less tendency to become ionized at pH values which are very close to their isoelectric point. The aggregated BSA molecules precipitated and formed the gel like product rapidly with the addition of the desolvating agent. The amount of time that the BSA particles spent in the solution while keeping contact with the dissolved cinnamon compounds was very little. Most of the aggregated BSA particles precipitated even before the complete addition of the recommended volume of ethanol/ cinnamon extract mixture in previous studies [Amirtham et al, (2016)] and the cross-linker. Therefore, the probability to entrap more cinnamon compounds is comparatively less for products C and D. That may be the reason for the observed low cinnamon entrapment percentage for products C and D.

Products A and B were synthesized at pH 9 which is far away from the isoelectric point of BSA (4.7). Hence BSA molecules were ionized and well separated from each other. At that pH value BSA particles do not move out from the solution phase rapidly. Therefore, most of the BSA particles get the opportunity to stay in the solution phase until the complete addition of the ethanol/ cinnamon extract mixture and the cross-linker. This enhances the probability to entrap more cinnamon compounds during the formation of microparticles. Hence the cinnamon entrapment efficiency of products A and B is greater than that of other products.

However, the cinnamon entrapment efficiency of product A is little bit greater than that of product B. This may be again due to the greater size of the microparticles

which were synthesized using citric acid as the cross-linking agent.

Since the alpha amylase inhibitory activity, solubility, cinnamon loading percentage and cinnamon entrapment efficiency of product A is greater than other products, product A was selected for further studies. Hence characterization using parameters such as particle size, surface charge, morphology, particle structure using FT-IR and cinnamon entrapment using UV-visible spectrophotometer were carried out only for product A.

Product A showed a mean particle diameter of 1.281 ± (0.004) µm (Table 5). According to Amighi *et al.* (2020), the particle size that is obtained under the conditions that were used in the synthesis of product A with citric acid as the cross-linking agent is 1.201 (±0.058) µm (Amighi *et al.,* 2020). As the mean particle size obtained for product A is very close to this value, it is clear that they have been formed effectively.

According to Danaei *et al.* (2018), the PDI of a sample with effective particle size distribution should be between 0.05-0.7. As per table 5, the PDI value of the product A falls within this range and therefore it can be stated that a homogenous population of microparticles have been synthesized which can act as safe, stable, and efficient microcarriers of cinnamon. The tendency of these BSA microparticles to accumulate in the target tissue, which depends on the particle size distribution, will be minimal (Danaei *et al.,* 2018).

Microparticles with a zeta potential between -10 and +10 mV are considered neutral, while microparticles with zeta potentials greater than +30 mV are considered strongly cationic and those with zeta potentials less than -30 mV are considered strongly anionic. Since most cell membranes are negatively charged, zeta potential can strongly affect a microparticle's tendency to penetrate membranes, with cationic particles displaying toxicity due to cell membrane disruption (Clogston and Patri, 2011). According to table 5, as the zeta potential of the synthesized microparticles is -1.09 mV, they are neutral and will easily penetrate the cell membranes without causing any toxicological effects.

Product A was also studied for morphology by SEM. Morphological analysis of the microparticles were carried out with FE-SEM, and the obtained image is shown in figure 3. The SEM micrograph revealed morphological aspects of microparticles with a spherical shape and uniform size.

The UV-absorbance spectra of the cinnamon extract and CLMP (Figure 4) showed a peak at 215 nm, proving that the active compounds of the cinnamon extract have been successfully loaded into the microparticles. A peak corresponding to citric acid cannot be observed as it has been completely washed away during the washing step. The pure BSA spectrum showed two characteristic peaks at 230 nm and 280 nm. However, these two peaks were slightly shifted to 235 nm and 285 nm respectively, in the absorbance spectrum of CLMP. This may have resulted due to the protein unfolding that may have occurred when the microparticle was dissolved in DMSO, an organic solvent. This agrees with the findings of Liu *et al*. (2009), which states that the spectrum shifts to shorter wavelengths as the polarity of the solvent decreases with the tryptophan residues buried in hydrophobic domains exhibiting a spectral shift of 5 to 20 nm. (Liu *et al.,* 2009).

FT-IR spectroscopy was performed to the product in order to find out if any chemical bond formation has occurred between citric acid and BSA as well as between the active compounds of cinnamon and BSA during the preparation of microparticles, resulting in any conformational changes in the protein structure of microparticles. Furthermore, it was performed to further verify if the cinnamon compounds have been successfully entrapped within the synthesized microparticles. The FT-IR spectrum of microparticles showed characteristic peaks that were also observed in the spectrum of the aqueous cinnamon extract (Figure 5). Peaks at 2750 cm-1 and 2850 cm-1 that may be due to the CHO stretch of cinnamaldehyde, a peak at 1056 cm-1 and a peak at 3000 cm-1 that may be due to the C-O and OH stretches respectively, of cinnamyl alcohol, benzoic acid, benzyl alcohol and methoxyeugenol were observed in the spectra of both aqueous cinnamon extract and CLMP. According to Wariyapperuma *et al.* (2020), these are the major compounds that are found in the CCSW aqueous extract, and therefore this further proves that the active compounds present in CCSW accession have been successfully extracted and encapsulated within the synthesized microparticles. (Wariyapperuma *et al.,* 2020 ; Jayawardena *et al.,* 2018). According to Xu *et al.* (2015), one to two carboxyl groups of one citric acid molecule can react with BSA, resulting in an increase in the total amount of carboxyl groups and a decrease in the total amount of amine groups in the cross-linked microparticles (Xu *et al.,* 2015). This explains the presence of a peak corresponding to the OH stretch and decrease in intensity of the peak at 3400 cm-1 corresponding to the N-H stretch in the microparticle spectrum. This means that interactions between BSA and citric acid have caused conformational changes in the protein structure of the synthesized microparticles. A similar observation has been made by Amighi *et al.* (2020) during the synthesis of BSA microparticles using citric acid as the cross-linking agent (Amighi *et al.,* 2020) Additionally, peaks at 1665 cm-1 and 1537 cm-1 observed in the pure BSA spectrum corresponding to the Amide I and Amide II stretches respectively, were absent in the microparticle spectrum. Hence, loading of active compounds of cinnamon have also induced conformational changes in the structure of the BSA protein. This means that the active compounds of cinnamon have interacted with the protein matrix of the synthesized microparticles via covalent bonds. This

result is similar to the results obtained by Rani (2016) (Rani, 2016).

CONCLUSIONS

This study has proven that the antidiabetic activity of the pressured water extract of the quills of *Cinnamomum zeylanicum* can be enhanced by micro-encapsulating the active compounds of the extract. For the micro-encapsulation of the bioactive compounds, BSA microparticles can be used as the microcarrier system.

Size of the microparticle, cinnamon loading percentage and cinnamon entrapment efficiency have a direct effect on the antidiabetic activity of the synthesized microparticles. If the size of the particle is in the microscale and the cinnamon loading percentage and cinnamon entrapment efficiency are higher, the activity of the particle becomes higher. The cross-linking agent, pH, and the concentration of the BSA solution which is used to prepare BSA microparticles can affect the above-mentioned parameters and the solubility of the product in a significant manner. Since the product ‘A’ has shown the highest alpha amylase inhibitory activity, cinnamon loading percentage, cinnamon entrapment efficiency and water solubility, it can be concluded that the CLMP with higher antidiabetic activity and desired properties can be synthesized at pH 9, if the concentration of the BSA solution is 20 mg/ mL and the cross-linking agent is citric acid.

Characterization of product A revealed to have a spherical morphology, uniform size with effective particle size distribution and a neutral surface charge. The UV-visible absorbance spectrum of the product showed the successful entrapment of the cinnamon compounds within the microparticles and the FT-IR spectrum showed that the cross-linking agent, citric acid had caused conformational changes in the protein structure of BSA. It also further proved that the active compounds were successfully loaded into the synthesized microparticles which interacted with the protein matrix via covalent bonds. Therefore, according to these characterization data of product A, it can be concluded that the CLMP have been synthesized effectively, which can be used as a powder form antidiabetic nutraceutical.

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