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MAKING OF CINNAMON (*CINNAMOMUM BURMANII*) BARK EXTRACT NANOPARTICLES WITH IONIC GELATION METHOD

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ABSTRACT

Cinnamon bark extract has high phenolic content which has potential as an antioxidant but has low bioavailability under conditions of large particle size. Therefore, it was carried out the making of cinnamon bark extract nanoparticles by ionic gelation method and characterization of the nanoparticles formed. This study aims to prepare and evaluate the characteristics of cinnamon bark extract nanoparticles. Chitosan-tripolyphosphate-cinnamon bark extract nanoparticles have a particle size of 454.2 nm with a zeta potential of -25.8 mV and functional groups that play a role in the formation of nanoparticles, namely hydroxyl groups and amide groups from chitosan and phosphate groups from tripolyphosphate and the topography was observed to be closer after the bioactive substances from the cinnamon bark extract were encapsulated and covered the surface of the nanoparticles formed.

KEYWORDS: ionic gelation, crosslinking, cinnamon, nanoparticles.

INTRODUCTION

Indonesia is very rich in biodiversity containing secondary metabolites and has been consumed for generations, both in the form of vegetables and fruits, which contain a lot of phenolic compounds and have high antioxidant activity. Govindappa (2015) managed to collect a number of literatures and list 419 species from 133 families of plants that have antioxidant activity, one of which is cinnamon (*Cinnamomum burmanii*) which contains flavonoid compounds (Darmadi *et al.*, 2017) and the parts used are generally in the form of bark (Maruthamuthu and Ramanathan, 2016)

Flavonoids are phenolic compounds that have the potential as antioxidants (Iskender *et al.*, 2016) and have bioactivity as antihyperglycemia (Tian-yang Wang *et al.*, 2018). Flavonoids are thought to play a role in regenerating damaged pancreatic-cells, increasing insulin secretion, and increasing antioxidant enzyme activity (Vinayagam and Xu, 2015). Flavonoids help antioxidant enzymes control blood sugar levels and prevent complications of hyperglycemia through free radical scavenging, reduction of oxidative stress and reactive oxygen species (Mohan and Nandhakumar, 2013). Therefore, cinnamon is used as a traditional medicine for people with hyperglycemia.

Natural bioactive substances which are classified as derivatives of polyphenolic compounds have low bioavailability under conditions of large particle size making it difficult to penetrate lipid membranes of body cells and are unstable to the influence of temperature and high light intensity so that they are easily oxidized (Desti *et al.*, 2020). One potential strategy that can be applied is to formulate it with a polymer into nanoparticle preparations that are more easily dispersed and stable in water (Liu *et al.*, 2019).

Ionic gelation is one of the methods implemented in producing polymer-based delivery systems (Ngadiwiyana *et al.*, 2018). Cinnamon bark extract can be negatively charged in water due to the presence of hydroxyl groups from polyphenol compounds and can hold ionic interactions with positively charged groups of chitosan amine groups (Roy *et al.*, 2019). The use of chitosan as a nanopolymer material requires the addition of sodium tripolyphosphate as a crosslinking agent to stabilize the formed nanoparticle polymer (Sorasitthiyanukarn *et al.*, 2018).

Based on the above background, the researchers conducted research on the preparation of nanoparticles of cinnamon bark extract using chitosan polymer and sodium tripolyphosphate as a stabilizer. Furthermore, the characterization of the cinnamon bark extract and the resulting chitosan-tripolyphosphate-cinnamon bark extract nanoparticles were carried out.

MATERIALS AND METHODS

Research Materials and Equipment

The ingredients used are cinnamon bark, chitosan, sodium tripolyphosphate (Merck), ethanol 96% (Merck), glacial acetic acid (Merck), $FeCl_3$ (Merck), Mg powder (Merck), HCl 36% (Merck), and aquadest.

The equipment used is a set of glassware, a set of maceration tools, a set of evaporators, filter paper, spatula, magnetic stirrer, a set of centrifuges, a fourier transform infrared (FTIR) spectrophotometer (Bruker-Tensor II), scanning electron microscopy (SEM) (Phenom-World), and particle size analyzer (PSA) (Malvern).

Method

Making cinnamon bark ethanol extract

Cinnamon bark powder was extracted using maceration method using 96% ethanol as solvent. The maceration process was carried out for 3 days and remaceration was carried out 2 times marked by the loss of maserate color. The filtrate obtained was then evaporated using a vacuum rotary evaporator at a temperature of 60^oC and a thick ethanol extract was obtained.

Preparation of cinnamon bark extract nanoparticles

The encapsulation process was carried out by making 50 mL of 1% cinnamon bark extract solution in ethanol and chitosan solvent dissolved with 1% glacial acetic acid until the volume became 100 mL. Both were mixed in a 500 mL beaker with stirring using magnetic stirrer at 3000 rpm for 60 minutes. Then 50 mL of 1% sodium tripoliphosphate was added, and stirring was continued for 60 minutes. Until a suspension of nanoparticles is formed. Furthermore, the suspension was dried in a refrigerator until nanoparticle crystals were obtained. The formed nanoparticles were analyzed using FTIR, SEM, and PSA to determine the characteristics of the nano encapsulant.

RESULTS AND DISCUSSION

Cinnamon bark extract

Cinnamon bark was taken in Belok Village, Petang District, Badung Regency, Bali Province. The results of the identification of plant determinations in the Botanical Gardens "Eka Karya" Bali - Indonesian Institute of Sciences, it is known that this cinnamon plant belongs to the *Cinnamomum burmanni* Blume species of *Lauraceae* (Letter of Determination Identification Results No. B-3529/III/KS.01.03/5/2021).

The moisture content of the cinnamon bark powder was obtained at 9.62%, this indicates that the percentage of water content in the cinnamon bark powder has met the simplicia standard. The water content that meets the simplicia standard should not be more than 10% (Kartini *et al*., 2019). The yield of maceration of cinnamon bark extract was 14.74% (w/w).

Phytochemical test of cinnamon bark extract

Phytochemical test is a qualitative analysis of secondary metabolite compounds that can be identified by reagents capable of providing the characteristics of each secondary metabolite group (Govindappa, 2015). The results of the phytochemical test of cinnamon bark extract are presented in Table 1.

Table 1: Phytochemical test results of cinnamon bark extract.

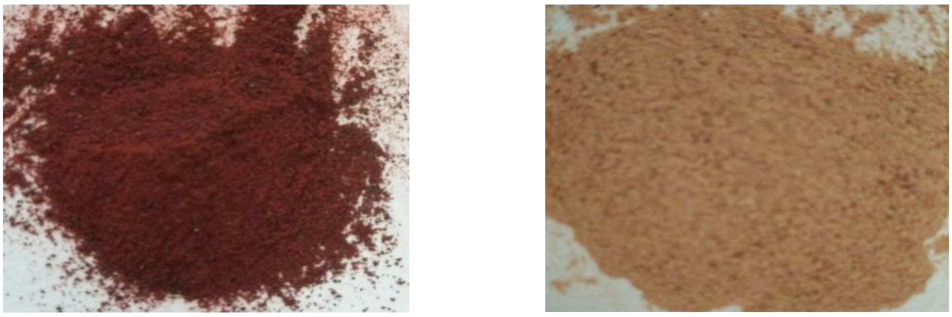
Phytochemical Test	Reagent	Results	Description
Polyphenol	$FeCl_3$	Color change from brownish red to black	Positive Polyphenol
Flavonoids	Mg Powder and HCl	Color change from brownish red to form a red precipitate	Positive Flavonoids

Phytochemical test results with several reagents showed that cinnamon bark extract was positive for polyphenols and flavonoids.

Nanoparticles

The nanoparticles were made by crosslinking method using chitosan and sodium tripolyphosphate as the basic ingredients of the nanoparticles (Manne *et al*., 2020). The bioactive substances contained in the cinnamon bark extract are trapped in the encapsulated crosslinked chitosan-tripolyphosphate nanoparticles (Ngadiwiyana *et al*., 2018).

The basic ingredients characterized by the making of nanoparticles are cinnamon bark extract (Cb) and chitosan-tripolyphosphate-cinnamon bark extract nanoparticles (CsTpp-Cb). The materials obtained are shown in Figure 1.



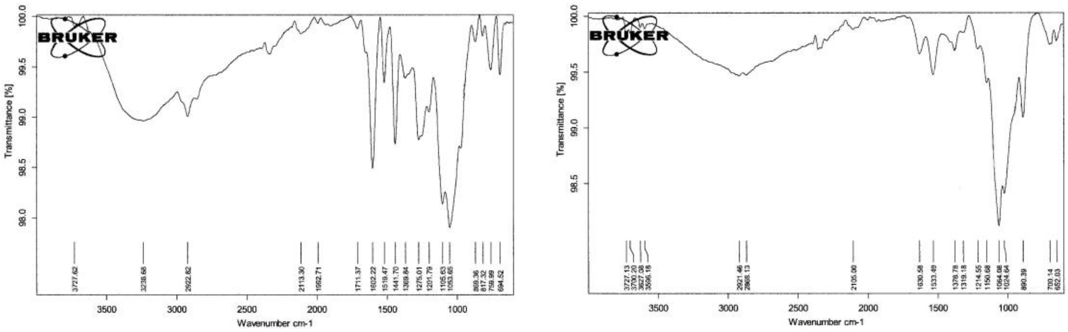
Cb CsTpp-Cb

Figure 1. Cinnamon bark extract and chitosan-tripolyphosphate nanoparticles-cinnamon bark extract.

The mechanism for the formation of CsTpp-Cb nanoparticles by the ionic gelation method is based on electrostatic interactions between the amine groups in chitosan and the negatively charged groups of polyanionic such as tripolyphosphate (Sardjono *et al.*, 2020). Sodium tripolyphosphate is a polyanionic which dissolves in water and then ionizes to form sodium ions and tripolyphosphate ions. Ionic crosslinking reaction occurs between the tripolyphosphate ion (polyanion) and the amine group $-NH_3^+$ (cation) of chitosan (Su *et al.*, 2020). As a result of complexation between different charges, chitosan undergoes ionic gelation and precipitation to form spherical particles such as spheres (Desti *et al*, 2020). The more ionic crosslinking reactions occur between chitosan and tripolyphosphate, the more nanoparticle molecules are formed that encapsulate the bioactive substances found in cinnamon bark extract (Tomaz *et al*., 2018).

Characterization with *fourier transform infrared* (FTIR)

The results of the FTIR analysis provide data about the functional groups contained in the sample (Nandiyanto *et al*., 2019). The interpretation of infrared spectra from the results of the FTIR analysis is carried out by referring to the functional group data bank found in several literatures or the results of interpretation by researchers. FTIR characterization was carried out to determine the functional groups in chitosan and cinnamon bark extract and tripolyphosphate for the making of CsTpp-Cb. The infrared absorption spectra of Cb and CsTpp-Cb samples are presented in Figure 2.



Cb CsTpp-Cb

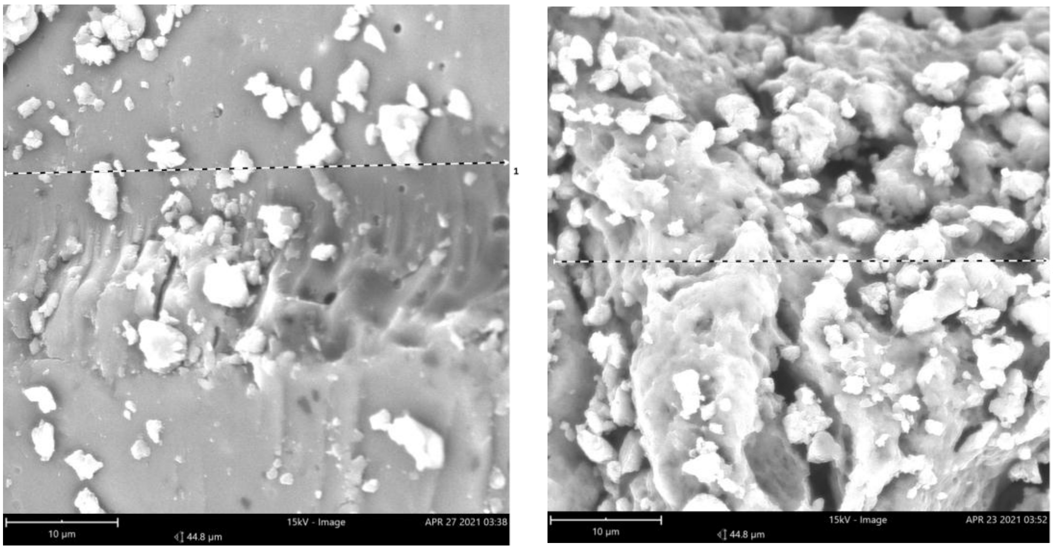
Figure 2. FTIR Spectra.

The infrared spectra of Cb show that the absorption that occurs at a wave number of 3238.68 cm^{-1} is caused by the stretching of O-H alcohol and phenol (Tian-yang Wang *et al*., 2018). The absorption at 2922.82 cm^{-1} indicates the presence of aromatic CH (Bilia *et al.*, 2014), while the absorption at wave numbers 1602.22 cm^{-1}, 1441.70 cm^{-1}, and 1275.01 cm^{-1} respectively, each revealed the presence of C≡C stretching of alkynes, was associated with C=C stretch of alkenes, CH bent of alkanes, and indicated the presence of C-C bonds (Tian-yang Wang *et al*., 2018). Absorption at wave number 1105.63 cm^{-1} indicates the presence of a C-O group (Darmadi *et al*., 2017), and absorption at wave number 1519.47 cm^{-1} indicates the presence of C=O (Bilia *et al*., 2014).

The interaction due to crosslinking formed in CsTpp-Cb can be seen through the shift in the wave number and intensity of each functional group. The O-H group was observed at a wave number of 3186.17 cm^{-1} in the CsTpp-Cb spectra and at a wave number of 3238.68 cm^{-1} in the Cb spectra. This indicates that there has been an interaction between Cb and CsTpp, which is marked by a shift in the wave number of the O-H group in CsTpp-Cb (Ngadiwiyana *et al*., 2018). In addition to the O-H group, chitosan also has an N-H functional group which can be seen at a wave number of 3596.18 cm^{-1} which indicates a stretched N-H secondary amide (Sorasitthiyanukarn *et al*., 2018) but in the Cb FTIR spectra, the wave number is not visible because the bond has not yet formed. between Cb and CsTpp. The cross-linking that occurs in CsTPP-Cb involves tripolyphosphate to stabilize the formed nanoparticles (Sardjono *et al*., 2020). The P-O stretching vibration was observed at a wave number of 892.08 cm^{-1} after Cb was encapsulated into a CsTpp-Cb structure (Su *et al*., 2020).

Characterization with *scanning electron microscopy* (SEM)

Scanning Electron Microscopy (SEM) is an optical microscope used to study the topographic texture and surface appearance of solids or powders (Kwon *et al*., 2019). The results of the characterization of Cs and CsTpp-Cb using SEM are presented in Figure 3.

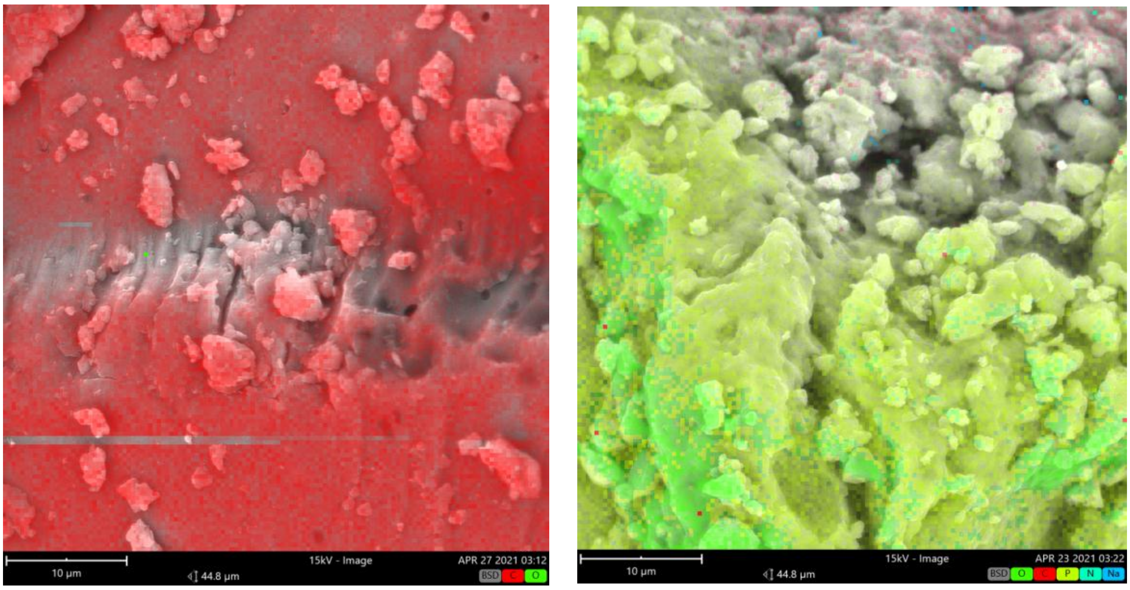


Cb CsTpp-Cb

Figure 3. Results of SEM imaging.

The results of this SEM imaging show topographic changes in CsTpp-Cb after the bioactive substances from Cb are encapsulated into CsTpp. The Cb bioactive substance enters and covers the CsTPP surface so that the resulting CsTpp-Cb structure becomes denser (Avelelas *et a*l., 2019).

Characterization with SEM-Mapping aims to determine the composition of the constituent elements of Cb and CsTpp-Cb by looking at the color differences of each constituent element because each element gives a distinctive color (Esfandiarpour-Boroujeni *et al*., 2017).

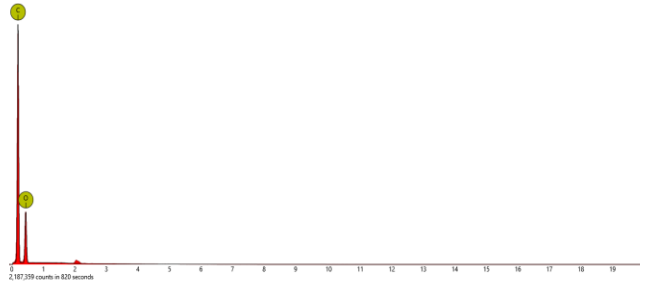


Cb CsTpp-Cb

Figure 4. Results of SEM-Mapping.

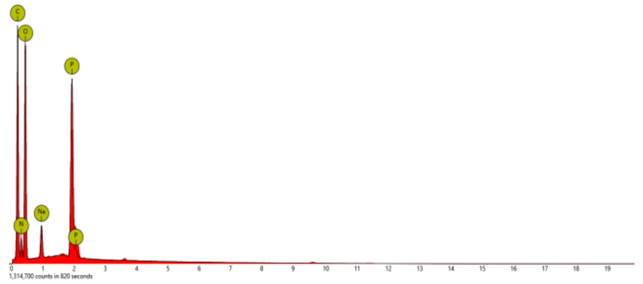
The results of the analysis using SEM-Mapping showed that an increase in the concentration of bioactive substances from Cb caused an increase in the amount of carbon and oxygen encapsulated in CsTpp-Cb (Avelelas *et al*., 2019).

Characterization with *Scanning Electron Microscopy-Energy Dispersive X-Ray* (SEM-EDX) showed a change in the composition of each element after the encapsulation process (Hao *et al.*, 2017). The results of the SEM-EDX analysis are presented in Figure 5.



Number Atomic	**Symbol Element**	**Name Element**	**% Lots Atom**	**% Amount Heavy**
6	C	Carbon	73.15	67.16
8	O	Oxygen	26.85	32.84

Cb



Number Atomic	**Symbol Element**	**Name Element**	**% Lots Atom**	**% Amount Heavy**
6	C	Carbon	49.54	41.13
8	O	Oxygen	33.74	37.32
7	N	Nitrogen	11.60	11.23
11	Na	Sodium	1.16	1.85
15	P	Phosphorus	3.96	8.47

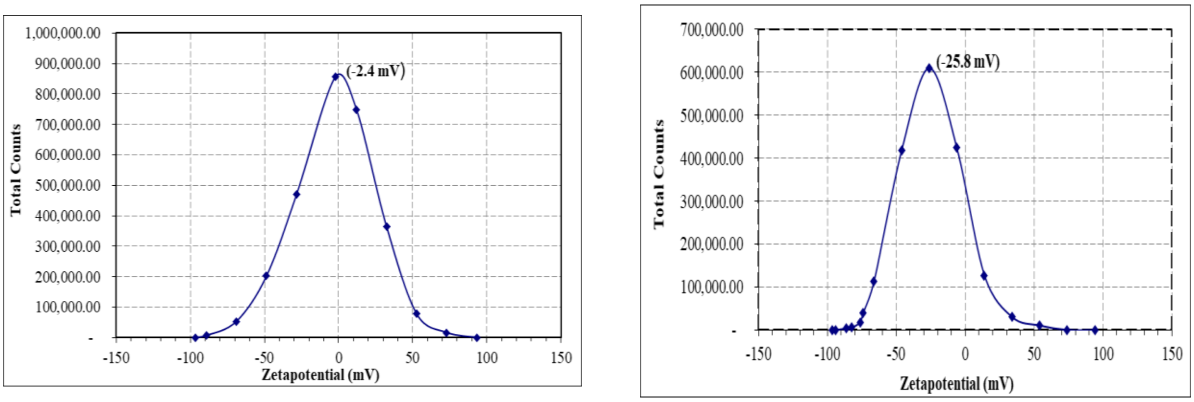
Cs-Tpp-Cb

Figure 5. Analysis results with SEM-EDX.

The results of the SEM-EDX analysis showed that after the formation of CsTpp-Cb there was an increase in C elements. The role of N and P elements is a cross-linking component in the ionic gelation process so that encapsulation can take place properly (Kwon *et al.*, 2019).

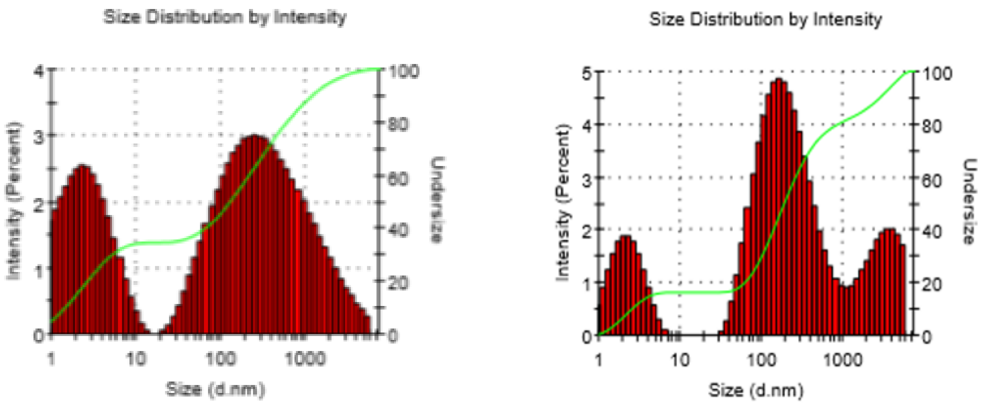
Characterization with particle size analyzer (PSA)

Particle size analyzer (PSA) is used to determine the value of the particle size and zeta potential of a material sample (Liu *et al*., 2019). Zeta potential and particle size are parameters used as a reference to determine the ability of a material as a drug compound and as a drug delivery system (Su *et al.*, 2020). Zeta potentials and particle sizes of Cb and CsTpp-Cb are presented in Figure 6, Figure 7, and Table 2.



Cb CsTpp-Cb

Figure 6. Zeta potential.



Cb CsTpp-Cb

Figure 7. Particle size.

Table 2: Zeta potential and particle size.

No	Sample	Zeta potential	Particle size
1	Cb	-2.4 mV	624.8 nm
2	CsTPP-Cb	-25.8 mV	454.2 nm

Table 2 shows that CsTpp-Cb has a potential zeta value of -25.8 mV which means it has a good ability as a bioactive agent (Su *et al*., 2020) and a particle size of 454.2 nm which indicates that CsTpp-Cb has an area of wider surface area compared to Cb (Tomaz *et al*., 2018).

The increase in particle size is due to agglomeration in chitosan molecules because the interactions of CsTpp and Cb are more and more dense so that the particles tend to cluster and agglomerate to form aggregates into larger particles (Desti *et al*., 2020). The making of nanoparticles that have smaller particle sizes will increase the surface area of the particles so that their permeability increases (Tomaz *et al*., 2018).

In the process of making CsTpp-Cb, there is an interaction between the positive charge of the protonated amine group of chitosan in an acidic atmosphere with the partially negatively charged atoms of the compounds contained in Cb (Desti *et al*., 2020). In this case, the partial negative charge of Cb can come from the hydroxyl group of phenolic compounds and its flavonoids so as to form chitosan-NH_3^+-O-phenolic/flavonoid interactions (Sardjono *et al*., 2020). In addition to interacting with the partially negatively charged group of Cb, the protonated amine group of chitosan allows chitosan to interact with negatively charged other compounds (Ngadiwiyana *et al*., 2018). The addition of tripolyphosphate aims to stabilize the nanoparticles formed through the interaction between the positive charge of chitosan on the surface of CsTpp-Cb with the negative charge of the tripolyphosphate.

CONCLUSION

Cinnamon bark extraction by maceration method with ethanol extract gave a yield of 14.00% and was predicted to contain flavonoid compounds. Cinnamon bark extract and chitosan-tripolyphosphate formed nanoparticles of chitosan-tripolyphosphate-cinnamon bark extract which gave results with a particle size value of 454.2 nm and a zeta potential value of -25.8 mV and the topography was observed to be more dense after the bioactive substances from the extract. Encapsulated and covered the surface of the formed nanoparticles.

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