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SYNTHESIS AND CHARACTERIZATION OF CHITOSAN NANOPARTICLES LOADED *CARICA PAPAYA* LEAVES EXTRACT AND TEST OF THEIR ACTIVITY IN KILLING OF YELLOW SNAILS

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ABSTRACT

In this study, chitosan nanoparticles (CTS NPs) have been sucessfully combined with *Carica papaya* leaves (CPLs) extract for 30 min at room temperature. The prepared chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract have been characterized by UV-vis, FTIR, SEM and TEM. The result showed these CTS NPs/CPLs extract obtained with the average particle size of ~20-50 nm. Moreover, the synthesized CTS NPs/CPLs extract also showed efficient their anti-organism activity for the killing of yellow snails occurred completely after 24 h. The presence of a small powder amount (500 mg) of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract in (5 L) water was enough to inhibit and kill yellow snails completely only after 24 h.

KEYWORDS: Chitosan nanoparticles (CTS NPs), *Carica papaya* leaves (CPLs) extract, yellow snails, biomaterial, anti-organisms.

1. INTRODUCTION

Effective delivery systems for the purpose of carrying a drug specifically, stably and safely to a desired site of action became the most challenging tasks of pharmaceutical formulation scientists.[1] Biopolymers such as chitosan, gelatin are the most widely used polymers in drug delivery.[2,3] Chitosan and its derivatives have proven wound healing properties individually and in combination with other drug materials.[4] Chitosan is widely used in various

pharmaceutical and medical applications because it is cheap, biodegradable and demonstrates good biocompatibility.[5,6]

Chitosan polysaccharide has taken on enormous importance in the control of postharvest pathogenic microorganisms through the development of biodegradable edible coatings and films containing natural antimicrobials; it also has elicitor properties that enhance the natural defenses of fruit, vegetables and grains. Chitosan is an excellent carrier of other functional substances. It has been use for encapsulation of different compounds such as essential oils,[7] RNA[8] antibiotics,[9] drugs for cancer therapy,[10] nutraceuticals[11] and vitamins,[12] among others, potentializing the combined properties of the encapsulating agent and chitosan.

Carica papaya leaves (CPLs) have great opportunities as a source of natural drug which can be used successfully in formulating various types of drugs for different applications because of its availability, effectiveness and safety. This research is conducted with the objective of studying selected in vitro parameters in killing of yellow snails from chitosan nanoparticles (CTS NPs) and *Carica papaya* leaves (CPLs) extract combination. *Carica papaya* leaves are well documented with the ability of yellow snails killing and was investigated for this purpose.

Nanoparticles are known to be more reactive and therefore more efficient in their antimicrobial activity,[13,14] due to the large area of contact with the microbial membrane and consequently the agglomeration on the surface of the cell wall of the fungus.[15]

Therefore, the incorporation of *Carica papaya* leaves extracts into chitosan nanoparticles may enhance the antimicrobial and the antifungical function. The aim of this work was to characterize and study the antimicrobial activity of chitosan nanoparticles (CTS NPs) incorporated with *Carica papaya* leaves (CPLs) extract applied for killing of yellow snails. As known, yellow snails are harmful organisms in agriculture (e.g, wet rice and vegetable cultivation,…). This is the first time that incorporation of *Carica papaya* leaves (CPLs) extracts into chitosan nanoparticles (CTS NPs) and their combined harmful anti-organisms activity has been reported in this work. Since, this eco-friendly method and a good product could be competitive and alternative to toxic the existing chemical products, which would be used for killing of yellow snails. Thus, the combined product of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract would be highly potential material to be used in biomedical and pesticides applications in the current time and in future.

2. MATERIALS AND METHODS

2.1. Materials

Carica papaya leaves were collected from papaya farm belongs to Residence 586 located in Can Tho City, Vietnam. Sodium tripolyphosphat (STPP, 99%) was purchased from India. Chitosan was bought from Vietnam's company. All solutions were prepared with deionized water (DI H_2O) from a MilliQ system.

2.2. Methods

2.2.1. Preparation of Carica papaya leaves extract

Carica papaya leaves were washed and dried in oven for 24 h at 70°C. The *Carica papaya* leaves (CPLs) powder obtained from dry leaves were broken by hands into small pieces and crushed in a ball mill with high speed for 15 min. Carica papaya leaves extract was conducted using ethanol and deion (DI) water in a ratio of 10:90, respectively. 10 g of the CPLs powder was placed in 1000 mL flask and the mixture of ethanol in DI water added in the ratio of 1:50. The mixture was filtered through filter paper (□□□□□□mm; hole size ~20-25 □m) using a funnel. After that, the mixture of *Carica papaya* leaves (CPLs) extract obtained through filtration was used for next steps.

2.2.2. Preparation of chitosan nanoparticles/Carica papa leaves extract

Chitosan nanoparticles (CTS NPs) loaded *Carica papaya* leaves (CPLs) extract were synthesized by a simple method using sodium tripolyphosphat (STPP) as a reducing agent at room temperature. In a typical synthesis, 2 mL of STPP (1 mg in 1 mL DI H_2O) was added to 10 mL of chitosan solution (1 mg/mL in acetic acid solution of 2%) and stirred for 10 min at room temperature. After that, 12 mL of *Carica papaya* leaves (CPLs) extract was also quickly added into the above solution and stirred for various reaction times (15 min, 30 min, 60 min, 90 min, and 120 min, respectively) at room temperature. The solution was then centrifuged (10000 rpm; 15 min) and washed with deionized water (DI water) to remove excess and then redisposed in DI water. The average particle size of the as-prepared chitosan nanoparticles (CTS NPs) combined with *Carica papaya* leaves (CPLs) extract is approximately 20-50 nm.

2.2.3. Characterization

For characterization of the chitosan nanoparticle loaded *Carica papaya* leaves extract, many examinations were conducted including the absorbance spectra of particle solutions examined by UV–vis spectrophotometry (UV-675; Shimadzu); the particle size and surface morphology

of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract investigated by transmission electron microscope (TEM) with a Philips Tecnai F20 G2 FEI-TEM microscope (accelerating voltage 200 kV). In addition, fourier transform infrared spectroscopy (FTIR) spectra of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract were found by using a Renishaw 2000 confocal Raman microscope system. The morphology of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract was performed by scanning electron microscope (SEM) with a JEOL JSM-6300F (SEMTech Solutions, Natick, Massachusetts, USA or Inspect S, FEI Ltd., Holland).

2.2.4. Preparation for the studying harmful anti-organisms activity of chitosan nanoparticles loaded Carica papaya leaves extract on yellow snails

Preparing for 4 trays containing 10 yellow snails for each tray. The solution is contained in each tray being deion water (DI H_2O) (5 L) (S1), deion water (DI H_2O) containing fish's food (5 g in 5 L DI H_2O) (S2), chitosan nanoparticles (CTS NPs) (500 mg in 5 L DI H_2O) (S3), and chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract (500 mg in 5 L DI H_2O) (S4), respectively. From the first feeding time after every 12 h, we will check the yellow snails' trays one time. If the yellow snail dies, its tail will turn up and float on the water's surface and be removed it from the tray. The observation was continuous conducted by replacing new water and the feeding (containing different solutions respective of (S1), (S2), (S3), and (S4)) into the yellow snails' trays until the yellow snails died completely.

3. RESULTS AND DISCUSSION

3.1. Characterization of the chitosan nanoparticles loaded Carica papaya leaves extract

As shown in Figure 1, the UV-vis spectra of chitosan nanoparticles (CTS NPs) loaded *Carica papaya* leaves (CPLs) extract exhibits with the maximum absorption peak at 672 nm, and appear two absorption peaks at 538 and 613 nm, respectively. Hence, it is demonstrated that *Carica papaya* leaves (CPLs) extract was successful combined in the chitosan nanoparticles' (CTS NPs') solution. The maximum absorption peaks of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extracts were measured in the range of ~538-672 nm, so the average particle size of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extracts can be predicted to be ~20-50 nm. As a result, the maximum absorption peak intensity of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract is the highest at 672 nm with reaction time of 30 min – see Figure 1(b). Thus, the optimal sample for the combination of chitosan nanoparticles (CTS NPs) with *Carica papaya* leaves (CPLs)

extracts will be conducted in 30 min at room temperature with volume amount of *Carica papaya* leaves (CPLs) extract being 12 mL.

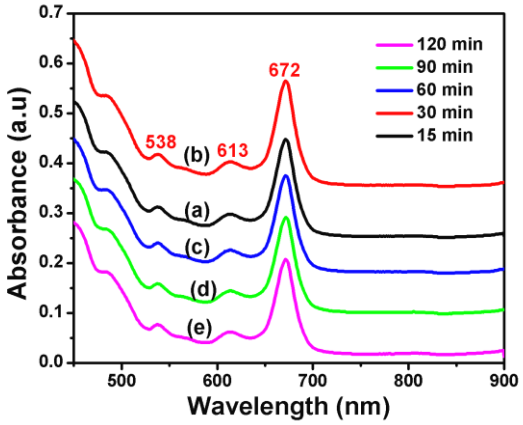

Figure 1: UV-vis spectra of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract at various reaction times: (a) 15 min, (b) 30 min, (c) 60 min, (d) 90 min, and (e) 120 min, respectively.

Scanning electron microscope (SEM) was used to observe the surface morphology of synthesized chitosan nanoparticles/*Carica papaya* leaves extract – see in Figure 2. Figure 2 shows that the SEM image of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract shows spherical shaped particles. The size of the particles is seen within 20-50 nm. The synthesized particles are in the form of dispersions.

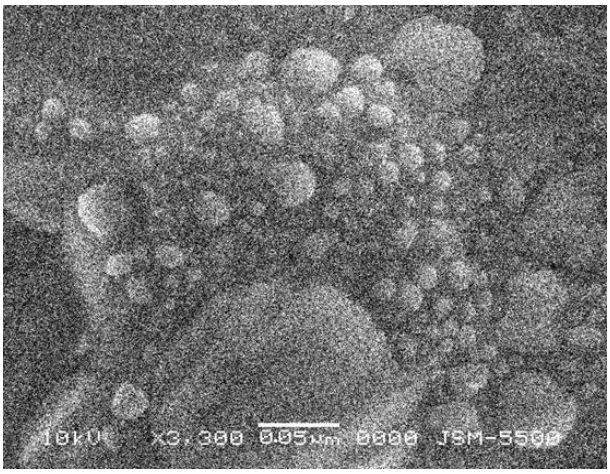

Figure 2: SEM images of chitosan nanoparticles/*Carica papaya* leaves extract with reaction time of 30 min at room temperature.

The surface morphology of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract has been observed by Transmission electron microscopy (TEM) – see Figure 3. The TEM images of the chitosan nanoparticles (CTS NPs)/Carica papaya leaves (CPLs) extract demonstrate that these nanoparticles are well-dispersed, uniform and spherical with the average particle size ~20-50 nm. There is no agglomeration of nanoparticles perhaps due to the presence of chitosan as a capping agent.

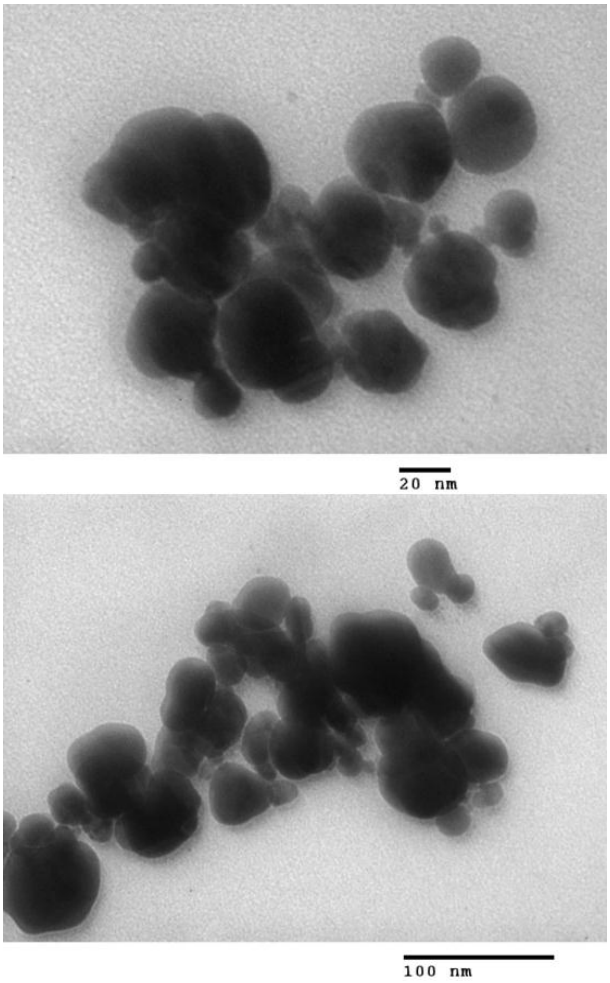

Figure 3: TEM images of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract with reaction time of 30 min at room temperature.

As shown in Figure 4(a), the FTIR spectrum of chitosan nanoparticles (CTS NPs) displays the presence of bands at ~3283 cm^{-1} (O-H stretching), C-H and C-N stretching at ~2910-2862 cm^{-1}, N-H bending at 1650-1563 cm^{-1}, N-H angular deformation in CO-NH plane at 1401-1650 cm^{-1} and C-O-C band stretching at 1063 cm^{-1}.[16] Furthermore, the peaks at 2356, 2109, and 1211 cm^{-1} demonstrate the C-C stretch in the carbohydrate structure.[17,18]

In the FTIR spectrum of chitosan nanocomposites (CTS NPs)/*Carica papaya* leaves (CPLs) extract – in Figure 4(b), the functional groups present in various biomolecules like carbohydrates, proteins, vitamins, and so on are illustrated by the peaks formed at 3368, 2910, 1650, 1563 and 1077 cm^{-1}, respectively.[19] The blending of *Carica papaya* leaves (CPLs) extract with chitosan nanocomposites (CTS NPs) resulted in intensity changes and the addition of new peaks in the FTIR spectra of the chitosan nanocomposites (CTS NPs)/*Carica papaya* leaves (CPLs) extract. The peak exhibited at 3368 cm^{-1} represents the characteristic N-H stretching of a primary amine. Notably, the peaks at 3368 cm^{-1} and 1077 cm^{-1} narrowed and elongated, which may express the addition of NH_2 group present in *Carica papaya* leaves (CPLs) – see in Figure 4(b).

As mentioned earlier, the *Carica papaya* leaves (CPLs) extracts are rich sources of antioxidants, anti-inflammatory, antimicrobial, and pain-relieving agents.[20] Hence, the successful blending confirmed by FTIR analysis indicates the chitosan nanoparticles (CTS NPs) loaded *Carica papaya* leaves (CPLs) extract.

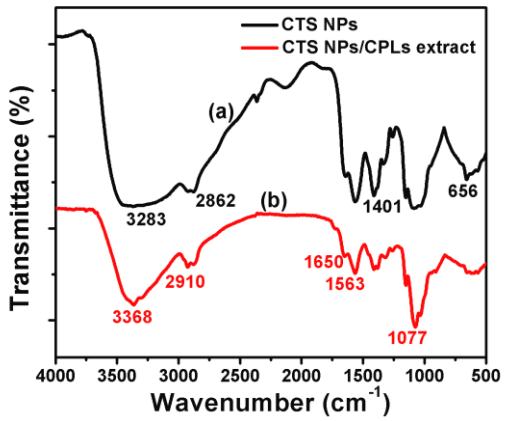

Figure 4. FTIR spectra of (a) chitosan nanoparticles (CTS NPs) and (b) chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract.

3.2. Harmful anti-organisms activity measurement of the chitosan nanoparticles/Carica papaya leaves extract for killing of yellow snails

As shown in Figure 5 and Table 1, the result of testing the harmful anti-organisms activity of deion water (DI H_2O) (S1), DI H_2O + fish's food (S2), DI H_2O + chitosan nanoparticles (CTS NPs) (S3), and DI H_2O + chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract (S4) for killing of yellow snails. Results showed that the chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract (S4) has killed yellow snails only after 24 h at

room temperature; while, the samples of (S3), (S2) and (S1) could kill yellow snails completely after 7 days, more 10 days, and 8 days, respectively for comparison. The observation showed that there has been a greater harmful anti-organisms efficiency (able to kill yellow snails only after 24 h of incubation) and higher of using the chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves extract solution for the killing of yellow snails as compared to those of (S1), (S2), and (S3) samples, respectively – see details in Table 1.

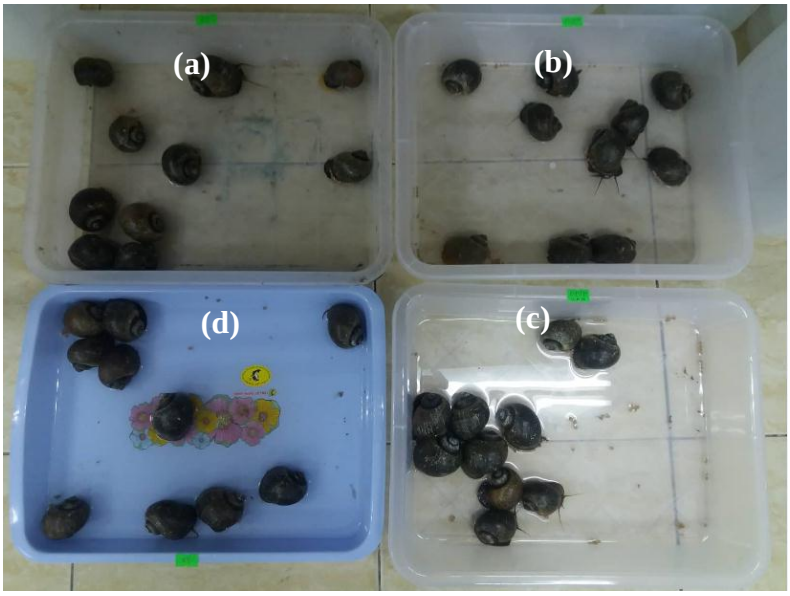

Figure 5: Images of yellow snails incubated in solutions of (a) deion water (DI H_2O) (S1); (b) DI H_2O + fish's food (S2); (c) DI H_2O + chitosan nanoparticles (CTS NPs) (S3); and (d) DI H_2O + chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract (S4), respectively.

Table 1: Amounts of yellow snails are killed during various times.

Day	(S1) sample	(S2) sample	(S3) sample	(S4) sample
0	0	0	0	0
1	0	0	0	10
2	0	0	0	-
3	0	0	2	-
4	0	0	0	-
5	2	0	3	-
6	4	0	3	-
7	1	0	2	-
8	3	0	-	-
9	-	0	-	-
10	-	0	-	-

4. CONCLUSIONS

A new and simple preparation of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract using sodium tripolyphosphat as a reducing agent for chitosan at room temperature have been successfully developed in this study. It proves to be an eco-friendly approach because its effectiveness and safety, and a cost effectiveness and an efficient route for the synthesis of chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract. The prepared CTS NPs/CPLs extract were determined their characterization and morphology by UV-vis, FTIR, SEM and TEM. Moreover, the obtained chitosan nanoparticles/*Carica papaya* leaves extract also showed their efficiently harmful anti-organisms activities for killing of yellow snails only after 24 h. It is demonstrated that chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract have sustainable antimicrobial, harmful anti-organism, antifungal activities and are safe in use. Therefore, the chitosan nanoparticles (CTS NPs)/*Carica papaya* leaves (CPLs) extract is a great and promising material to be used for biomedical and pesticides applications in the future.

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