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Chitosan Nanoparticles in Combination with *Houttuynia cordata* Leaves Extract Application in Preservation of Oranges

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Abstract*—Chitosan nanoparticles (CTS NPs) loaded Houttuynia cordata leaves (HCLs) extract possess highly antibacterial activity. The aim of the present study was to investigate in combination of chitosan nanoparticles (CTS NPs) and Houttuynia cordata leaves (HCLs) extract with reaction time of 30 min at room temperature. The prepared chitosan nanoparticles (CTS NPs)/Houttuynia cordata leaves (HCLs) extract have been characterized by UV-vis, FTIR, SEM and TEM. The obtained result showed these CTS NPs/HCLs extract have been gained with the average particle size of ~10-25 nm. Moreover, the synthesized CTS NPs/HCLs extract also showed efficient their antibacterial and antifungal activity on the preservation of Oranges. The result showed that the presence of a small powder amount (500 mg) of chitosan nanoparticles (CTS NPs)/Houttuynia cordata leaves (HCLs) extract in (2 L) water was enough to inhibit against bacterial and fungal on preservation of oranges due to the vitamin C content in the observed orange sample still remaining about 85.36% after 19 days of storage at room temperature as compared to that of original orange sample. Thus, it would be highly potential material to be used in biomedical, antimicrobial and antifungal applications in future.*

***Keywords*—** *Chitosan nanoparticles (CTS NPs), Houttuynia cordata leaves (HCLs) extract, Orange, biomaterial, antibacterial, antifungal.*

I. INTRODUCTION

In many last decades, with the advance of modern medicine and drug research, chemical synthesis has replaced plants as the primary source of medicinal agents in industrialized countries. However, in 1985, the World Health Organization estimated that about 80% of the world's population relied on traditional medicines including herb medicines for their primary health care needs [1]. 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 [2].

Biopolymers such as chitosan, gelatin are the most widely used polymers in drug delivery [3, 4]. As known, 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. Moreover, chitosan is an excellent carrier of other functional substances. It has been use for encapsulation of different compounds such as essential oils [5], RNA [6], antibiotics [7], drugs for cancer therapy [8], nutraceuticals [9] and vitamins [10], among others, potentializing the combined properties of the encapsulating agent and chitosan. Thus, chitosan is widely used in various pharmaceutical and medical applications because it is cheap, biodegradable and demonstrates good biocompatibility.

Houttuynia cordata Thunb. (Saururaceae) is widely distributed in eastern Asia, including China, Korea, Japan, and Vietnam, and is used in folk medicine for diuresis and detoxification. It contains many flavonoids (quercitrin, isoquercitrin, rutin, etc.), alkaloids (aristolactam B, norcepharadione B, splendidine, etc.), and volatile components of essential oils (methyl-*n*-nonyl ketone, lauraldehyde, β-myrcene, etc.) [11-13]. Therefore, the extracts and components of *Houttuynia cordata* have been demonstrated to exhibit antioxidative, antiviral, antibacterial, antihypertensive and anti-inflammatory effects [14-18]. However, these extracts have not yet been reported to apply in preservation of Oranges.

Herein, the incorporation of *Houttuynia cordata* leaves (HCLs) extracts into chitosan nanoparticles (CTS NPs) 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 *Houttuynia cordata* leaves (HCLs) extract for applying in the preservation of Oranges. This is the first time that incorporation of *Houttuynia cordata* leaves (HCLs) extracts into chitosan nanoparticles (CTS NPs) and studied their antibacterial activity on the preservation of Orange 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 the preservation of agricultural products. Thus, the combined product of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract would be highly potential material to be used in biomedical, cosmetic and bio-preservative applications in the current time and in future.

II. MATERIALS AND METHODS

2.1. Materials

Houttuynia cordata leaves (HCLs) and oranges were collected and chosen from farm belongs to Residence 586 located in Can Tho City, Vietnam. Sodium tripolyphosphat (STPP, 99%) was purchased from HiMedia, Mumbai, 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 Houttuynia cordata leaves extract

Houttuynia cordata leaves were washed and dried in oven for 18 h at 50°C. The *Houttuynia cordata* leaves (HCLs) powder obtained from dry leaves were broken by hands into small pieces and crushed in a ball mill with high speed for 15 min. *Houttuynia cordata* leaves extract was conducted using ethanol and deion (DI) water in a ratio of 10:90, respectively. 10 g of the HCLs 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 (ϕ=110 mm; hole size ~20-25 μm) using a funnel. After that, the mixture of *Houttuynia cordata* leaves (HCLs) extract obtained through filtration was used for next steps.

2.2.2. Preparation of chitosan nanoparticles/Houttuynia cordata leaves extract

Chitosan nanoparticles (CTS NPs) loaded *Houttuynia cordata* leaves (HCLs) extract were synthesized by a simple method using sodium tripolyphosphat (STPP) as a reducing agent for chitosan 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, various amounts (2 mL; 6 mL; 8 mL; 9 mL and 12 mL, respectively) of *Houttuynia cordata* leaves (HCLs) extract were also quickly added into the above solution and stirred for 30 min 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) loaded *Houttuynia cordata* leaves (HCLs) extract is approximately 10-25 nm.

2.2.3. Characterization

For characterization of the chitosan nanoparticle loaded *Houttuynia cordata* 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)/*Houttuynia cordata* leaves (HCLs) 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)/*Houttuynia cordata* leaves (HCLs) extract were found by using a Renishaw 2000 confocal Raman microscope system. The morphology of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) 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 testing the antibacterial and antifungal activity of chitosan nanoparticles combined with Houttuynia cordata leaves extract on preservation of oranges

Firstly, choose fresh oranges (just picked from the garden). These oranges should be relatively equal in the size, the petiole, the green shell, and the shade. It has to make sure that the oranges are not damaged by other pests before it is used.

Secondly, the chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract solution is prepared for embedding oranges. Typical, 2 L of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract solution (250 mg CTS NPs/HCLs extract in 1000 mL DI H_2O) is put in 2 beakers (1000 mL). Oranges are dipped in the chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract solution for 1-3 min so that oranges can be covered by CTS NPs)/HCLs extract solution on their surface completely. After that, oranges are preserved and stored in cartons for investigation with various storage times of 3 days, 9 days, 14 days, and 19 days, respectively. All orange samples are observed for preservation without presence (S1 sample) and with presence of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract solution (S2 sample), which are repeated 3 times for each survey factor.

Finally, the observation and evaluation for the efficency of oranges preservation using chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract solution is determined through parameters such as color, petiole dropping, pH, vitamin C content, and mass loss.

III. Results and Discussion

3.1. Characterization of the chitosan nanoparticles combined with Houttuynia cordata leaves extract

As shown in Figure 1, the UV-vis spectra of chitosan nanoparticles (CTS NPs) loaded *Houttuynia cordata* leaves (HCLs) extract exhibits with the maximum absorption peak at 670 nm, and appear two absorption peaks at 538 and 613 nm, respectively. Hence, it is demonstrated that *Houttuynia cordata* leaves (HCLs) extract was successful combined in the chitosan nanoparticles' (CTS NPs') solution.

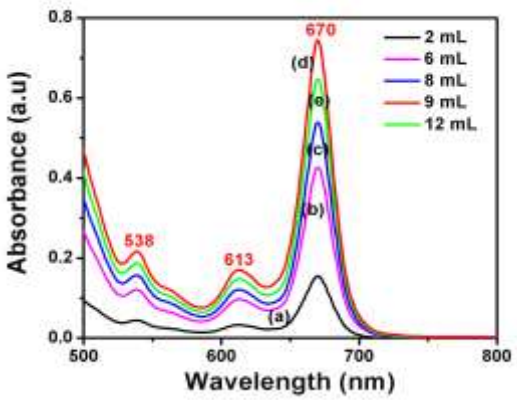

Fig. 1. UV-vis spectra of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract with reaction time of 30 min at various volume amounts of *Houttuynia cordata* leaves (HCLs) extract: (a) 2 mL, (b) 6 mL, (c) 8 mL, (d) 9 mL, and (e) 12 mL, respectively.

The maximum absorption peaks of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extracts were measured in the range of ~538-670 nm, so the average particle size of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract can be predicted to be ~10-25 nm. As a result, the maximum absorption peak intensity of chitosan

nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract is the highest at 670 nm with volume of *Houttuynia cordata* leaves (HCLs) extract being 9 mL – see Figure 1(d). When the volume amount of *Houttuynia cordata* leaves (HCLs) extract is increased, leading to the maximum absorption peak at 670 nm gradually reduced. This may be due to the HCLs extract's solution volume increased, which leads to agglomeration in the solution of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract. Thus, the optimal sample for the combination of chitosan nanoparticles (CTS NPs) with *Houttuynia cordata* leaves (HCLs) extract will be conducted in 30 min at room temperature with volume amount of *Houttuynia cordata* leaves (HCLs) extract being 9 mL.

As shown in Figure 2, the surface morphology of chitosan nanocomposites (CTS NPs) combined with *Houttuynia cordata* leaves (HCLs) extract has been observed by Transmission electron microscopy (TEM). The TEM images of the chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract demonstrate that these nanoparticles are well-dispersed, uniform and spherical with the average particle size of about 10-25 nm. There is no agglomeration of nanoparticles perhaps due to the presence of chitosan as a capping agent covered completely *Houttuynia cordata* leaves (HCLs) extract in their combination.

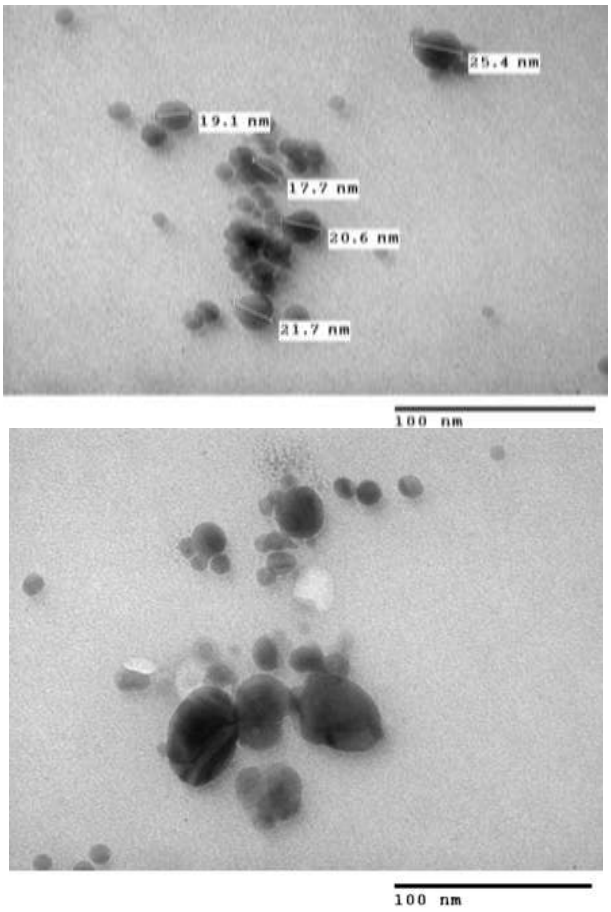

Fig. 2. TEM images of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract with reaction time of 30 min at room temperature.

Scanning electron microscope (SEM) was used to observe the surface morphology of synthesized chitosan nanocomposites (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract – see in Figure 3. Figure 3 shows that the SEM image of chitosan nanocomposites (CTS NPs) combined with *Houttuynia cordata* leaves (HCLs) extract exhibits spherical shaped particles. The size of the particles is seen within 10-25 nm. The synthesized particles are in the form of dispersions and deagglomerations.

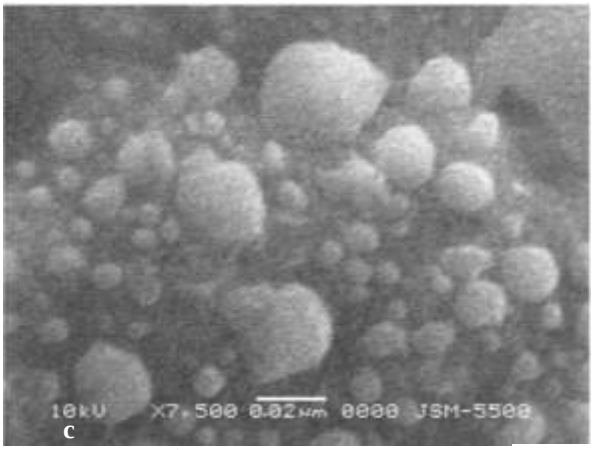

Fig. 3. SEM images of chitosan nanoparticles combined with *Houttuynia cordata* leaves extract with reaction time of 30 min at room temperature.

Figure 4 shows the FTIR spectra of chitosan nanoparticles (CTS NPs) and their ingredients. As shown in Figure 4(a), the FT-IR spectrum of chitosan nanoparticles (CTS NPs) displays the presence of bands at 3323 cm^{-1} (O-H stretching), C-H and C-N stretching at ~2922-2870 cm^{-1}, N-H bending at 1646-1562 cm^{-1}, N-H angular deformation in CO-NH plane at 1409-1562 cm^{-1} and C-O-C band stretching at 1084 cm^{-1}. The long-chain bond is seen in 655 cm^{-1} [19]. In the FTIR spectrum of chitosan nanocomposites (CTS NPs)/*Houttuynia cordata* leaves (HCLs) 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 3362, 3290, 2922, 2870, 1646, 1562 and 1074 cm^{-1}, respectively [20].

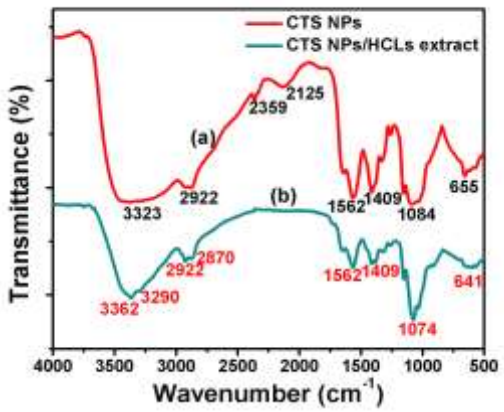

Fig. 4. FTIR spectra of (a) chitosan nanoparticles (CTS NPs) and (b) chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract.

The blending of *Houttuynia cordata* leaves (HCLs) 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)/*Houttuynia cordata*

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leaves (HCLs) extract. The addition peak exhibited at 3362 cm^{-1} represents the characteristic N-H stretching of a primary amine. Notably, the peaks at 3362 cm^{-1} and 1074 cm^{-1} narrowed and elongated, which may express the addition of NH_2 group present in *Houttuynia cordata* leaves (HCLs) extract – see in Figure 4(b).

3.2. Antibacterial and antifungal activity investigation of the chitosan nanoparticles/Houttuynia cordata leaves extract for the preservation of Oranges

As shown in Figure 5 and Table I, the result of testing the antibacterial and antifungal activity of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract applied in the preservation of oranges. Orange samples were preserved by using without presence (S1) and with presence of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract (S2) solution.

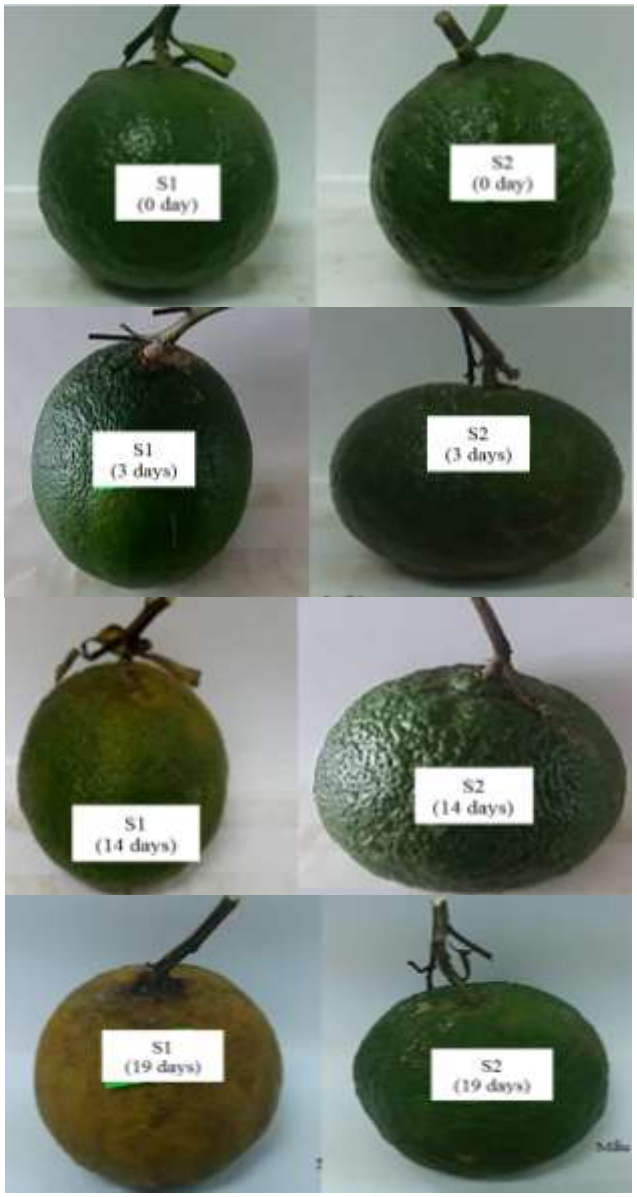

Fig. 5. Images of orange samples with different storage times at room temperature without presence (S1 sample) and with presence of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract (S2 sample) for preservation, respectively.

Results showed that the chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract (S2) can keep orange still fresh, delicious and glossy after 19 days of storage at room temperature. While, the orange sample (S1) without presence of chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract, which is damaged after 19 days for comparison. The observation showed that chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract used for preserving oranges that have been brought extremely high efficiency (oranges are still fresh, deliciuos and glossy after 19 days of storage at room temperature with vitamin C content remaining ~85.36% as compared to original orange sample) – see details in Figure 5 and Table I.

TABLE I. Results of the investigation and evaluation for oranges' preservation using chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract solution

Time (day)		Color shell	Petiole dropping	pH	Vitamin C content (mg/mL)	Mass loss (g)
0 day (Orange picking day)		Fresh green, relatively glossy	Intact and strong	2.8	0.3326	-
3 days	S1	Green shell, slightly wrinkled	Intact petiole	2.9	0.3424	11.98
	S2	Green shell, still glossy	Intact petiole	2.675	0.4125	10.04
9 days	S1	Several yellow spots, wrinkled shell	Intact petiole, weak grip	2.8	0.362	26.26
	S2	Still green, relatively glossy	Intact petiole, relatively good adhesion	2.45	0.427	28.33
14 days	S1	Very wrinkled shell, yellow spots	Intact petiole but weak	3.4	0.2563	50.43
	S2	Still green and glossy shell	Intact petiole	3.2	0.2898	31.87
19 days	S1	Yellow shell, soft, corrupt	Weak, near falling out	3.5	0.2219	42.57
	S2	Still green shell	Intact petiole	3.3	0.2839	41.56

IV. CONCLUSION

A new, simple and green method of chitosan nanoparticles (CTS NPs) combined with *Houttuynia cordata* leaves (HCLs) 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)/*Houttuynia cordata* leaves (HCLs) extract. The prepared CTS NPs/HCLs extract were determined their characterization and morphology by UV-vis, FTIR, TEM and SEM. Moreover, the obtained chitosan

nanoparticles/*Houttuynia cordata* leaves extract also showed their efficiently antimicrobial and antifungal activities for preservation of Oranges. These oranges are still good and glossy after 19 days of storage. It is demonstrated that CTS NPs/HCLs extract have sustainable antimicrobial activities and are safe in use. Therefore, the chitosan nanoparticles (CTS NPs)/*Houttuynia cordata* leaves (HCLs) extract has great and promising potential material to be used for preservation of agricultural and aquatic products, pharmaceuticals, and cosmetics in the future.

REFERENCES

[1] N. R. Farnsworth, O. Akerele, A. S. Bingel, D. D. Soejarto and Z. Guo, Bulletin of the World Health Organization 1985, 63, 965-981.
[2] G. Orive, R. M. Hernández, A. R. g. Gascón, A. Domínguez-Gil and J. L. Pedraz, Current Opinion in Biotechnology 2003, 14, 659-664.
[3] H. Tan, R. Ma, C. Lin, Z. Liu and T. Tang, International Journal of Molecular Sciences 2013, 14, 1854.
[4] S. Shankar, X. Teng, G. Li and J.-W. Rhim, Food Hydrocolloids 2015, 45, 264-271.
[5] M. E. Sotelo-Boyás, Z. N. Correa-Pacheco, S. Bautista-Baños and M. L. Corona-Rangel, LWT 2017, 77, 15-20.
[6] H. Ragelle, R. Riva, G. Vandermeulen, B. Naeye, V. Pourcelle, C. S. Le Duff, C. D'Haese, B. Nysten, K. Braeckmans, S. C. De Smedt, C. Jérôme and V. Préat, Journal of Controlled Release 2014, 176, 54-63.
[7] K. G. Desai, 2016, 33, 107-158.
[8] A. Khdair, I. Hamad, H. Alkhatib, Y. Bustanji, M. Mohammad, R. Tayem and K. Aiedeh, European Journal of Pharmaceutical Sciences 2016, 93, 38-44.
[9] H. Zhang, J. Jung and Y. Zhao, Carbohydrate Polymers 2016, 137, 82-91.
[10] D. de Britto, M. R. de Moura, F. A. Aouada, L. H. C. Mattoso and O. B. G. Assis, Food Hydrocolloids 2012, 27, 487-493.
[11] G. Z. Li, O. H. Chai, M. S. Lee, E.-H. Han, H. T. Kim and C. H. Song, Biological and Pharmaceutical Bulletin 2005, 28, 1864-1868.
[12] K. Hayashi, M. Kamiya and T. Hayashi, Planta Med 1995, 61, 237-241.
[13] L.-C. Chiang, J.-S. Chang, C.-C. Chen, L.-T. Ng and C.-C. Lin, The American Journal of Chinese Medicine 2003, 31, 355-362.
[14] J.-S. Chang, L.-C. Chiang, C.-C. Chen, L.-T. Liu, K.-C. Wang and C.-C. Lin, The American Journal of Chinese Medicine 2001, 29, 303-312.
[15] L.-T. Ng, F.-L. Yen, C.-W. Liao and C.-C. Lin, The American Journal of Chinese Medicine 2007, 35, 465-475.
[16] Y. H. Chen, Liu, J., Chen, C., Chao, P., & Chang, T., Journal of nutritional science and vitaminology 2003, 49, 327-333.
[17] R. Bauer, A. Pröbstle, H. Lotter, W. Wagner-Redecker and U. Matthiesen, Phytomedicine 1996, 2, 305-308.
[18] S.-K. Kim, S. Y. Ryu, J. No, S. U. Choi and Y. S. Kim, Archives of Pharmacal Research 2001, 24, 518-521.
[19] G. Saraswathy, S. Pal, C. Rose and T. P. Sastry, Bulletin of Materials Science 2001, 24, 415-420.
[20] P. C. Nagajyothi and K. D. Lee, Journal of Nanomaterials 2011, 2011.