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Evaluation of the antibacterial activity of encapsulated d-limonene inβ-cyclodextrin-based nanosponge

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ABSTRACT

Following the increasing resistance of bacteria to antibiotics and increasing public awareness of the relationship between food and human health, several studies have been conducted to find potential antimicrobial agents. D-limonene(L) is a liquid and a colorless bioactive compound of the terpene family that has high antioxidant and antimicrobial activity. Due to its low solubility, high volatility, and sensitivity to light, its applications in the food and pharmaceutical industries are limited. In this study, L was incorporated in β-cyclodextrin nanosponges (CD-NS) to overcome these limitations. For the preparation of L-NS, different molar ratios of β-CD and Diphenyl carbonate (DPC) as cross-linker (1:4, 1:6, and 1:8 of CD:DPC) and different ratios (w/w) of L: NS (1:2, 1:4, and 1:6) were prepared. The results showed the solubility of L increased after encapsulation in the nanosponges. Encapsulated L showed higher antibacterial activity compared to free L and minimum inhibitory concentration (MIC) and minimum bactericidal concentration of L decreased 324 and 648 times after encapsulation. Our results propose that CD-NS is a suitable carrier for hydrophobic and sensitive compounds and L-NS can be used as a potential preservative with enhanced antibacterial activity in food applications. It is important to note that the results of this study were obtained in vitro and further studies related to their toxicity (in vivo) are needed for confirmation of their application in the era of nutrition and genomics.

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1. Introduction

With the increase in the resistance of bacteria to antibiotics and the increase in people's awareness of the relationship between food and human health, many studies have been conducted to find potential antibacterial agents. For this reason, natural compounds, especially essential oils and extracts obtained from plants, have recently received special attention. The obtained results have shown that antimicrobial compounds isolated from natural compounds can increase the efficiency of antibiotics, improve their mechanisms and prevent the adaptation of microorganisms to natural conditions. There are many low-soluble and insoluble bioactive and nutritious compounds that are essential for human health. Among these compounds, we can mention terpenes that act separately or synergistically with other compounds in order to improve the efficiency of bioactive compounds [1]. Dilimonene is a liquid and colorless bioactive compound of the monoterpene family [2, 1]. This compound is relatively stable in the dark and at room temperature. But it is unstable in the presence of light, air, moisture and high temperature [3]. D-limonene is easily subjected to oxidative decomposition, which eventually leads to the loss of its aroma and flavor and the formation of epoxide, ketone and alcohol [4]. Also, this substance has hydrophobicity[1] This characteristic causes its poor solubility in liquid phases. Therefore, today researchers are looking to use some new methods to increase the solubility and protect this volatile compound during the process. One of the important methods that can be used in this field is the use of nano-encapsulation method [5]. Nanoencapsulation with hydrophilic carriers is one of the ways to increase solubility and bioavailability as well as protect these compounds. Nanoencapsulation will protect unstable compounds against inappropriate conditions during processing, storage and transportation and increase their quality, stability and bioavailability in the body. Nanosponges can potentially be widely used in the food industry. Cyclodextrin nanosponges are suitable carriers for sparingly soluble and insoluble compounds due to having hydrophobic cavities and a hydrophilic shell as well as high loading efficiency. On the other hand, since nanosponges are solid, it is easier to use them in food and pharmaceutical products. The easy method of producing nanosponges, as well as the ease of production on an industrial scale, is another advantage of using nanosponges as nanocarriers for sparingly soluble and insoluble compounds [6]. In recent years, extensive research has been done using nanoencapsulation to increase the antimicrobial properties of d-limonene. In a research The combined effect of two monoterpene dilimonene and carvacrol on Leishmania disease[2] They have reviewed. The obtained results showed that the combined effect of these two can be a suitable option for the nanotherapy of this disease[7]. In another study, the antibacterial effect of d-limonene when complexed with beta-cyclodextrin and when it is combined with an antibiotic was investigated and compared.Based on the results obtained, the antimicrobial effect of d-limonene when complexed with beta-cyclodextrin did not show a significant increase against gram-positive and gram-negative strains, and when it was combined with norfloxacin and imipenem, the effects were opposite or insignificant against bacterial strains. shows. It should be noted that as long as d-limonene was used with gentamicin, the effect was synergistic[3] showed on Bacillus cereus and E. coli bacteria[1]. Another study showed that gum arabic can significantly increase the physical stability of dilimonene and protect it from oxidation even at high temperature (55 degrees Celsius). On the other hand, the antimicrobial property and the positive effect of dilimonene on reducing and inhibiting the growth of microorganisms were mentioned [4].Also, several studies have been conducted on encapsulating natural compounds sensitive to light, temperature and low solubility with low bioavailability in nanosponges. In a research, by encapsulating flutamide in beta-cyclodextrin-based nanosponges, researchers succeeded in improving its solubility. Flutamide is an anticancer drug in the treatment of the prostate,

1. Hydrophobic

2. leishmania

3. synergic

which has low oral bioavailability[8]. It is also stated in a report that by incorporating erlotnib in cyclodextrin nanosponges, its solubility and stability increased. Erlotnib is an anticancer drug that has poor bioavailability due to its low solubility and instability in the stomach and intestines.[9]. In a research, by encapsulating meloxicam in cyclodextrin nanosponges, its solubility, stability and controlled release increased.[10].

During a research, by encapsulating cinnamon essential oil in cyclodextrin nanosponges and evaluating their antimicrobial activity against food pathogens, they stated that the produced complex not only protects the essential oil, but also improves the antibacterial performance of the essential oil, and this introduced compounds as encapsulating agents for active food packaging applications[11]. Also, by encapsulating ferulic acid in beta-cyclodextrin nanosponge and using it in a pomegranate juice drink and investigating its antimicrobial effect, as well as monitoring color changes and the amount of antioxidants in pomegranate juice in a certain period of time, they concluded that the antimicrobial property of ferulic acid after The encapsulation increases and also preserves the antioxidant compounds of pomegranate juice and preserves its color. According to the obtained results, they acknowledge that ferulic acid encapsulated in beta-cyclodextrin nanosponge can potentially be used in anthocyanin-containing fruit juices to improve their stability during storage.[12]. In another study, by encapsulating coriander essential oil in beta-cyclodextrin nanosponge, in addition to creating a sustained controlled release system, the researchers improved its antimicrobial activity against food-borne pathogens and stated that this compound is a potential new strategy to overcome Due to the poor packaging of current food, it can be presented in terms of antimicrobial properties[13]. Since so far no research has been done to nanoencapsulate dilimonene in beta-cyclodextrin nanosponge to increase its stability and solubility, therefore, in this research, we have decided to produce beta-cyclodextrin nanosponges containing dilimonene, which is a bioactive compound, and evaluate the changes in solubility, encapsulation efficiency. To investigate the antibacterial properties by the diffusion method from the well, to determine the minimum concentration of growth inhibition and the minimum concentration of its lethality. It should be noted that food quality and safety control plays a vital role in human health and is always an essential element in proper nutrition, parallel to which, accurate and appropriate safety control in the production process to consumption is also important. Food and everything that is used in food production can be useful for humans and satisfy their needs if it is free from any kind of danger, otherwise it will cause irreparable losses to the individual and society from various aspects. slow Since the standards and laws regarding beta-cyclodextrin nanosponges have not been established by the Iranian Standards Organization and also the American Food and Drug Administration (FDA) and due to the fact that during the complex synthesis process, the particles may become nanosized or have toxicity. Therefore, their use in food formulation requires toxic tests such as MTT, checking their possible apoptosis and necrosis rate on different cell lines, and also using animal models with the aim of being free of toxic risks and safe. .

2- Materials and methods

2-1- Chemicals

Beta-cyclodextrin and diphenyl carbonate from Sigma-Aldrich[4] United States of America and dilimonene essential oil from Merck, Germany, and tryptone soy broth (TSB)[5]Muller Hinton AgarMHA)[6] and Muller Hinton BrathMHB)[7] From Kiolub[8] Canada was purchased. Bacterial strains of Escherichia coli (ATCC: 35150), Shigadysentery (ATCC: 12022, Staphylococcus aureus) 25923: (ATCC, Enterococcus (ATCC: 29212) were obtained from Pasteur Institute of Iran.

2-2-Production of betadextrin nanosponge using melt method

Melt method was used to produce beta-cyclodextrin nanosponge**.** For this purpose, beta-cyclodextrin and diphenylcarbonate were used

4. Sigma-Aldrich
5. Tryptone Soy Broth
6. Mueller Hinton Agar
7. Muller Hinton Broth
8. QUELAB

as cross-linking agents with different molar ratios of nanosponge: binder 1:4, 1:6 and 1:8. First, diphenyl carbonate was poured and melted in a beaker as a cross-linking agent, then a specific amount of beta-cyclodextrin was slowly added to it and stirred for 5 hours at a temperature of 90-100 degrees Celsius by a magnetic stirrer. After the reaction was completed, a white substance was obtained, which was washed with water (removal of cyclodextrin) and ethanol (removal of diphenyl carbonate) using filter paper to remove unreacted substances. Then the samples were dried and stored in an oven at 45 degrees Celsius [14].

2-3- Encapsulating dilimonendrano-sponge beta-cyclodextrin

First, beta-cyclodextrin nanosponge was dissolved in 10 cc of water and placed on a magnetic stirrer. Then, different weight ratios of dilimonene: nanosponge 1:2, 1:4 and 1:6 were added to the solution and ultrasonicated for 10 minutes to reduce the size of the particles. Then the desired solution was stirred for 24 hours at a temperature of 25 degrees Celsius on a magnetic stirrer. In order to prevent light radiation, the solution was covered with aluminum foil. After spending the desired time, the suspension was centrifuged for 10 minutes at 2000 rpm in order to separate the loaded and unloaded dilimonene in the nanosponge.Mpw nstrument - Poland) became. Then motherhood[9] at -40°C in a freeze dryer[10] It dried [15].

2-3-1- Encapsulation efficiency

10 mg of the sample was dispersed in 10 ml of ethanol. then for 10 minutes in a centrifuge (Mpw nstrument - Poland) was placed at 2000 rpm, after which the absorbance of the supernatant was read using a UV spectrophotometer at a wavelength of 231 nm. The loading capacity of nanoencapsulated dilimonene in nanosponge was calculated using equation 1 and 2, respectively [15].

1)

Loading rate = weight of d-limonene loaded in nano sponge - loading rate/Total weight of nano sponge - total weight of d-limonene ×100

2) essential oil= The weight of the/loaded in the nanosponge ×100

2-3-2- Solubility

For this purpose, 10 mg of the complex was dispersed in 10 ml of distilled water and stirred for 24 hours at room temperature. Then the samples were centrifuged at 2000 rpm for 10 minutes.Mpw nstrument - Poland) were placed, in the next step, the amount of absorption of the surfactant was read using a UV spectrophotometer at a wavelength of 231 nm [5].

2-4- Evaluation of antimicrobial properties

2-4-1- Preparation of standard bacteria

First, the tested bacteria were removed from the freezer at -80 degrees Celsius and transferred to the TSB environment and kept in a greenhouse for 18-24 hours. In the next step, the bacteria were transferred from the TSB medium to the MHA medium and were cultured linearly and kept in a greenhouse at 37°C for 24 hours. Plates containing bacteria were kept at 4 degrees Celsius for use during different experiments.

2-4-2- Diffusion method from well in agar

In order to measure the antimicrobial property of dilimonen alone and dilimonene encapsulated in beta-cyclodextrin nanosponge, diffusion method from well in agar was used. For this reason, the tested bacteria are diluted with half McFarland[11] by sterile swap on the culture mediumMHA were cultivated In each plate, five wells with a diameter of 6 mm using a crack borer[12] Created. In the next step, concentrations of 1.56, 3.12, 6.25, and 12.5 mg/ml of dilimonen alone and dilimonene encapsulated in nanosponge were prepared. Then, 100 microliters of each dilution was poured into the well of each bacterium and the plates were kept in a greenhouse at 37°C for 24 hours. After a maximum of 24 hours, the diameter of the halo of non-growth of bacteria was measured with a caliper with an accuracy of 0.01 mm. In this test, 100 microliters of the

9. supernatant
10. Freez dryer
11. 0.5 McFarland (108 CFU/ml)
12.Cork borer

antibiotic meropenem (with a dilution of 16 µg/ml) and penicillin (with a dilution of 32 µg/ml) were used for gram-negative and gram-positive bacteria, respectively, as a positive control in the middle well. All the tests related to the evaluation of the halo diameter of the non-growth of bacteria for each sample and each bacteria were repeated three times[16].

2-4-3- Determining the minimum inhibition concentration[13] (MIC) and minimum bacterial growth lethal concentration (MBC)[14]

The minimum amount of antimicrobial substance that inhibits the visible growth of microorganism is known as MIC. In order to determine the minimum growth inhibitory concentration of dilimonen alone and dilimonene encapsulated in nanosponge, the microdilution broth method was used. To perform this test, first 100 microliters of MHB culture medium was added to each well of the 96-well plate. Then, in the first column of each row, 100 microliters of BetterTib was added at a concentration of 420 (v/v 50) and 1.3 mg/ml for dilimonen and encapsulated dilimonen. Then different two-two dilutions while mixing the samples with MHB culture medium until reaching the concentrations of (50-0.097 v/v) 420-0.82 and 1.3-0.0025 mg/ml of Dilimonen and Dilimonen encapsulated by BetterTib in Microplates were prepared. In the next step, 5 microliters of the suspension of each bacteria with a dilution of 10 CFU/ml[7] (The dilution equivalent of CFU/ml $10^5$5x) was added to each of the wells containing MHB and dilimonen alone or encapsulated dilimonene. Then the microplates were incubated at 37°C for 24 hours. Optical density[15] (OD) of microplates before and after incubation was determined at a wavelength of 630 nm in a Microplate Reader (Biotek - USA). Comparing the amount of turbidity measured in both stages was determined as the minimum concentration of bacterial growth inhibition**.** In this experiment, culture medium was used for negative control and culture medium and bacteria were used for growth control. For the positive control, the nanosponge alone was used. After determining the MIC, from each of the wells of the 96-house plate that did not show the appearance of turbidity caused by the growth of bacteria, with a loop[16] The sterile sample was removed and cultured on the surface of MHA culture medium and at temperature **37** degrees Celsius for 24 hours. The minimum concentration of dilimonen alone and nano-encapsulated dilimonene that inhibited the growth of 99.9% of bacteria on the plate was reported as MBC. All experiments related to MBC determination were done in three replicates for each sample and each bacterium [16].

2-4-4- Statistical analysis

In this research, the data were reported as mean ± standard deviation and after collection, they were entered into SPSS 20 software. To determine a significant difference, paired T.test (for parametric variables) and Wilcoxon (for non-parametric variables) were used to compare parameters before and after encapsulation. It should be noted that in this study, significance was considered at the $P \leq 0.05$ level.

3- Results

3-1- Encapsulation efficiency, loading rate and solubility rate

In this research, different molar ratios of diphenyl carbonate to nanosponge (1:4, 1:6, 1:8) and different weight ratios of dilimonene to nanosponge (1:2, 1:4, 1:6) were used to obtain the best encapsulation ratio of dilimonene. used. As can be seen in table (1-3), the solubility of dilimonene in water after encapsulation in nanosponge has increased in all proportions due to the trapping of dilimonene in the hydrophobic cavities of the nanosponge. Of course, the solubility decreased with increasing the ratio of dilimonene to nanosponge (from 1:2 to 1:6), which is probably due to the saturation solubility of dilimonene in nanosponge at higher ratios. Also, connectors can form complex nanochannels and prevent the encapsulation of dilimonene. It should be noted that the solubility of free dilimonene is 7.5 mg, which increased 154 times after encapsulation, which shows that dilimonene is well encapsulated in the nanosponge. It should be noted that other

13. Minimum Inhibitory Concentration
14. Minimum Bactericidal Concentration
15. Optical density
16. Loop

researchers have obtained results of increasing the solubility of poorly soluble compounds in nanosponges. The highest amount of loading and encapsulation efficiency was observed in the ratio of 1:2. Probably, as mentioned in the case of solubility, increasing the ratio of binder to nanosponge has caused a decrease in encapsulation efficiency and loading efficiency. The results related to loading rate and encapsulation efficiency are given in table (1-3). Based on the results obtained from the solubility, loading and encapsulation efficiency, a molar ratio of 1:4 (nano sponge to binder) and a weight ratio of 1:2 (dlimonene to nanosponge) was selected as the optimal sample for further tests. was used [17-20 ،15 ،9]. According to some reports, researchers succeeded in improving their solubility by encapsulating bioactive compounds such as flutamide, cinnamon, and erlotinib in beta-cyclodextrin nanosponge.[17, 9 and 8].

Table 1 Encapsulation efficiency, Loading capacity and solubilityof limonene loaded nanosponge(L-NS).

Proportion of CD[a]:DPC[b] (molar ratio)	Proportion of L[c]:NS[d] (weight ratio)	Encapsulation efficiency of L (%)	Loading capacity of L (%)	Solubility of L-NS
	1:2	52.95 ± 1.16	26.4 ± 0.10	1161.60
1:4	1:4	15.65 ± 0.46	3.9 ± 0.04	362.58
	1:6	11.36 ± 0.74	1.8 ± 0.06	312.73
	1:2	10.74 ± 0.35	5.37 ± 0.08	217.12
1:6	1:4	12.24 ± 0.44	3.06 ± 0.05	254.96
	1:6	8.96 ± 0.21	1.4 ± 0.03	915.46
	1:2	13.12 ± 0.80	6.56 ± 0.07	381.26
1:8	1:4	11.46 ± 0. 65	2.8 ± 0.05	366.08
	1:6	8.4 ± 0.30	1.3 ± 0.03	141.43

β-cyclodextrin (a), Diphenyl carbonate (b), limonene(c), nanosponge(d)

3-2- Diffusion from the well in agar[17]

The results of diffusion method from the well in agar for dilimonene in free and nanoencapsulated form are presented in Table 2. The halo diameter of dilimonen lack of growth in concentrations of 1.56, 3.12, 6.25, 12.5milligrams per milliliterrespectively for bacteria *Escherichia coli*0, 10/5, 14, 15/5 mm, for *Shigadysentery* 0, 10, 13 and 15 mm, for *Enterococcus*0, 0, 12.5 and 14 mm and for A*Staphylococcus aureus* 0, 0, 12 and 13 mm. Also, the inhibition diameter of dilimonene loaded in nanosponge at concentrations of 1.56, 3.12, 6.25, and 12.5 milligrams per milliliter respectively for bacteria*Escherichia coli* 5.11, 13, 5.19 and 5.20 mm, for*Shigadysentery*17, 19, 5.23 and 5.26 ml, for*Enterococcus* 12/5, 12/5, 16/5 ml and for*Staphylococcus aureus* It was 12.5, 17.5, 19 and 21.5 mm. Based on the obtained results, dilimonene in free and encapsulated form prevents the growth of the mentioned Gram-positive and Gram-negative strains, and a more significant effect was observed in encapsulated dilimonene ($P \leq 0.05$). Other researchers succeeded in reducing the MIC value of this essential oil compared to its free form by encapsulating thyme essential oil in beta-cyclodextrin nanosponge.]21[. alsoOther researchers encapsulated essential oils such as cinnamon in beta-cyclodextrin nanospongessucceeded in improving the antibacterial activity of the intended essential oil]17[. Also, by encapsulating coriander essential oil in beta-cyclodextrin nanosponge, the researchers succeeded in increasing its antimicrobial properties.[13].

17. Agar well diffution

Table 2 Mean and standard deviation of the inhibition zone of limonene(L) and limonene loaded in nanosponge (L-NS)

Bacterium	Concentration (mg/ml)	Inhibition zone of positive control in plates containing L (mm)	Inhibition zone of free L (mm)	Inhibition zone of positive control in plates containing L-NS (mm)	Inhibition zone of L-NS (mm)	p.value
Escherichia coli	12.50	28.00 ± 0	15.50 ± 0.5	27.00 ± 0.5	20.50 ± 0.5	0.001[a]
	6.25		14.00 ± 0		19.50 ± 0.5	0.000[b]
	3.12		10.50 ± 0.5		13.00 ± 1	0.000[b]
	1.56		-		11.50 ± 0.5	0.000[a]
Shigella flexneri	12.50	34.00 ± 1	15.00 ± 1	37.00 ± 1	26.50 ± 0.5	0.000[a]
	6.25		13.00 ± 0		23.50 ± 0.5	0.000[a]
	3.12		10.00 ± 0		19.00 ± 1	0.000[b]
	1.56		-		17.00 ± 1	0.000[a]
Staphylococcus aureus	12.50	40.00 ± 1	14.00 ± 0	42.00 ± 1	21.50 ± 0.5	0.000[a]
	6.25		12.50 ± 0.5		19.00 ± 1	0.000[a]
	3.12		-		17.50 ± 0.5	0.000[b]
	1.56		-		12.50 ± 0.5	0.000[b]
Enterococcus	12.50	39.00 ± 0.5	13.00 ± 0	38.00 ± 0.5	16.50 ± 0.5	0.000[b]
	6.25		12.00 ± 0		12.50 ± 0.5	0.122[a]
	3.12		-		12.50 ± 0.5	0.000[a]
	1.56		-		10.00 ± 0	0.000[a]

[a] paired samples T.test
[b] Wilcoxon test
(P-value < 0.05)

3-3- The results of the minimum concentration of growth inhibition and the minimum concentration of lethality

The results of minimum growth inhibitory concentration (MIC) and minimum lethal concentration (MBC) of free dilimonen and nanoencapsulated dilimonene are presented in Tables 3 and 4. The results showed the MIC of dilimonene in free form for bacteria*Escherichia coli*And*Shigadysentery* 25/26 mg/ml and for *Staphylococcus aureus*And*Streptococcus* It was equal to 50.52 mg/ml. While the MIC of encapsulated dilimonene for bacteria*Escherichia coliand Shigadysentery* respectively equal to 0.162 and 0.081 mg/ml and for *Staphylococcus*Oreos and*Streptococcus* It was equal to 0.650 milligrams per milliliter respectively. These values show a significant decrease in the MIC of encapsulated dilimonene compared to its free form. MBC test results of dilimonene in free form for*Escherichia coli, Shigladysantri, Staphylococcus saurus* And*Enterococcus* respectively equal to 25/26, 50/52, 210,*420* mg/ml was obtained. After encapsulating dilimonene in the nanosponge, the MBC values for*Escherichia coli*And*Shigadysentery* respectively equal to 0.162 and 0.081 mg per milliliter, and for*Staphylococcus aureus* And *Enterococcus* A value of 0.65 mg/ml was obtained. Based on the mentioned values, it can be concluded that dilimonene is well encapsulated in the nanosponge. It should be noted that the most sensitive bacteria*Shigadysentery* showed that its MIC and MBC values were reduced by 324 and 648 times, respectively, compared to the free form of dilimonene. In this experiment, the nanosponge alone did not show antimicrobial properties.Also, in other studies conducted on ferulic acid encapsulated in beta-cyclodextrin nanosponge, it was observed that the MIC of ferulic acid decreased after encapsulation compared to its free form.]22[. In another study, by encapsulating thyme essential oil in beta-cyclodextrin nanosponge, researchers succeeded in reducing its MIC and MBC compared to its free form.]21[.

Table 3 Minimal inhibitory concentration (MIC) of L and L-NS

MIC			Bacterium
P-value	L-NS	L	
0.00 *	0.162	26.25	*Escherichia coli*
0.00 *	0.081	26.25	*Shigella flexneri*
0.00 *	0.65	52.5	*Staphylococcus aureus*
0.00 *	0.65	52.5	*Enterococcus*

*(P-value < 0.05)

Table 4 Minimal bactericidal concentration (MBC) of L and L-NS

MBC			Bacterium
P-value	L-NS	L	
0.00 *	0.162	26.25	*Escherichia coli*
0.00 *	0.081	52.5	*Shigella flexneri*
0.00 *	0.65	210	*Staphylococcus aureus*
0.00 *	0.65	420	*Enterococcus*

* (P-value < 0.05)

4 - Conclusion

In this study, nanoencapsulation of dilimonene inside beta-cyclodextrin-based nanosponges was successfully performed. Our results showed that the solubility of dilimonene after encapsulation increased 154 times compared to its free form, which is probably due to the decrease in the crystallinity of dilimonene and its entrapment in the nanosponge matrices. The study of antimicrobial properties showed that dilimonen alone and in encapsulated form has antimicrobial activity against Escherichia coli, Shigladysentery, Staphylococsaurus and Enterococcus strains, and encapsulated dilimonen showed more significant antimicrobial properties. It should be added that the nanosponge alone did not show antibacterial activity against the tested strains.Thus, our results show that beta-cyclodextrinic nanosponge is a suitable carrier for hydrophobic and sensitive compounds and the synthesized complex can be used as a potential compound as a preservative or in food formulations with increased antibacterial activity in food programs.Due to the fact that during the synthesis process of the complex, the particles may be nanosized or have toxicity, so their use in food formulation requires toxic tests such as MTT, checking the amount of apoptosis and possible necrosis on the cells. different cell types as well as the use of animal models with the aim of being free of toxic risks and safe.

5-Resources

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ارزیابی اثر ضد باکتریایی دی لیمونن نانوانکپسوله شده در نانواسفنج بتاسیکلودکسترین

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چکیده

با افزایش مقاومت باکتری ها به آنتی بیوتیک ها و افزایش آگاهی مردم از ارتباط بین غذا و سلامتی انسان، مطالعات و بررسی های متعددی جهت پیدا کردن ترکیبات آنتی باکتریال بالقوه صورت گرفته است. دی لیمونن یک ترکیب زیست فعال مایع و بدون رنگ از خانواده ترپنها است که فعالیت آنتی اکسیدانی و ضد میکروبی بالایی دارد. با این حال به دلیل فراریت بالا حلالیت پایین و حساسیت نسبت به نور کاربردهای آن در صنایع غذایی و دارویی محدود شده است. در این پژوهش، برای غلبه و رفع این محدودیت ها دی لیمونن در نانواسفنج بتاسیکلودکسترین نانوانکپسوله شد. به همین منظور، از نسبت مولی ۱:۴، ۱:۶و ۱:۸ بتاسیکلودکسترین به دی فنیل کربنات به عنوان اتصال دهنده عرضی و همچنین از نسبت های وزنی ۱:۲، ۱:۴و ۱:۶ دی لیمونن به نانواسفنج استفاده شد. نتایج نشان داد حلالیت دی لیمونپس از انکپسوله شدن در نانواسفنج بتاسیکلودکسترین ۱۵۴ برابر افزایش یافت. دی لیمونن انکپسوله شده فعالیت ضد باکتریایی بالاتری را در مقایسه بادی لیمونن آزاد نشان داد و حداقل غلظت بازدارندگی رشد (MIC) و حداقل غلظت کشندگی (MBC) دی لیمونن پس از انکپسوله شدن در نانواسفنج به ترتیب ۳۲۴ و ۶۴۸ مرتبه کاهش یافت. نتایج نشان داد که نانواسفنج بتاسیکلودکسترینیک حامل مناسب برای ترکیبات آبگریز و حساس است وکمپلکس سنتز شده می تواند به عنوان یک ترکیب بالقوه به عنوان نگهدارنده با افزایش فعالیت ضد باکتریایی در برنامه های غذایی مورد استفاده قرار گیرد. توجه به این نکته ضروری است که نتایج این مطالعه در شرایط آزمایشگاهی به دست آمده است و مطالعات بیشتری در مورد سمیت آنها (in vivo) جهت تأیید کاربرد در حوزه صنایع غذایی مورد نیاز است.