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Encapsulation of *Tribulus terrestris* and *Valeriana officinalis* Extracts in Nanoliposomes and Evaluation of its Antibacterial and Antioxidant Properties

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ABSTRACT: One of the traditional medicine treatments is the use of medicinal herbs. Nanoliposomes have been utilized to improve the therapeutic properties. *Tribulus terrestris* and *Valeriana officinalis* are valuable native herbs in Iran. The aim of the present study was to improve and compare the functional properties of these herbs and in their nanoliposome forms. After extracting and production of nanoliposomes, antimicrobial and physicochemical, morphological properties and antioxidant activity were determined by disk diffusion and DPPH method, respectively. The loading capacity and encapsulation efficiency of the samples were 11.5 ± 0.6 % to 16.2 ± 0.3 % and 18.3 ± 0.7 % to 23.0 ± 0.2 %, respectively. The mean size of nanoliposomes particles containing *Valeriana officinalis* extract was 65.93 ± 4.1 nm, which were the largest particles with a significant difference. The highest mean of total phenolic content belonged to nanoliposomes containing *Tribulus terrestris* extract was 120.6 ± 1.5 (mg gallic acid/g extract). Among the microorganisms, *Streptococcus iniae* (20.51 ± 0.14 mm) and *Staphylococcus aureus* (19.17 ± 1.21 mm) had a higher inhibition zone as compared with other microorganisms. The results indicated that nanoliposomes increased the functional properties of *Tribulus terrestris* and *Valeriana officinalis* medicinal herbs.

Keywords: *Antibacterial, Antioxidant, Nanoliposomes, Tribulus terrestris, Valeriana officinalis*.

Introduction

In recent years, herb extracts have attracted major attention due to the lack of side effects, and their use is increasing in the food and drug industries as antimicrobial, antioxidant and preservative compounds (Nouri, 2020). The antimicrobial properties of herb extracts have been proven against the pathogenic and spoilage microorganisms (Hematian *et al*., 2020). Some studies have confirmed the possibility of using cinnamon and clove extracts as natural antimicrobial compounds in food products, such as milk and fish or the use of common sage and thyme to preserve cheese. Herb extracts and their compounds are active against a wide variety of microorganisms, including gram-negative and gram-positive bacteria, mold and yeast (Lu *et al.*, 2017).

Tribulus terrestris is an annual herb distributed in warm and humid regions, including the mediterranean, warm regions of Europe, Asia, Africa and Australia. *Tribulus terrestris* belongs to the *Zygophyllaceae* family, which has 10 to 60 cm height, 4 to 10 mm wide flowers and 5 yellow petals in each flower, its fruit is 10 mm long and 4 to 6 mm wide. *Tribulus*

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terrestris has reciprocal and often unequal leaves that are composed of small leaflets. Leaflets are 3 to 6 pairs on both sides of the main petiole and have small single yellow flowers (Naseri *et al*., 2019). *Tribulus terrestris* is a common weed that grows in many countries and considers as a noxious weed in some places. This plant has been used in Chinese and Indian medicines and considered as one of the most important aphrodisiacs (Semerdjieva and Zheljazkov, 2019). Active components of *Tribulus terrestris* are protodioscin, terrestrosins A-E, desgalactotigonin, Fgitonin, desglucolanatigonin, gitonin, tigogenin, furostanol glycosides, β-Sitosterol, spirosta-3,5-diene, stigmasterol, diosgenin, hecogenin, ruscogenin, Kaempferol, quercetin and tribulusamides (Akram *et al*., 2011). *Tribulus terrestris* has various benefits, such as antimicrobial, antibacterial and antioxidant properties, cyclooxygenase inhibition, free radicals scavenging, lipid peroxidation inhibition and modulation of inflammatory factors (Georgiev *et al*., 2010). *Tribulus terrestris* is used in the treatment of various diseases, such as kidney stones, hypotension, diabetic, cardiovascular diseases, gastrointestinal disorders, enhancement of male sexual function and liver diseases (Rogerson *et al*., 2007).

Valeriana officinalis is a native and perennial herb with height up to 150 m in Iran, belongs to the Valerianaceae family and finds in North America, Europe, and Asia. *Valeriana officinalis* has pinnately-separated leaves with 6 to 10 pairs of lance-shaped leaflets and small white or pink flowers in a dense head of many stalked clusters (Nandhini *et al*., 2018). The active constituents of different species essential oils include monoterpene, polyphenols and flavonoids, saponins, coumarins, terpenoids and steroids (Nouri, 2020). Terpenes are identified as chemically monoterpenes and sesquiterpenes. Valeric, isovaleric, valerenic, isovalerenic and acetoxyvalerenic acids, bornyl acetate, bornyl isovalerenate, 1-pinene, 1-comphene, 1- borneol, terpineol, valeranone and cryptofauronol are the organic compounds of *Valeriana officinalis* (Pilerood and Prakash, 2013). Pharmacological studies indicated that these plants possessed various biological activities, especially in antioxidant, anti-inflammatory, anticancer, anticonvulsive, anti-parkinson's and anti-alzheimer's disease, etc. Regarding the compounds contributed to therapeutic values, the findings indicated that *Valeriana officinalis* extracts are main compounds for the treatment of epilepsy, depression, parkinson and alzheimer diseases (Nandhini *et al*., 2018). Studies have shown that the type and amount of compounds in essential oil and *Valeriana officinalis* extract are a function of geographical location, seasonal changes and vegetative or reproductive phase of the herb.

Despite the beneficial effects of *Tribulus terrestris* and *Valeriana officinalis* compounds, their use is limited due to their lack of proper identification, instability, environmental intolerances during the production and storage (Pooyamanesh *et al*., 2019). Therefore, the use of new methods seems necessary to the selective protection of natural compounds during the production and storage. For this purpose, various methods are proposed, such as encapsulation of nanoparticles, microparticles and liposomes.

Nanoencapsulation is a new method to increase the physical stability of bioactive compounds against adverse environmental factors and interference compounds. This method involves the manipulation of atoms and molecules, which leads to the formation of nanoscale structures (often 100 nm or less) and maintenance the optimal properties of the extract during the shelf life. Nanoencapsulated compounds have higher biological activity due to high surface area. This method is also used to maintain active compounds against environmental factors,

such as oxygen, light, moisture and pH variation (Nouri and Khodaiyan, 2021).

Valeriana officinalis and *Tribulus terrestris* are valuable native herbs in Iran, so the aim of this study is to increase and preserve their functional properties. This goal was carried out to compare the antioxidant and antimicrobial properties of control compounds (*Valeriana officinalis* and *Tribulus terrestris* extracts) with nanoliposome compounds. However, field studies indicated that nanoparticles containing the extracts are not prepared, therefore it is necessary to conduct this research.

Materials and Methods

- *Materials*

Valeriana officinalis and *Tribulus terrestris* were collected from the surrounding areas of Tabriz and Jafarabad in Qom province in April 2019, respectively, and both plants were identified and approved by a botanist (Figure 1). *Bacillus cereus*, *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (PTCC 1399), *Listeria monocytogenes* (PTCC 1163), *Staphylococcus aureus* (PTCC 1431) and *Streptococcus iniae* (PTCC 1887) were obtained from the Razi Vaccine and Serum Research Institute, Iran. All other chemicals used were of analytical grade.

- *Extraction process*

These plants were pulverized away from direct sunlight and in a dry shade by a mill. Aqueous extract of *Valeriana officinalis* and *Tribulus terrestris* was prepared by mixing powder with distilled water (1: 10 w/v, 100 °C), boiled for 10 min and its solid particles were taken up by filtration. The extract was dried in an oven at 60 °C and stored at 4 °C until use (Nouri, 2020). Control extract containing *Valeriana officinalis* (VO), *Tribulus terrestris* (TT) and their mixtures were used in later stages (equal proportions of both extracts: C).

- *Preparation of nanoliposomes containing TT (NTT), VO (NVO) and their composition of nanoliposomes (NC)*

Multilayer liposomes were prepared by a thin-film hydration method. For this purpose, 90 mg of lecithin was completely dissolved in ethanol solvent. A thin layer was formed by solvent evaporation in a rotary evaporator (Heidolph, Germany) at 140 rpm and 50 °C. Then, hydration was performed by adding TT with VO at a ratio of 1: 5 and NC at a ratio of 1: 1 in distilled water. The sample was homogenized by a homogenizer (Heidolph, Germany) at 20,000 rpm and a temperature higher than the transfer phase for 10 min to degrade the multilayered liposomes and convert them into single-layer liposomes. Finally, liposome was fragmented using a probe sonicator for 5 min, during 5 cycles of 1 min on 1 min off (Materials & Sonics, vibracell, UK). Thus, monolayer liposomes were produced at the nanometer scale (Xia and Xu, 2005).

Tribulus terrestris *Valeriana officinalis*

Fig. 1. An overview of the *Tribulus terrestris* and *Valeriana officinalis* herbs.

- ***Physicochemical and morphology properties of NTT and NVO***

In present study, the physicochemical and morphology properties of nanoliposomes were investigated, including encapsulation efficiency, loading capacity, particles size, particles size distribution (dispersion percentage) and zeta potential.

To determine the encapsulation efficiency and loading capacity of nanoparticles, the dispersions were mixed and heated with hydrochloric acid solution at 95 °C for 30 min. In this process, the structure was broken and the encapsulated nanoparticles were released. The solution was cooled for a period time and mixed with methanol solution before centrifugation (9000 rpm, 2 min), (Nouri, 2020). Standard calibration curve was obtained by plotting the desired concentrations (0.1, 0.4, 0.7, 1 and 1.3 mg/ml) of the control extracts in methanol solution (vertical axis) versus absorbance values (horizontal axis). The absorbance of the samples was measured according to their concentration at 415 nm and then equations 1 and 2 were used.

Equation 1: Encapsulation efficiency (%) = (amount of extract loaded /amount of initial extract) × 100

Equation 2: Loading capacity (%) = (amount of extract loaded / amount of sample) × 100

Nanoliposome particles were measured by a dynamic light scattering (DLS) (Nanophox Sympatec GmbH, made in Germany) at 25 °C. The samples were diluted with deionized water to have a number of particles in a certain range (between 200 and 2000 kCPS). Zeta sizer was used to determine the zeta potential of liposomes containing essential oils. The zeta potential of liposomes was measured at pH 7.4, temperature 25 °C and power 149 W (Moghimi *et al*., 2016).

- ***Antioxidant performance of NVO, NTT and NC***

The antioxidant capacity of TT and VO solutions (giving hydrogen atom or electron) was measured before and after encapsulation in nanoliposomes by assessing the purple reduction of DPPH methanolic solution. Spectrophotometric evaluation was performed using DPPH reagent. 50 µl of TT and VO extracts added to 5 µl of DPPH solution (0.004 % methanolic solution) before and after encapsulation (different concentrations). After 30 min at ambient temperature, the absorbance was read at 517 nm and the free radical scavenging percentage of sample was calculated according to equation 3. Finally, the results are reported as IC_{50} (IC_{50} is a concentration of antioxidant that traps 50 % of DDPH free radicals), (Dehghani *et al*., 2019).

Equation 3: Absorbance (%) = (control sample absorbance/sample absorbance - control sample absorbance) × 100

- ***Antimicrobial performance of NVO, NTT and NC***

Antimicrobial activity of NVO and NTT was determined by disk diffusion according to a standard method (Standard, 1999). This experiment was carried out on 6 pathogenic microorganisms in food such as *Bacillus cereus, Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (PTCC 1399), *Listeria monocytogenes* (PTCC 1163), *Staphylococcus aureus* (PTCC 1431) and *Streptococcus iniae* (PTCC 1887). In order to maintain the activity of strains, new cultures were prepared from them every month and stored in the refrigerator. At this stage, the target microorganisms were inoculated into BHI broth and diluted to 10^6 CFU/ml in saline solution. Then, 0.1 ml of them was cultured superficially on nutrient agar plates. After culture, wells (8 mm) were made in agar and filled with 70 µl of NVO and NTT (1 %). The prepared plates were

incubated for 2 h at 37 °C. All plates were examined for zone of inhibition and the results were announced as an average.

- ***Statistical analysis***

LSD test at 99% level was used to analyze the data from a completely randomized design and compare the mean of the data. Statistix software version 8 was used for statistical analysis and all the mentioned tests were performed in three replications for each sample.

Results and Discussion

- ***Physicochemical and morphology properties of NVO, NTT and NC***

Physicochemical and morphology properties including encapsulation efficiency, loading capacity, particle size, particle size distribution, dispersion percentage, and zeta potential were evaluated. These nanoparticle properties are a scale of particles capacity in storage and their release power that process conditions such as purity of the raw material, temperature, mixing time and energy, type and salt content, zeta potential and gelling point affect them (Nouri and Khodaiyan, 2021).

Using the mentioned equations (Equations 1 and 2) in Table 1 of the encapsulation efficiency, the loading capacity is shown. Loading capacity and encapsulation efficiency of the samples range from 11.5 ± 0.6 % to 16.2 ± 0.3 % and 18.3 ± 0.7 % to 23.0 ± 0.2 %, respectively. Table 1 indicated that NVO had a significantly higher percentage of loading capacity and encapsulation efficiency than other samples. Factors such as wall material, capsule structure, cholesterol, lipid and the reaction between the material in the wall, and inside the nucleus affect the encapsulation efficiency (Fan *et al*., 2008). The results of Table 1 indicated that loading capacity percentage of VO extract is the highest ($p<0.05$). The loading capacity of VO extract occupies a larger volume of initial sample, therefore the total particle values are higher, and the loading capacity fraction indicates a smaller number due to a larger denominator. TT extract containing less fat, more hydrophilicity, and a smaller average particle size, leads to more water absorbance and shrinkage. The numerical (extract loaded) is smaller than the denominator, so the factor values became lower (Wisuitiprot *et al*., 2011). Encapsulation efficiency percentage of VO extract has higher values than TT extract (Table 1). A significant decrease of this percentage was observed in the other two samples ($p <0.05$). There is a limitation in loading capacity of TT nanoparticles due to the smaller particle, and larger amounts of the extract are not able to encapsulate. The reduction in this factor may also be related to shrinkage in loading capacity. The highest encapsulation efficiency percentage is observed when the most suitable links between hydroxyl groups are obtained in the extract.

The dispersion index and zeta potential results are shown in Table 1. In present study, the effect of extract addition on system stability was monitored through changes in zeta potential. This factor in any environment indicates the stability, non-sedimentation of particles and hydrocolloid systems that by reducing it, the stability of the system is reduced. In Table 1, the zeta potential ranged from -11.05 ± 0.4 to -9.64 ± 0.7, and among the samples, nanoliposomes with zeta potential and dispersion index were in the same range without significant differences ($p \geq 0.05$). Zeta potential is a function of surface charge in lipid vesicles, the adsorbed layers, and the particles nature, which the liposomes are dispersed in it. The higher surface charge in liposome prevents the accumulation of vesicles due to more repulsive forces, and the liposome stability increased. Surface charge increases cell and liposome interactions and a bioactive

compound. The results of Table 1 indicated that all samples were stable under normal conditions and did not precipitate. According to DLS results, the vesicles had an acceptable dispersion, and a low dispersion index was obtained. The difference between the present study results and other studies is probably due to the difference in the amount of mechanical force, if more force is used, smaller particles are obtained (Gulseren and Corredig, 2013; Zou *et al*., 2014). The use of ethanol has also changed the loading capacity, ester stability and reduced the particle size (Hematian *et al*., 2020). In present study, the zeta potential results of nanoliposomes are different from the previous results, which may be due to phospholipids and cholesterol used in nanoliposomes production, however, they are the main nanoliposome constituents (Gulseren and Corredig, 2013).

Particle size is one of the determinative properties in the application of nanoparticles (Nouri and Khodaiyan, 2020). Particle size changes of the samples were shown in Figure 2, and the liposomes were vesicles with spherical shapes and adequate dispersion. Average particle size of NVO, NTT and NC were 65.93 ± 4.1 nm, 30.21 ± 3.6 nm, and 51.7 ± 1.9 nm, respectively. According to Figure 2, the type of extract used in the process had a significant effect on the particle size distribution index. The mean of this index in NVO extract (Figure A) is significantly higher than other samples ($p < 0.05$). Significant increase in the average particle size at 0.01 level of sample NVO compared with other samples can be attributed to the increase in particle size after the process according to the this index. In this treatment, the extract particles came together and hydrogen bonds created, since the extract is more lipophilic than the other. The bonds occupy a larger volume, which nanoparticles produce compared with the corresponding points in NTT and NC diagrams. Figure 2 indicated average particle size decreased in equal proportions of two extracts. In this treatment, due to the creation of new links between two extracts and the reduction of encapsulation efficiency, there is less extract in the nanoparticles, which reduces the particle size.

Table 1. The results of loading capacity (%), encapsulation efficiency (%), particle dispersion (nm) and zeta potential (mV), (mean ± standard error)

Samples	Loading capacity (%)	Encapsulation efficiency (%)	Particle dispersion (nm)	Zeta potential (mV)
NVO	$16.2^{c} \pm 0.3$	$23.0^{b} \pm 0.2$	$0.182^{a} \pm 0.2$	$-11.05^{a} \pm 4.0$
NTT	$5.11^{a} \pm 0.6$	$2.19^{a} \pm 0.5$	$201.0^{a} \pm 1.0$	$-64.9^{a} \pm 0.7$
NC	$14.4^{b} \pm 0.1$	$18.3^{a} \pm 0.7$	$0.193^{a} \pm 0.04$	$-10.32^{a} \pm 0.6$

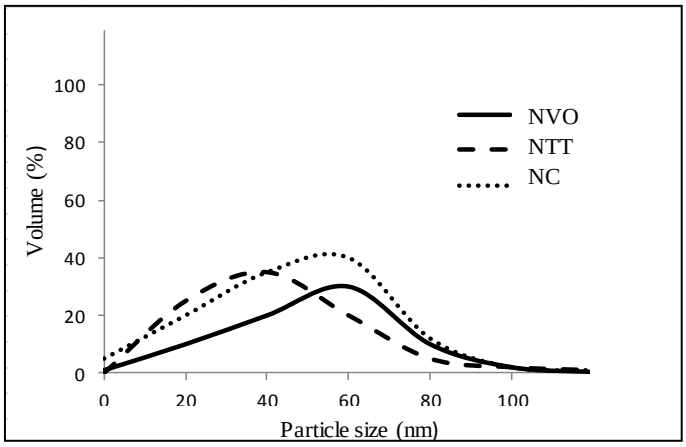

Fig. 2. The results of particles size of NVO, NTT and NC

- ***Antioxidant performance of NVO, NTT and NC***

Total phenolic content and antioxidant capacity of NVO and NTT are shown in Figure 3, based on the inhibition of DPPH and IC_{50} in accordance with Equation 3. The aim was to investigate the differences between the properties of C and NC, these factors were measured for both extracts. As shown in Figure 3, the mean total phenolic content of VO and NVO, TT and NTT, C and NC were 48.12 ± 3.2 and 73.90 ± 2.1 (mg gallic acid/g extract), 98.40 ± 0.3 and 120.6 ± 1.5 (mg gallic acid/g extract), 1.1 ± 93.12 and 110.81 ± 2.4 (mg gallic acid/g extract), respectively. Figure 3 indicated that IC_{50} value of VO was 28.51±0.7 (µg/ml). The antioxidant activity of VO significantly increased after encapsulation in nanoliposomes, therefore IC_{50} of NVO decreased to 20.14 ± 1.4 (µg/ml). This treatment was observed in TT, and the antioxidant activity of TT and NTT samples were 18.20 ± 1.2 and 14.4 ± 0.9 (µg/ml), respectively, which is similar to NC. The antioxidant activity of TT and VO extracts was determined by DPPH free radical elimination method. There is a linear equation between antioxidant activity and phenolic content of herb extracts, fruits, *etc*., which is in line with the results of present study (Luximon Ramma *et al*., 2002). Figure 3 indicated that the average total phenolic content and antioxidant properties of TT have better results with significant differences than VO, however, TT has more phenolic compounds and their movement is better than VO in hydrophilic environment. On the other hand, the results indicated that each of the nanoliposomes with significant differences compared with their free extract have better antioxidant properties, which is due to the more phenolic compounds in a higher surface to volume ratio (Akbrian *et al*., 2017). Among the samples, NC and NTT hve the best antioxidant properties. The results are consistent with the findings of many researchers, nanoliposomal encapsulation improves the antioxidant activity of phenolic compounds against lipid oxidation, by better spreading it in the environment and making it more available (Spigno *et al*., 2013). However, the results of present study are inconsistent with the results of some researchers; they reported the antioxidant properties of free phenolic compounds and encapsulation of milled olive remain without much difference (González-Paredes *et al*., 2011).

- ***Antimicrobial Performance of NVO, NTT and NC***

The antimicrobial activity of NVO, NTT and NC tested on 6 types of pathogenic bacteria and the results reported in Table 2. All samples had inhibitory properties on bacteria, *Bacillus cereus*, *Salmonella*

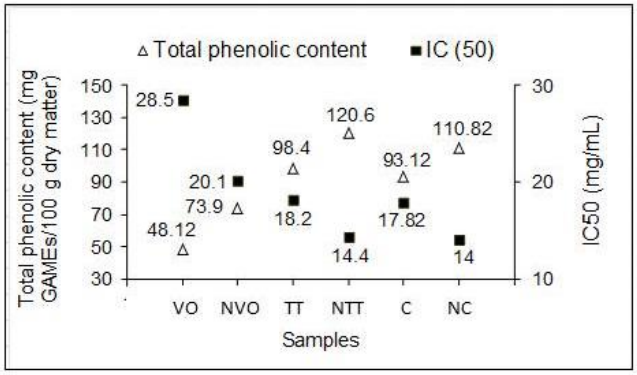

Fig. 3. Mean total phenolic content (mg gallic acid / g extract) and antioxidant activity of samples in terms of DPPH (IC_{50} in µg / ml)

typhimurium, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus iniae*, gram-positive and gram-negative bacteria. Among the microorganisms available, *Streptococcus iniae* (20.51 ± 0.14 mm) had a higher inhibitory zone as compared to other bacteria, while *Salmonella typhimurium* (6.10 ± 0.20 mm) indicated the lowest sensitivity. NVO has the least inhibitory effect on *Listeria monocytogenes* (10.30 ± 0.12 mm) and the most destructive effect *on Streptococcus iniae* compared to other bacteria. The inhibitory zone indicated no significant difference between *Escherichia coli* (18.07 ± 0.30 mm) and *Staphylococcus aureus* (18.73 ± 1.10 mm). *Salmonella typhimurium* (13.10 ± 0.18 mm) was significantly more resistant to NVO than the previous bacteria. The results indicated that when examining the effect of NTT on microorganisms, *Staphylococcus aureus* (19.17 ± 1.21 mm) and *Streptococcus iniae* had a higher zone of inhibition compared to other bacteria, while *Escherichia coli* (6.02 ± 0.09 mm) indicated the lowest sensitivity. In the case of other bacteria, the inhibition zone was from highest to lowest, including *Bacillus cereus*, *Listeria monocytogenes* and *Salmonella typhimurium*, respectively.. In previous studies, ethanolic extract of TT has shown antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Helicobacter pylori* and *Klebsiella pneumoniae* (Mohammed, 2008; Al Bayati and Al Mola, 2008; Hakemi *et al*., 2013).

The antimicrobial activity of VO and TT increased significantly after encapsulation in the nanoliposome. These results indicate the effectiveness of encapsulation. The use of nanoliposomes can improve cell transport and release active compounds into bacterial cells (Liolios *et al*., 2009).

These results can be due to the reaction between nanoliposomes and bacterial cells, which is carried out in variety ways such as intramembrane transport, release power, binding, absorbance, and phagocytosis. Antimicrobial activity of biological materials is reduced by nanocapsulation because of the inhibition of the main material release within the nanoliposomes, the reaction with proteins and the formation of sedimentation (Zou *et al*., 2014). In the present study, the nanoparticles composition and the extract structure have led to more antimicrobial properties, therefore antimicrobial nanoparticles always have a greater inhibitory effect on microbial growth than antimicrobial particles in larger dimensions. However, nanoparticles have a higher surface to volume ratio than larger particles, more surfaces to attach to microstructures, disorder membrane permeability, and make changes in microbial cells.

Different processes have been proposed on the effect of inhibitory properties extractions on bacteria. Terpene and phenolic compounds, in addition to damaging the cell membrane of microorganisms, which cause the destruction of the layer and its permeability, have a destructive effect on performance of the layer such as disruption of the electron transfer, protein and nucleic acid synthesis or cause the destruction of cell membranes and

Table 2. Mean of antimicrobial activity of VO, NVO, TT, NTT, C and NC (mean ± standard error)

Bacterial species	Antimicrobial activity of VO		Antimicrobial activity of TT		Antimicrobial activity of NVO and NTT	
	VO	NVO	TT	NTT	C	NC
Listeria monocytogenes	$1.6^{a} \pm 0.20$	$10.03^{b} \pm 0.12$	$9.70^{b} \pm 0.09$	$14.01^{cd} \pm 0.08$	$9.01^{b} \pm 0.21$	$13.00^{c} \pm 0.05$
Bacillus cereus	$12.20^{c} \pm 0.03$	$14.18^{e} \pm 0.26$	$13.01^{c} \pm 1.05$	$15.03^{d} \pm 1.02$	$12.76^{c} \pm 0.15$	$17.20^{de} \pm 0.18$
Salmonella typhimurium	$9.01^{b} \pm 0.15$	$10.13^{c} \pm 0.18$	$5.71^{a} \pm 0.04$	$8.40^{ab} \pm 0.06$	$8.23^{ab} \pm 1.01$	$11.01^{bc} \pm 1.05$
Escherichia coli	$10.00^{b} \pm 0.07$	$18.07^{e} \pm 0.30$	$4.92^{a} \pm 1.10$	$6.02^{a} \pm 0.09$	$10.06^{b} \pm 0.83$	$9.04^{b} \pm 0.96$
Staphylococcus aureus	$12.05^{c} \pm 0.09$	$18.73^{e} \pm 1.10$	$15.21^{d} \pm 0.03$	$19.17^{ef} \pm 1.21$	$12.24^{c} \pm 0.92$	$17.90^{de} \pm 0.07$
Streptococcus iniae	$15.22^{d} \pm 0.40$	$20.51^{f} \pm 0.14$	$12.34^{c} \pm 0.94$	$19.16^{ef} \pm 0.13$	$14.07^{cd} \pm 1.30$	$19.81^{ef} \pm 0.46$

leakage of intracellular material into the extracellular membrane and ultimately lead to inhibition of bacterial activity (Khosravi Zanjani *et al*., 2015). Various reports indicate the relationship between phenolic compounds and antimicrobial activity. In a study, the several medicinal herbs extracts, flavonoids and phenol of extracts have shown that there is a significant relationship between antimicrobial activity and polyphenol compounds in the studied herbs (Zakerin *et al*., 2015). In some cases, the results indicate that despite the high or low phenolic compounds, the antimicrobial properties are not commensurate with these compounds. In the present study, no significant relationship was observed between the two mentioned factors. In order to justify this issue, numerous factors such as climatic conditions, including water, air, soil, height, amount of chemical compounds and other environmental factors also led to changes in amount and variety of active ingredients medicinal plants (Nandhini *et al*., 2018).

Conclusion

The results of present study indicated that the loading capacity and encapsulation efficiency of the samples ranged from 11.5 ± 0.6 % to 16.2 ± 0.3 % and 18.3 ± 0.7 % to 23.0 ± 0.2 %, respectively. The mean particle size of NVO, NTT and NC were 65.93 ± 4.1 nm, 30.21 ± 3.6 nm and 51.7 ± 1.9 nm, respectively. Microbial results indicated that all samples had inhibitory properties on *Bacillus cereus*, *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Streptococcus iniae*. In general, the results of the present study indicated that NVO and NTT can increase the functional properties and this method is recommended for craftsmen.

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