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RESEARCH PAPER

Study of the Physicochemical Properties and Antimicrobial Activities of Nanoparticles Containing β-Cyclodextrin and Geranial

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ABSTRACT

In general, β-cyclodextrin (β-CD) is widely used in various technologies of the food industries. The aims of this study were preparation, characterization and optimization of a novel nanosize formulation of β-CD NPs loaded with geraniol (GR). In the current study, the optimum conditions for maximum encapsulation efficiency and loading of geraniol were assessed using response surface methodology (RSM). Furthermore, in vitro antimicrobial activities against Staphylococcus aureus, Bacillus cereus, Salmonella entritidis, Escherichia coli, Candida albicans and Aspergillus niger were studied. The present study is the first study to investigate antimicrobial activity of the GR inclusion complexes in nanosize formulations. The GR complexes were assessed using scanning electron microscopy (SEM), infrared (IR) spectroscopy and differential scanning calorimetry (DSC). Furthermore, antimicrobial activity of the inclusion complexes (IC) against bacteria and fungi were assessed. Minimum inhibitory concentrations (MIC) and inhibition zones of the GR-β-CD inclusion complexes were calculated using agar/broth dilution and agar well-diffusion methods. Encapsulation efficiency (EE) and loading values of the optimized formulation included 87.25 and 10.45%, respectively, with a size distribution of 117 nm ±1 and appropriate particle size distribution (PDI). Moreover, SEM, IR and DSC verified fabrication of inclusion complexes between GR and β-CD. The inhibition zones of β-CD-GR complexes were recorded as the following order: Bacillus cereus > Staphylococcus aureus > Salmonella entritidis > Escherichia coli. The RSM helped prepare geraniol nanoinclusion complexes using β-cyclodextrin with optimum responses. The antimicrobial activity of GR highly enhanced after efficient complexation. This study provides appropriate information on use of inclusion complexes of GR.

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INTRODUCTION

Geraniol (GR) is a monoterpene alcohol with pharmacological properties that is derived from the essential oils of aromatic plants such as Kansho-shochu, sweet basil, hopped beer and green tea [1–3]. The US Food and Drug Administration (FDA) has certified geraniol as a food additive for flavoring foods such as beverages, candies and ice creams [4]. Promising bioactive substances such as essential oils (EO) are used as preservatives in foods or medicines due to the various functional activities of these compounds, including antioxidant,

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antimicrobial and anticancer effects [5, 6]. Biological activities of the EOs against foodborne pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enteritidis*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* have extensively been investigated [2, 7]. Encapsulation simultaneously increases antimicrobial potencies of the aromatic oily components of EOs such as geraniol and their certainly controlled/sustained releases, facilitating their close interactions with microorganisms. Nanobiomaterials used in designation of formulations include various advantages and important functional properties, which are achieved by increased surface areas and binding of the bioactive molecules, enhanced permeabilities and retention effects [8–10]. When encapsulated in β-cyclodextrin (β-CD), EOs and their components present high stabilities against degradative actions of oxygen and temperature as well as promising various uses in pharmaceutical and food industries. Moreover, better retention and increased protection of EOs in colloidal matrices depend on the type of the technique [11, 12]. In the last few decades, various formulation approaches have been introduced for the development of β-cyclodextrin with various EOs compounds [11–14]. For poorly soluble compounds such as geraniol, β-CD is a useful material for complexation because of its various advantages [15, 16]. Several studies have reported that geraniol includes antimicrobial activities against foodborne pathogens. A summary of these studies is provided in Table 1. These studies have majorly included pure forms of GR instead of GR/CD molecular-inclusion complexes [17–28].

As previously reported by Menezes et al., and Hadian et al., GR can be loaded in β-cyclodextrin using precipitation in nanoparticle (NP) complex forms [29, 30]. Use of systemic experimental designs with a mathematical optimization approach such as RSM helps determine acceptable formulations of flavor compounds [31]. Advances of using experimental design methods include decreases in number of experiments, identification of interactions between multiple factors and detection of optimal responses within the experimental regions by decreases in variability [32–34]. Development of encapsulated holy basil essential oil in gelatin using RSM with a maximum encapsulation efficiency has been reported by Sutaphanit et al. [35]. Alves et al., have successfully optimized encapsulation of the cocoa waste extracts using chitosan, maltodextrin and RSM as a nutrient source for aquatic animal feeds [33]. Nowadays, use of natural preservatives in food industries is a major concern of the global public health. Thus, a novel plan for the development of effective carriers of bioactive compounds with various advantages can be the use of nanotechnology for the inclusion complex formation with β-CDs. Based on the previous reports by the authors [30], simultaneous use of β-CD complexation based nanocarriers for the development of natural antimicrobial delivery systems has been investigated in this study. The objective of the current study was to investigate encapsulation efficiency and loading of GR in β-CDs molecular-inclusion complexes using RSM. Furthermore, *in vitro* antimicrobial activities of GR inclusion complexes against *S. aureus*, *B. cereus*, *S. enteritidis*, *E. coli*, *C. albicans* and *A. niger* were studied. The present study is the first study to report antimicrobial activities of the GR inclusion complexes in nanosize formulations.

MATERIALS AND METHODS

Materials

Geraniol (98% purity) and β-cyclodextrin (≥ 97% purity), menthol and tetramethylsilane (TMS) were purchased from Sigma-Aldrich, Steinheim, Germany. Anhydrous sodium hydroxide, hydrochloric acid and dimethyl sulfoxide-d6 (DMSO-d6, at least 99.5% purity) were supplied by Merck, Darmstadt, Germany. Demineralized water (pH 7.6 ±0.2) was used in the study. Other solvents included HPLC grades. *Staphylococcus aureus* PTCC 1112, *Bacillus cereus* PTCC 1015, *Salmonella enteritidis* PTCC 1709, *Escherichia coli* PTCC 1330, *Candida albicans* PTCC 5027 and *Aspergillus niger* PTCC 5012 were provided by the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran.

Preparation and optimization of the GR-β-CD inclusion complexes

The GR-β-CD NPs were prepared using precipitation method with minor modifications according to Rachmawati et al. [36]. Briefly, 500 mg of the β-CD were dissolved in 20 mL of ethanol and distilled water (DW) (20:80 v/v) for 30 min at 45 °C. Then, GR was added to the hydro-alcoholic solution of β-CD with continuous agitation. The mixture was stirred at 300 rpm for 1 h at 37 ºC and set for 4 h at room temperature. After

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Table 1- Summary of antimicrobial activities of geraniol from previous studies

Type pf Microorganism	Method	Inoculum (CFU/mL)	Minimum inhibitory concentration /EC50*	Minimum bactericidal concentration	Reference
E. coli	Microdilution	10^8	1386.8 µg/mL	2773.6 µg/mL	17
S. aureus	Microdilution	10^8	2773.6 µg/mL	2773.6 µg/mL	
B. cereus	Microdilution	5×10^5	0.07 mg/mL	Bacterial growth	18
E. coli	Microdilution	5×10^5	0.06 mg/mL	0.25 mg/mL	
S. aureus	Microdilution	5×10^5	0.03 mg/mL	Bacterial growth	
E. coli O157:H7	Microdilution	5×10^5	0.6 µL/mL	-**	19
S. aureus	Microdilution	5×10^5	0.6 µL/mL	-	
P. aeruginosa	Microdilution	5×10^5	>2.4 µL/mL	-	
L. monocytogenes	Microdilution	5×10^5	0.5 µL/mL	-	
S. aureus	Broth Microdilution	10^5	2.44 µg/mL	-	20
S. aureus	Microdilution	10^7,10^8	0.25 mg/mL	-	21
S. aureus	Disk diffusion	10^7,10^8	19 mm	-	
E. coli	Microdilution	10^7,10^8	>8 mg/mL	-	
E. coli	Disk diffusion	10^7,10^8	15 mm	-	
B. cereus	Microdilution	10^8	1386.8 µg/mL	-	22
S. aureus	Microdilution	10^8	2773.6 µg/mL	-	
E. coli	Microdilution	10^8	1386.8 µg/mL	-	
S. enteritidis	Microdilution	10^8	2773.6 µg/mL	-	
E. coli	Microdilution	5×10^5	250 mg/L	-	23
S. aureus	Microdilution	5×10^5	300 mg/L	-	
C. albicans	Microdilution	10^5 cells/mL	130 µg/mL	160 µg/mL	24
A. niger	Radial growth technique	-	128.7	-	25
A. flavus	Mycelial Radial growth inhibition technique	-	287mg/L	-	23
C. albicans	Microdilution	1-5×10^6CFU/mL	16-32 µg/mL	-	26
Candida spp.	Microdilution	5×10^3 cells/mL	37.5-1000 µg/mL	-	27
Candida spp.	Microdilution	0.5 McFarland	72-128 µg/mL	-	28

*EC50= The concentration causing 50% mycelial growth inhibition. **. Not determined

sonication, solution was centrifuged at 3000 rpm for 15 min to precipitate free GR. The supernatant was filtered using 0.2-µm filters. Then, sample was transferred to a vacuum chamber at 40 °C for 6 h. In this study, RSM and rotatable central composite design (RCCD) with two independent variables were used to optimize formulations of the GR-β-CD complexes [37]. Using multivariate quadratic equation, main and reciprocal effects of the independent variables on each dependent variable were separately assessed. The experimental design and data analysis were carried out using Design-Expert Software v.8.0.5 (Stat-Ease, Minneapolis, USA). All experiments were carried out in triplicate. Independent variables included β-CD (X_1) and GR (X_2) and dependent variables included loading (Y_1) and encapsulation (Y_2) efficiencies. Design of the statistical test was carried out to determine optimal conditions for the achievement of the highest percentage of dependent variables. Five coded levels of the two variables were incorporated into the design, resulting in 13 experiments. Based on the number of independent variables and their levels, number of the test samples included 13 treatments with five central and eight non-centered points. Equations 1 and 2 show effects of the variables on the percentage of loading and GR encapsulation, respectively. In analysis of variance (ANOVA), the second power of independent variable B^2 (GR^2) was significant at 0.01. The regression model was as follows:

$$Y_1 = +32.76 + (-0.69)X_1 + (-0.15)X_2 + 4.8X_1X_2 + 4.48X_1^2 + (-0.01)X_2^2 \quad (1)$$

For encapsulation efficiency, the quadratic model was significant at 0.01 (< 0.0002). In ANOVA, the second power of independent variable B^2 (GR^2) was significant at 0.01. The regression model was as follows:

$$Y_2 = +476.39 + (-9.93)X_1 + (-7.62)X_2 + 0.14X_1X_2 + 0.056X_1^2 + (-0.19)X_2^2 \quad (2)$$

Coefficient of determination (R^2) and adjusted and predicted coefficients of determination (R^2-adj and R^2-pred) were used to compare various models and select an appropriate model for the response. Based on the preliminary tests, ranges of the changes were assessed at 5–23.11% by weight in GR and 76.89–95% by weight in β-CD.

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Characterization of GR-β-CD complexes

Assessment of the geraniol EE and loading

Total quantity of the GR in GR-β-CD inclusion complex powders and surface oil extractions was measured using gas chromatographer (Agilent, Waldbrornn, Germany) Hewlett-Packard Model 6890 equipped with an FID detector and stationary phase with HP-5 Column (30 m length × 0.25 mm ID × 0.25 µm film thickness), according to Bhandari et al. [38, 39]. Concentration of the GR was quantified using standard calibration curves. Calibration curves were plotted for each compound using six various concentrations of GR and the internal standard in hexane in the range of 0.5–10 µg mL^{-1}. Loading and EE of the GR-β-CD complexes for GR were respectively calculated using Equations 1 and 2 according to Tao et al. [40]:

$$EE\ (\%) = A\ (mg) / B\ (mg) \times 100 \quad (1)$$

$$Loading\ (\%) = A\ (mg) / C\ (mg) \times 100 \quad (2)$$

Where, A, B and C were quantities of the trapped GR (differences between the total oil and surface oil) in the complexes, primary feeding quantities of the GR and number of the produced particles, respectively. Calibration curves were plotted for each compound using six various concentrations of geraniol and the internal standard in hexane in the range of 0.5–10 µg mL^{-1}.

Particle size distribution, Zeta-potential and polydispersity index assessment

Particle size and polydispersity index (PDI) of the optimized GR-β-CD inclusion complexes were assessed using photon-correlation spectroscopy (PCS). Suspension was serially diluted in MiliQ water and analyzed using Malvern ZetaSizer Nano Series (Malvern, UK) [36].

Infrared spectroscopy

Geraniol, β-CD, physical mixture and nanoinclusion complexes of the GR were studied using infrared (IR) spectroscopy. Geraniol was scanned in IR spectra of 4,000–400 cm^{-1} (Spectrum One, Perkin Elmer, Waltham, MA, USA). Spectra included an average of 32 scans at a resolution of 4 cm^{-1}. The IR spectral analysis was carried out using KBr pellet press method and the IR spectra were recorded using Nicolet Magna-IR System 550 equipped with Nicolet OMNIC Software [41].

Scanning electron microscopy

Hitachi SU 3500 Scanning Electron Microscopy (SEM) (Hitachi, Japan) instrument was used for the surface morphology. Samples were attached to an aluminum stub using double-sided carbon tape and were electrically conductive by coating with thin layers of gold (33 Aº thickness) prior to study with SEM.

Differential scanning calorimetry

Thermal analysis of β-CD, geraniol and their physical mixture and inclusion complex was carried out using Mettler Toledo DSC System (DSC, 823E, Mettler Toledo, Switzerland). Mettler Stare Software v.9.x was used for data acquisition and indium for calibrating the instrument. Sample (2 mg) was sealed in the aluminum pans and heated at the rate of 10 °C min^{-1} from -20 to 300 °C under 8-kPa nitrogen atmosphere [36]. Physical mixtures of the GR and β-CD were prepared using simple mechanical mixing. The mole ratio of GR to HP-β-CD was 1:2.

Antimicrobial activity assay

Bacteria were cultured on soybean-casein digest agar and incubated at 30–35 °C for 20–24 h. A loopful of bacteria was suspended in sterile normal saline and homogenized using vortex. Density of the microbial suspension was adjusted to 0.5 McFarland turbidity standard. The 0.5 McFarland standard included an absorbance reading of 0.08–0.1 at 625 nm [42]. Furthermore, *C. albicans* was cultured on Sabouraud dextrose agar (SDA) and incubated at 35 °C for 24 h. Then, a suspension of 1-5 × 10^6 cells mL^{-1} was prepared using 0.5 McFarland turbidity standard (light transmittance 76% at 530 nm). The *A. niger* was cultured on SDA and incubated at 20–25 °C until spore formation (72 h). The spore suspension was prepared by washing surface of the cultured slant using normal saline containing 0.05% Tween 80 and sterile glass beads. Suspension was transferred to a sterile tube. After 3-5 min, rough particles were precipitated and the supernatant was transferred to another sterile tube. Turbidity of the spore suspension was adjusted to transmittance of 80-82% at 530 nm (equal to 4 × 10^5 to 5 × 10^6 spore mL^{-1}) [43]. Minimum inhibitory concentrations (MICs) of the GR and GR-β-CD against the bacteria were assessed using agar dilution method [44].

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Statistical analysis

In this study, all experiments were carried out in triplicate and mean ±SD (standard deviation) was reported. The ANOVA, regression coefficient calculation and performance stepwise procedure were used to simplify the models and generating of three-dimensional surface plots using Design-Expert Software v.7.1.1 (Stat-Ease, Minneapolis, USA). Significance of the equation parameters for each response was reported using *F*-value at a probability (*P*) of 0.05. In addition, adequacy of the models was calculated using model analysis, lack-of-fit test and coefficient of determination (R^2) analysis.

RESULTS AND DISCUSSION

Characterization of GR-ß-CD inclusion complexes

To overcome problems such as time and cost linked to the formulation, effective and reliable RSM was used to assess consequences of the independent variables [β-CD (X_1) and GR (X_2)] on the responses [loading (Y_1) and encapsulation efficiency (Y_2)] of the NP formulation. Effects of the proportions of GR and β-CD on the encapsulation efficiency and loading of the guest molecules (GR) were the major objectives of the first part of this study. Correlation coefficient of the model for the loading and encapsulation efficiency included 0.83 and 0.88, respectively. The *P*-value for the loading and encapsulation proportions shows appropriately and efficiency of the computational model; hence, it can be used as a completely successful model for the calculation and estimation of the proportions of loading and encapsulating. Using quadratic equation of multivariate, main and reciprocal effects of the independent variables were tested separately for each dependent variable. Significance of each term was reported using *F*-value and *P*-value; where, small *P*-values and large *F*-values illustrate further significant effects on the respective independent variables (Table 2). For loading and EE proportions, the quadratic model was significant at 0.05 (< 0.0001).

The EE% of GR-β-CD formulations was calculated using various GR to β-CD ratios. Total oil and surface oil were detected using GC/FID. Based on the results of early stages and available resources, effects of the two independent variables, β-CD and GR were considered as 80–95 and 5–20% w/w, respectively (Table 1). Three-dimensional response surface plots of the β-CD on encapsulation of GR are shown in Fig. 1a. As stated, effects of the independent variables (GR and β-CD) on loading and EE proportions showed a descending trend to the central point and then changed.

As seen in Fig. 1, GR included obvious effects on loading and encapsulation efficiencies of the NP formulation. Based on the model, values of loading and encapsulation efficiency respectively included 10.45 and 87.25% under optimal conditions. To investigate effects of independent variables on dependent ones, surface response plots of the quadratic polynomial models were created by varying two of the independent variables within the experimental ranges (Fig. 1b). As Figs. 1a and 2b illustrate, loading and encapsulation efficiencies increased by increasing the weight proportion of β-CD to GR; therefore, interaction of these two variables was significant. Previously, several attempts have been made to complexation of constituent of several EOs with cyclodextrin [12, 14, 29, 30, 41]. The highest responses were achieved for F3 formulation at 95 and 12.50% for β-CD and GR, respectively. Furthermore, the lowest loading (28.5%) and encapsulation (2.2%) efficiencies were achieved at 1.89:87.5 GR-β-CD ratio for F4 formulation. GR, as a primary component of the rose, palmarosa and citronella oils is able to increase transdermal penetration due to its lipophilic nature [14, 29]. Zhang et al. reported the formation of inclusion compounds in terms of baicalin-ß-CD ratio, time and temperature to achieve the maximum encapsulation efficiency using RSM [45]. A similar report was published by Menezes et al., demonstrating that the EE of GR encapsulated in β-CD was nearly 60% at the presence of surfactants [29]. It has been concluded that differences in proportion of EE may be due to the method of formulation and the solvent used for extraction. Based on the recent studies, optimized nano GR-β-CD inclusion complexes using RSM have shown a higher encapsulation efficiency, compared to the previous studies [30]. The current results are partly similar to the results by other researchers. As shown in Figs. 1c and 1d, the mean particle size and zeta-potential of optimized nanoinclusion complexes (F3 formulation) was in the range of 117 nm ±1 and -17.7 mV ±2.3, respectively. Furthermore, NPs were uniform and monodispersed and PDI was in the range of 0.14 ±0.03.

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Table 2. Effects of various ratios of β-CD and GR on loading and encapsulation efficiencies of the nanoparticle formulation using analysis of variance of regression coefficients from the fitted response surface quadratic model

Formulation	Independent variable		Response		Source	Loading (Y1)		Encapsulation efficiency (Y2)	
	X_1	X_2	*F*-value	*P*-value		*F*-value	*P*-value	*F*-value	*P*-value
F1	87.50	12.50	87.50	12.50	Model	78.35	< 0.0001	89.74	< 0.0001
F2	87.50	12.50	87.50	12.50	A-β-CD	244.37	< 0.0001	7.44	0.0414
F3	98.11	12.50	98.11	12.50	B-Gr	2.61	0.1674	308.61	< 0.0001
F4	87.50	1.89	87.50	1.89	AB	5.12	0.0732	7.04	0.0452
F5	80.00	5.00	80.00	5.00	A2	11.16	0.0206	4.989E-003	0.9464
F6	87.50	23.11	87.50	23.11	B2	51.06	0.0008	241.70	< 0.0001
F7	87.50	12.50	87.50	12.50	Lack of fit	1.30	0.3171	0.53	0.5056
F8	80.00	20.00	80.00	20.00	R^2	0.83		0.88	
F9	76.89	12.50	76.89	12.50					
F10	87.50	12.50	87.50	12.50					
F11	95.00	5.00	95.00	5.00					
F12	95.00	20.00	95.00	20.00					
F13	87.5	12.50	87.5	12.50					

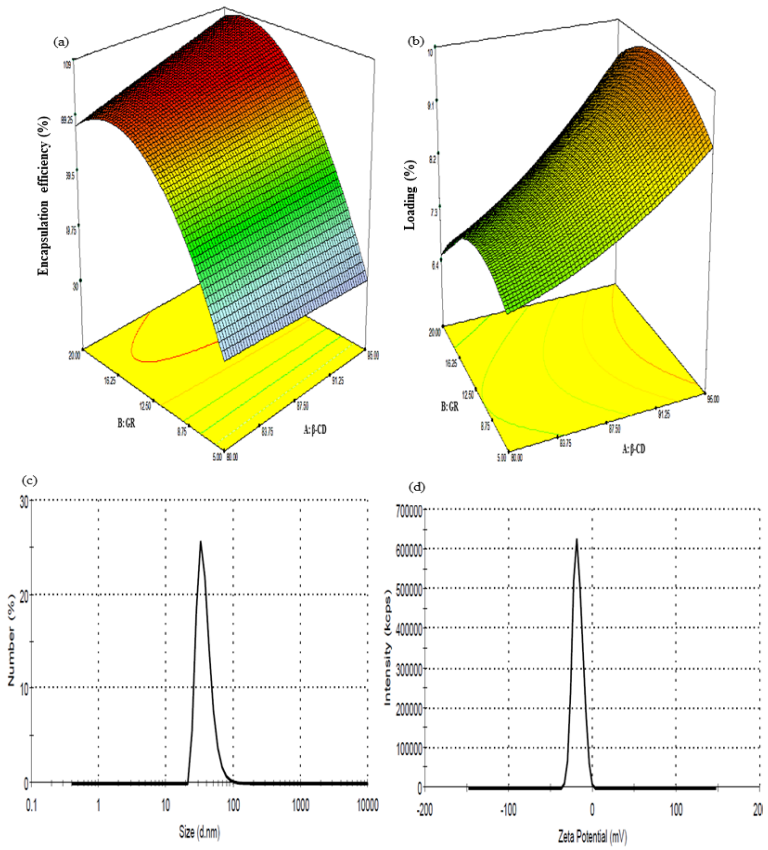

Fig. 1. Response surface plots of nano-inclusion complexes on encapsulation efficiency (1a) and loading (1b) as two functions of β-CD and GR variables and particle size (1c) and zeta potential (1d) of β-CD-GR nano-inclusion complexes (F3 formulation).

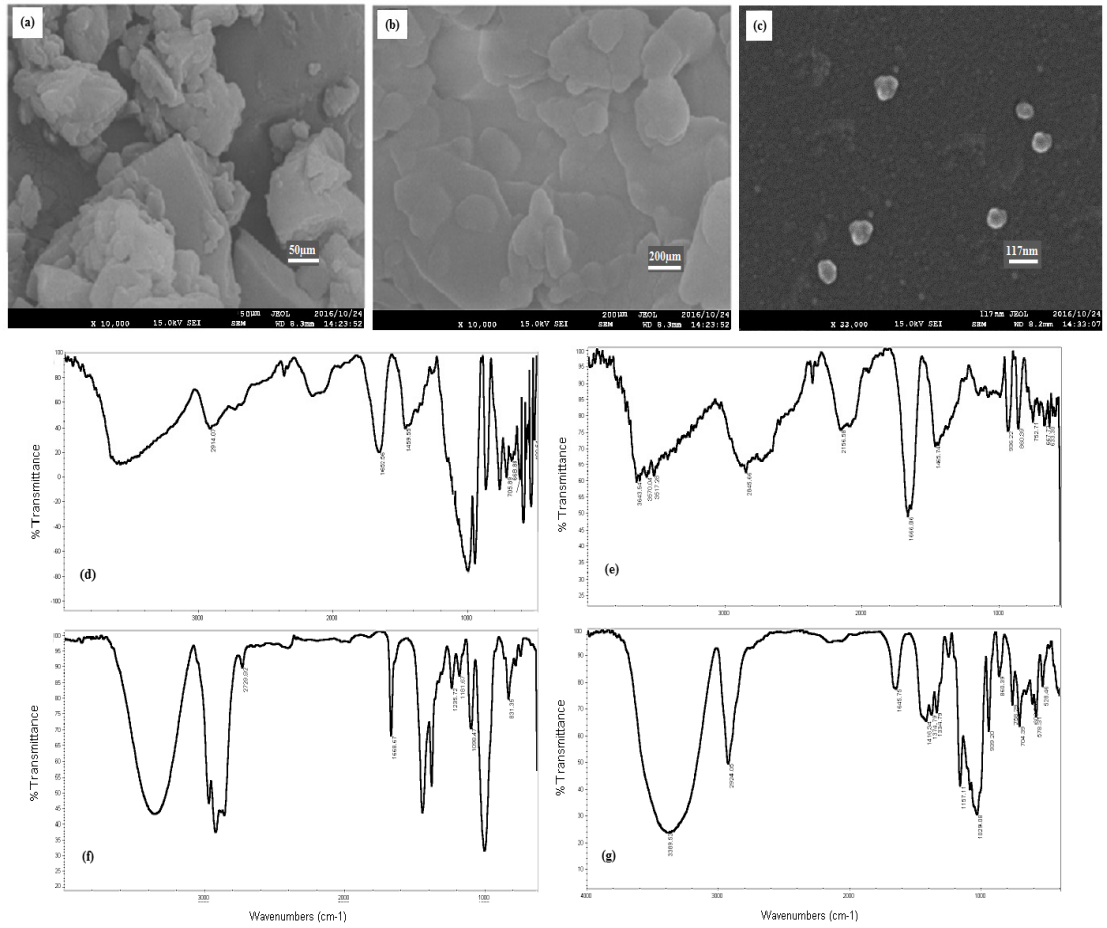

Fig. 2. SEM images of pure β-CD (2a), β-CD-GR physical mixture (2b) and nano-inclusion complexes formulation (2c) and IR spectra of β-CD (2d), GR (2e), β-CD-GR physical mixture (2f) and β-CD-GR nano-inclusion complexes (2g).

Scanning electron microscopy and infrared spectroscopy

Fig. 2 demonstrates images of the pure β-CD, GR-β-CD physical mixture and F3 formulation. Pure β-CD showed crystalline particles with various sizes with no characteristic shapes (Fig. 2a). As illustrated in Fig. 2c, particle shape and morphology of F3 formula exhibited various forms, compared to that of physical mixture of GR-β-CD (Fig. 2b). Pellets of the GR-β-CD included a uniform porous surface (Fig. 2c).

The IR spectra for GR (Fig. 2d) showed absorption bands at 3354 (O-H stretching vibration), 2968, 2919 and 2729 (C-H stretching vibration), 1668 and 1444 (C=C stretching vibration), 1235 (C-C stretching vibration) and 1118, 1098 and 1001 (C-O stretching vibration) cm^{-1}. The IR spectra for β-CD (Fig. 2e) included absorption bands at 3582 (O-H stretching vibration), 2914 (C-H stretching vibration), 1652 (H-O-H stretching vibration), 1159 (C-O stretching vibration) and 1091 (C-O-C stretching vibration) cm^{-1}. As shown in Fig. 2f, changes were detected in shape, position and intensity of the absorption bands of pure β-CD, the physical mixture and the inclusion complexes. The physical mixture (Fig. 2f) included a combination of the individual patterns of β-CD and GR (F3 formulation). In IR spectrum the peak at 3000 cm^{-1} corresponding to the C-H group stretching [12, 29]. Although obvious changes were seen in spectra of the inclusion complex of GR and β-CD (Fig. 2g), absorption peaks shifted to lower frequencies at 3389, 2924, 1645 and 1416 cm^{-1}. These changes could demonstrate formation of the intramolecular hydrogen bonds in GR-β-CD inclusion complex. When the functional groups of the drug were included within the apolar cavity of β-CD in the complex an apparent decrease of

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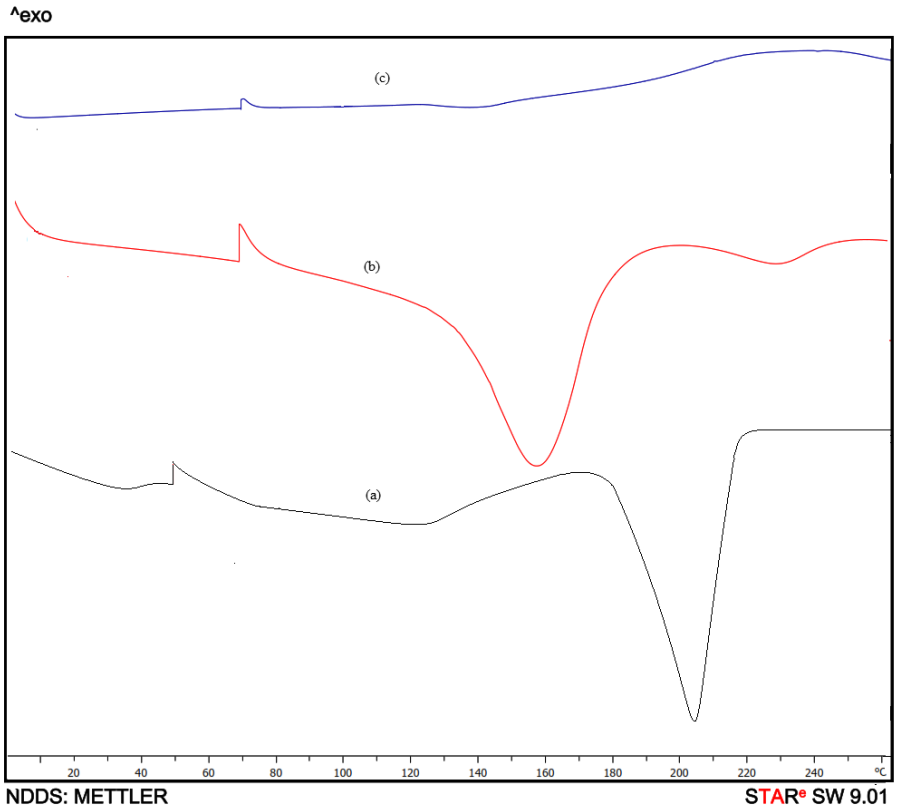

Fig. 3. DSC thermograms of (3a) geraniol, (3b) β-CD/ geraniol physical mixture and (3c) β-CD/ geraniol inclusion complex (F3 formulation)

the absorption intensities of the corresponding bands is appeared [32]. This results are similar to other findings [12, 28]. In solid state complexes, the only host-guest interactions show are O H· · ·O hydrogen bonds between the hydroxyl groups of geraniol and cyclodextrins [16]. As previously reported by Chadha et al., the present IR results have demonstrated that the inclusion complex prepared using coprecipitation method changed overall intensity of the IR absorption patterns of GR complex with β-CD and presence of interactions between GR and β-CD (46). The SEM micrographs have verified interactions between the matrix and the guest in the inclusion complexes. A similar phenomenon has been reported in literatures [12, 13, 29, 30].

Differential scanning calorimetry

In fact, DSCs are used for the investigation of stability and physical and chemical interactions between β-cyclodextrin and guest molecules. If the guest molecules are well placed in polymer matrices, no exothermic or endothermic peak should be observed in guest-polymer or bioactive-polymer thermograms [9, 45]. The DSC thermograms of samples are presented in Fig. 3. The guest substance might change by melting, evaporation, decomposition, oxidation or polymorphic transition. It has generally been reported that disappearance of endothermic peaks, appearance of new peaks and peak broadening or shifting to various temperatures indicate transition in crystal-lattice, melting, boiling and sublimation points of the inclusion complexes formation. In this study, DSC thermograms of geraniol (Fig. 3a) showed endothermic melting peaks at 248 and 155 °C, possibly due to the oxidation or elimination of water [9, 47].

Thermograms of β-CD and geraniol physical mixture (Fig. 3b) have shown two endothermic peaks at nearly 130 and 240 °C, which were close to those of pure β-CD and geraniol. These may indicate less or no interactions between the drug and β-CD in the physical mixture. In contrast, DSC curves for F1 formulation (Fig. 3c) have shown no endothermic peaks, which suggest that the guest was incorporated into the NPs and verify formation of a host-guest inclusion complex. As

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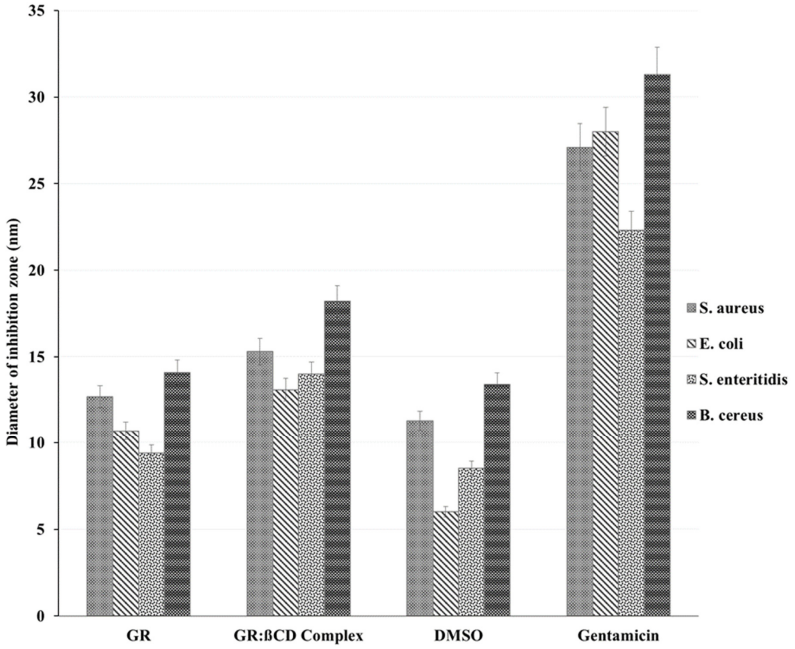

Fig. 4. Diameter of inhibition zones (mm) produced by the studied bacteria against GR and β-CD-GR nano-inclusion complexes (F3 formulation).

reported by the previous studies, elimination of the free-geraniol and β-cyclodextrin peaks demonstrates formation of a complex between these two compounds [12, 41, 47].

Antimicrobial activity of the GR-β-CD inclusion complexes

In this study, antimicrobial properties of F3 formulation against two Gram-positive bacteria of *S. aureus* and *B. cereus* and two Gram-negative bacteria of *S. enteritidis* and *E. coli* were investigated. As illustrated in Fig. 4, diameters of the GR inhibition zones were significantly larger than those of the DMSO inhibition zones, indicating that antibacterial effects of GR against Gram-positive and Gram-negative bacteria were significantly greater than those of DMSO and water.

In all experiments, diameters of the inhibition zones of gentamicin showed gentamicin as an effective antibiotic against the highlighted bacteria. This has verified that the current method for the assessment antimicrobial activity was carried out correctly. Moreover, MIC of the encapsulated GR was significantly larger than that of its free form, representing a stronger antibacterial effect of the GR-β-CD inclusion complexes ($P < 0.05$) (Table 3). Comparisons between the antibacterial activities of free and complexed GR and the gentamicin showed significant differences ($P < 0.001$). This study showed that the MIC of GR for Gram-positive bacteria was mostly lower than that for Gram-negative bacteria. The MIC varied from 3×10^3 to 5×10^3 μg mL^{-1} for free GR, while it varied from 2×10^3 to $\times 10^3$ μg mL^{-1} for the GR-β-CD inclusion complex.

Considering MIC values and diameters of the inhibition zones (Fig. 4 and Table 3), *B. cereus* and *S. entritidis* were the most sensitive and the most resistant bacteria to GR either in complex or free form, respectively. These results are likely linked to the fact that further resistance of Gram-negative bacteria to GR can be attributed to the presence of their outer membranes, preventing penetration of lipophilic EOs into the bacterial cells [48, 49]. The β-CD is a relatively large molecule with a number of hydrogen donors and acceptors in its chemical structure. When poorly soluble components such as essential oils are complexed with β-CD, aqueous solubility and efficacy of the poorly soluble components are improved [44, 45]. Results from the current study are similar to results from Hill et al. study, who showed that the antimicrobial efficiency of GR in β-cyclodextrin complexes was higher than that in its free forms since it increased water solubility

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Table 3. Minimum inhibitory concentration of free GR and GR-β-CD* inclusion complexes against the highlighted microorganisms using agar and broth dilution methods

Microorganism	MIC (μg mL^{-1})		Control		
	Geraniol	GR-βCD	DMSO	Gentamicin (2 μg mL^{-1})	Amphotericin-B (2.5 μg mL^{-1})
Staphylococcus aureus PTCC 1112	$5 \times 10^{3a**}$	$4 \times 10^{3b*}$	+	-	
E. coli PTCC 1330	$3 \times 10^{3a*}$	$2 \times 10^{3b*}$	+	-	
Salmonella enteritidis PTCC 1709	$5 \times 10^{3a*}$	$3 \times -10^{3b*}$	+	-	
Bacillus cereus PTCC 1015	$3 \times 10^{3a*}$	$2 \times 10^{3b*}$	+	-	
Candida albicans PTCC 5027	$4 \times 10^{3a*}$	$3 \times 10^{3b*}$	+		-
Aspergillus niger PTCC 5012	$4 \times 10^{3a*}$	$3 \times 10^{3b*}$	+		-

MIC, minimum inhibitory concentration; DMSO, dimethyl sulfoxide; *F3 formulation; **different letters show statistically significant differences ($P < 0.05$, replicates = 2); growth (+); no growth (-)

Table 4. Diameters* of inhibition zones of free GR and GR-β-CD inclusion complexes** against *Candida albicans* PTCC 5027 and *Aspergillus niger* PTCC 5012 using agar well diffusion method

Fungus	Geraniol (Mean ±SD)	GR-β-CD (Mean ±SD)	DMSO control	Growth contrc
Aspergillus niger	7.7 ±1.2 a***	15.8 ±0.6 b	+	+
Candida albicans	12 ±1.0 a	14.1 ±1.7 b	+	+
Amphotericin-B	2.5		+	+
	-	-		

*Diameters of the wells included 7 mm (replicates = 2); **F3 formulation; ***different letters show statistically significant differences ($P < 0.05$); growth (+); no growth (-)

of the lipophilic compounds and hence helped its easier penetration into the bacterial cells [13]. In another study, it was indicated that antibacterial activity of the monoterpenoid alcohols such as linalool, nerol, citronellol and GR was stronger than antifungal activity of these compounds [50]. Wang et al. reported that β-CD inclusion complexes improved antimicrobial activity of the aromatic oily liquids extracted from EOs such as eugenol through increasing its accessibility to susceptible sites such as membrane and cytoplasm of the microorganisms by enhancing EO solubility [12]. However, advantages of the β-CD encapsulation are not limited to increasing solubility of the EO components. Improvement of the antimicrobial mode of action, decrease of the minimal concentration needed for antimicrobial inhibition and protection of EOs against degradation are other important advantages [12, 41]. The greater susceptibility of Gram-positive bacteria to EOs has been reported severally in literatures [49, 50]. Results of the current study have suggested that the MIC of free GR and βCD-GR nanoinclusion complexes against the Gram-positive and Gram-negative bacteria is much higher than 100 μg mL^{-1} indicating its weak antimicrobial activity; as previously reported by other studies [48, 51].

In the present study, antifungal activities of GR and GR-β-CD inclusion complexes against *C. albicans* and *A. niger* were assessed using microdilution method at concentrations of 1000–6000 μg mL^{-1}. Free GR and GR-β-CD complexes showed antifungal activities at completely different concentrations ($P < 0.05$). The MIC values of free GR and GR-β-CD complexes against *C. albicans* were seen at concentrations greater than 4000 and 3000 μg mL^{-1}, respectively (Table 4). A similar result was observed for *A. niger* at concentrations greater than 4000 and 3000 μg mL^{-1}, respectively. These MIC values were significantly greater than those of amphotericin B. As Table 3 demonstrates, diameters of the antifungal inhibition zones against *C. albicans* and *A. niger* induced by GR-β-CD complexes were greater than those induced by its free form ($P < 0.05$).

This may support inhibitory effects of nanocomplex and free GR against *C. albicans* growth as 3×10^3 and 4×10^3 μg mL^{-1}, respectively. These values are much higher, compared to the value of 100 μg mL^{-1}. This finding was previously reported by Ayala-Zavala et al. for the inhibitory effects of EO inclusion complexes against fungal growth [52]. Singh et al. showed that disruption of membrane and lose of its integrity were the possible action mechanisms of GR against *C. albicans* cells using micrographic images [53]. According to Zanetti et

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al. who studied the antibacterial effects of pure geraniol and cinnamic acid, GR showed a greater antimicrobial activity against a broad spectrum of pathogenic bacteria, similar to that demonstrated in the present study. They suggested this function as a principal mechanism; by which, geraniol and the nerol included double bonds in their molecules. Therefore, they showed a greater antimicrobial activity against the highlighted bacteria, compared to that the other essential oils with single bonds did [54]. Other studies support relationships of the chemical structure of EOs and associated compounds such as position of the OH group in structure of geraniol, possibly showing roles in their antimicrobial activities. Nanoinclusion complexes of geraniol included a higher antimicrobial activity than that the pure or free form of geraniol did. Nanoinclusion complex containing bioactive compounds is an efficient methodology approach to increase physical stability of the active substances, retain them from interactions with other ingredients and increase their bioactivity [47, 54–56]. In general, it seems that biomolecules in nanoparticle structures, as appropriate carrier systems, have improved their delivery characteristics such as antimicrobial activity, compared to single forms of the compounds.

CONCLUSION

In the present study, precipitation method were used for the fabrication of GR-β-CD inclusion complexes with a size distribution of 117 nm ±1. Physical characteristics of GR-β-CD inclusion complexes were verified using SEM, IR spectroscopy and DSC analysis. Response surface design has been shown to be effective for optimization of factors involved in developing of GR-β-CD inclusion complexes with high loading and encapsulation efficiencies. Furthermore, the optimized formulation has demonstrated significant antimicrobial activities against two Gram-positive and two Gram-negative bacteria, compared to free geraniol with a similar concentration. Based on the optimized GR-β-CD nanoinclusion complexes (F3 formulation), the maximum and the minimum inhibition zones of the GR-β-CD complexes have been recorded for *B. cereus* and *E. coli,* respectively. Based on these results and biological activities of the developed formulation, further *in vivo* studies on the use of this compound in food industries are necessary.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Chen F, Li W, Jiang L, Pu X, Yang Y, Zhang G, et al. Functional characterization of a geraniol synthase-encoding gene from Camptotheca acuminata and its application in production of geraniol in Escherichia coli. Journal of Industrial Microbiology and Biotechnology. 2016;43(9):1281-92.
2. Jin X, Sun J, Miao X, Liu G, Zhong D. Inhibitory effect of geraniol in combination with gemcitabine on proliferation of BXPC-3 human pancreatic cancer cells. Journal of International Medical Research. 2013;41(4):993-1001.
3. Babukumar S, Vinothkumar V, Sankaranarayanan C, Srinivasan S. Geraniol, a natural monoterpene, ameliorates hyperglycemia by attenuating the key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats. Pharmaceutical Biology. 2017;55(1):1442-9.
4. Dehsheikh AB, Sourestani MM, Dehsheikh PB, Mottaghipisheh J, Vitalini S, Iriti M. Monoterpenes: Essential Oil Components with Valuable Features. Mini-Reviews in Medicinal Chemistry. 2020;20(11):958-74.
5. Kringel DH, Antunes MD, Klein B, Crizel RL, Wagner R, de Oliveira RP, et al. Production, Characterization, and Stability of Orange or Eucalyptus Essential Oil/β-Cyclodextrin Inclusion Complex. Journal of Food Science. 2017;82(11):2598-605.
6. de Assis KMA, de A. Rêgo RI, de Melo DF, da Silva LM, Oshiro- Júnior JA, Formiga FR, et al. Therapeutic Potential of Melaleuca alternifolia Essential Oil in New Drug Delivery Systems. Current Pharmaceutical Design. 2020;26(33):4048-55.
7. Rota C, CarramiÑAna JJ, Burillo J, Herrera A. In Vitro Antimicrobial Activity of Essential Oils from Aromatic Plants against Selected Foodborne Pathogens. Journal of Food Protection. 2004;67(6):1252-6.
8. Haloci E, Toska V, Shkreli R, Goci E, Vertuani S, Manfredini S. Encapsulation of Satureja montana essential oil in β-cyclodextrin. Journal of Inclusion Phenomena and Macrocyclic Chemistry. 2014;80(1-2):147-53.
9. Guimarães AG, Oliveira MA, Alves RdS, Menezes PdP, Serafini MR, de Souza Araújo AA, et al. Encapsulation of carvacrol, a monoterpene present in the essential oil of oregano, with β-cyclodextrin, improves the pharmacological response on cancer pain experimental protocols. Chemico-Biological Interactions. 2015;227:69-76.
10. de Oliveira JL, Campos EVR, Pereira AES, Nunes LES, da Silva CCL, Pasquoto T, et al. Geraniol Encapsulated in Chitosan/Gum Arabic Nanoparticles: A Promising System for Pest Management in Sustainable Agriculture. Journal of Agricultural and Food Chemistry. 2018;66(21):5325-34.
11. Wang J, Cao Y, Sun B, Wang C. Physicochemical and release

characterisation of garlic oil-β-cyclodextrin inclusion complexes. Food Chemistry. 2011;127(4):1680-5.
12. Wang T, Li B, Si H, Lin L, Chen L. Release characteristics and antibacterial activity of solid state eugenol/β-cyclodextrin inclusion complex. Journal of Inclusion Phenomena and Macrocyclic Chemistry. 2011;71(1-2):207-13.
13. Hill LE, Gomes C, Taylor TM. Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. LWT - Food Science and Technology. 2013;51(1):86-93.
14. Mourtzinos I, Kalogeropoulos N, Papadakis SE, Konstantinou K, Karathanos VT. Encapsulation of Nutraceutical Monoterpenes in β-Cyclodextrin and Modified Starch. Journal of Food Science. 2007;73(1):S89-S94.
15. Kayaci F, Sen HS, Durgun E, Uyar T. Functional electrospun polymeric nanofibers incorporating geraniol–cyclodextrin inclusion complexes: High thermal stability and enhanced durability of geraniol. Food Research International. 2014;62:424-31.
16. Ceborska M, Szwed K, Asztemborska M, Wszelaka-Rylik M, Kicińska E, Suwińska K. Study of β-cyclodextrin inclusion complexes with volatile molecules geraniol and α-terpineol enantiomers in solid state and in solution. Chemical Physics Letters. 2015;641:44-50.
17. Miladinović DL, Ilić BS, Kocić BD, Miladinović MD. An in vitro Antibacterial Study of Savory Essential Oil and Geraniol in Combination with Standard Antimicrobials. Natural Product Communications. 2014;9(11):1934578X400901.
18. Guimarães AC, Meireles LM, Lemos MF, Guimarães MCC, Endringer DC, Fronza M, et al. Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. Molecules. 2019;24(13):2471.
19. Tomadoni B, Cassani L, Moreira MR, Ponce A. Efficacy of vanillin and geraniol in reducing Escherichia coli O157:H7 on strawberry juice. LWT - Food Science and Technology. 2015;64(2):554-7.
20. Coutinho HDM, de Freitas MA, Gondim CNFL, de Albuquerque RS, de Alencar Ferreira JV, Andrade JC. In vitro antimicrobial activity of Geraniol and Cariophyllene against Staphylococcus aureus. Revista Cubana de Plantas Medicinales. 2015;20(1):98-105.
21. Pontes EKU, Melo HM, Nogueira JWA, Firmino NCS, de Carvalho MG, Catunda Júnior FEA, et al. Antibiofilm activity of the essential oil of citronella (Cymbopogon nardus) and its major component, geraniol, on the bacterial biofilms of Staphylococcus aureus. Food Science and Biotechnology. 2018;28(3):633-9.
22. Ilić BS, Kocić BD, Ćirić VM, Cvetković OG, Miladinović DL. AnIn VitroSynergistic Interaction of Combinations ofThymus glabrescensEssential Oil and Its Main Constituents with Chloramphenicol. The Scientific World Journal. 2014;2014:1-12.
23. Badawy MEI, Marei GIK, Rabea EI, Taktak NEM. Antimicrobial and antioxidant activities of hydrocarbon and oxygenated monoterpenes against some foodborne pathogens through in vitro and in silico studies. Pesticide Biochemistry and Physiology. 2019;158:185-200.
24. Sharma Y, Khan LA, Manzoor N. Anti-Candida activity of geraniol involves disruption of cell membrane integrity and function. Journal de Mycologie Médicale / Journal of Medical Mycology. 2016;26(3):244-54.
25. Marei GIK, Abdel Rasoul MA, Abdelgaleil SAM. Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. Pesticide Biochemistry and Physiology. 2012;103(1):56-61.
26. Leite MCA, de Brito Bezerra AP, de Sousa JP, de Oliveira Lima E. Investigating the antifungal activity and mechanism(s) of geraniol against Candida albicans strains. Medical Mycology. 2014;53(3):275-84.
27. Singulani JL, Pedroso RS, Ribeiro AB, Nicolella HD, Freitas KS, Damasceno JL, et al. Geraniol and linalool anticandidal activity, genotoxic potential and embryotoxic effect on zebrafish. Future Microbiology. 2018;13(15):1637-46.
28. Miron D, Battisti F, Silva FK, Lana AD, Pippi B, Casanova B, et al. Antifungal activity and mechanism of action of monoterpenes against dermatophytes and yeasts. Revista Brasileira de Farmacognosia. 2014;24(6):660-7.
29. Menezes PP, Serafini MR, Santana BV, Nunes RS, Quintans LJ, Silva GF, et al. Solid-state β-cyclodextrin complexes containing geraniol. Thermochimica Acta. 2012;548:45-50.
30. Hadian Z, Maleki M, Abdi K, Atyabi F, Mohammadi A, Khaksar R. Preparation and characterization of nanoparticle β-Cyclodextrin:Geraniol inclusion complexes. Iranian Journal of Pharmaceutical Research. 2018;17(1):39-51.
31. Haidong L, Fang Y, Zhihong T, Changle R. Study on preparation of β-cyclodextrin encapsulation tea extract. International Journal of Biological Macromolecules. 2011;49(4):561-6.
32. Meyers RH, Montgomery DC, Anderson-Cook CM. Response Surface Methodology: Process and Product Optimization Using Designed Experiments: John Wiley & Sons; 2016.
33. Gabbay Alves TV, Silva da Costa R, Aliakbarian B, Casazza AA, Perego P, Carréra Silva Júnior JO, et al. Microencapsulation of Theobroma cacao L. waste extract: optimization using response surface methodology. Journal of Microencapsulation. 2017;34(2):111-20.
34. Venkateswarlu C, Jujjavarapu SE. Stochastic Global Optimization Methods and Applications to Chemical, Biochemical, Pharmaceutical and Environmental Processes. 1st ed: Elsevier; 2020.
35. Sutaphanit P, Chitprasert P. Optimisation of microencapsulation of holy basil essential oil in gelatin by response surface methodology. Food Chemistry. 2014;150:313-20.
36. Rachmawati H, Edityaningrum CA, Mauludin R. Molecular Inclusion Complex of Curcumin–β-Cyclodextrin Nanoparticle to Enhance Curcumin Skin Permeability from Hydrophilic Matrix Gel. AAPS PharmSciTech. 2013;14(4):1303-12.
37. Cornell JA. Experiments with mixtures: designs, models, and the analysis of mixture data: John Wiley & Sons; 2002 978-0-471-39367-2.
38. Bhandari BR, D'Arc BR, Thi Bich LL. Lemon Oil to β-Cyclodextrin Ratio Effect on the Inclusion Efficiency of β-Cyclodextrin and the Retention of Oil Volatiles in the Complex. Journal of Agricultural and Food Chemistry. 1998;46(4):1494-9.
39. Bhandari BR, D'Arc BR, Padukka I. Encapsulation of Lemon Oil by Paste Method Using β-Cyclodextrin: Encapsulation Efficiency and Profile of Oil Volatiles. Journal of Agricultural and Food Chemistry. 1999;47(12):5194-7.
40. Tao F, Hill LE, Peng Y, Gomes CL. Synthesis and characterization of β-cyclodextrin inclusion complexes

of thymol and thyme oil for antimicrobial delivery applications. LWT - Food Science and Technology. 2014;59(1):247-55.
41. Nait Bachir Y, Nait Bachir R, Hadj-Ziane-Zafour A. Nanodispersions stabilized by β-cyclodextrin nanosponges: application for simultaneous enhancement of bioactivity and stability of sage essential oil. Drug Development and Industrial Pharmacy. 2018;45(2):333-47.
42. Forbes BA. Laboratory methods in basic mycology. Bailey & Scott's diagnostic microbiology. 2002:711-97.
43. Şahin F, Karaman İ, Güllüce M, Öğütçü H, Şengül M, Adıgüzel A, et al. Evaluation of antimicrobial activities of Satureja hortensis L. Journal of Ethnopharmacology. 2003;87(1):61-5.
44. Andrews JM. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy. 2001;48(suppl_1):5-16.
45. Zhang L, Xu W, Sun Y, Li L, Gao C, Wang J. Response surface optimization of the preparation of baicalin-β-Cyclodextrin inclusion compound. Latin American Journal of Pharmacy. 2013;32(8):1146-51.
46. Chadha R, Arora P, Bhandari S, Jain DVS. Effect of hydrophilic polymer on complexing efficiency of cyclodextrins towards efavirenz-characterization and thermodynamic parameters. Journal of Inclusion Phenomena and Macrocyclic Chemistry. 2011;72(3-4):275-87.
47. Abarca RL, Rodríguez FJ, Guarda A, Galotto MJ, Bruna JE. Characterization of beta-cyclodextrin inclusion complexes containing an essential oil component. Food Chemistry. 2016;196:968-75.
48. Zhao J, Zhao G, Liu Y. Antibacterial activity of a hexahydro-β-acids/methyl-β-cyclodextrin inclusion complex against bacteria related to foodborne illness. Journal of Food Safety. 2019;39(5).
49. Ayala-Zavala JF, Soto-Valdez H, González-León A, Álvarez-Parrilla E, Martín-Belloso O, González-Aguilar GA. Microencapsulation of cinnamon leaf (Cinnamomum zeylanicum) and garlic (Allium sativum) oils in β-cyclodextrin. Journal of Inclusion Phenomena and Macrocyclic Chemistry. 2007;60(3-4):359-68.
50. Suppakul P, Miltz J, Sonneveld K, Bigger SW. Antimicrobial Properties of Basil and Its Possible Application in Food Packaging. Journal of Agricultural and Food Chemistry. 2003;51(11):3197-207.
51. Ozturk S, Ercisli S. The chemical composition of essential oil and in vitro antibacterial activities of essential oil and methanol extract of Ziziphora persica Bunge. Journal of Ethnopharmacology. 2006;106(3):372-6.
52. Ayala-Zavala JF, del-Toro-Sánchez L, Alvarez-Parrilla E, González-Aguilar GA. High Relative Humidity In-Package of Fresh-Cut Fruits and Vegetables: Advantage or Disadvantage Considering Microbiological Problems and Antimicrobial Delivering Systems? Journal of Food Science. 2008;73(4):R41-R7.
53. Singh S, Fatima Z, Hameed S. Insights into the mode of action of anticandidal herbal monoterpenoid geraniol reveal disruption of multiple MDR mechanisms and virulence attributes in Candida albicans. Archives of Microbiology. 2016;198(5):459-72.
54. Zanetti M, Ternus Z, Dalcanton F, De Mello M, De Oliveira D, Araujo P, et al. Microbiological characterization of pure geraniol and comparison with bactericidal activity of the cinnamic acid in gram-positive and gram-negative bacteria. J Microb Biochem Technol. 2015;7(4):186-93.
55. Prakash B, Kujur A, Yadav A, Kumar A, Singh PP, Dubey NK. Nanoencapsulation: An efficient technology to boost the antimicrobial potential of plant essential oils in food system. Food Control. 2018;89:1-11.
56. Assadpour E, Mahdi Jafari S. A systematic review on nanoencapsulation of food bioactive ingredients and nutraceuticals by various nanocarriers. Critical Reviews in Food Science and Nutrition. 2018;59(19):3129-51.

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