RESEARCH PAPER

In vitro cytotoxicity of *Cuminum cyminum* essential oil loaded SLN nanoparticle

Massoud Tavassolirajaee [1], Maryam Tatari [2]*, Malihe Samadi Kazemi [3], Seyedeh Faezeh Taghizadeh [4, 5]

[1]Department of Horticultural Science, Faculty of Agriculture, Shirvan Branch, Islamic Azad University, Shirvan, Iran
[2]Department of Agronomy, Faculty of Agriculture, Shirvan Branch, Islamic Azad University, Shirvan, Iran
[3]Department of Chemistry, Faculty of Sciences, Bojnourd Branch, Islamic Azad University, Bojnourd, Iran
[4]Department of Horticultural Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
[5]Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

Objective(s): Encapsulation of essential oils (EOs) into the nano-carrier leads to reduced EOs volatility and oxidation, as well as increased therapeutic efficiency. This study assessed the chemical composition and cytotoxic effects of cumin essential oil (CEO) and nano-encapsulation cumin essential oil (NECEO) against three cancer cell lines.

Materials and Methods: Solid lipid nanoparticle (SLN) formulations were evaluated for their size, zeta potential and encapsulation efficiency (EE). Isolation of the CEO and NECEO and their chemical composition were analyzed by gas chromatography-mass spectrometry (GC/MS). Cytotoxicity of CEO and NECEO against human lung cancer (A549), human breast cancer (MCF7), and human prostate cancer (PC3) was examined using MTT assay.

Results: Our findings showed that in CEO and NECEO, the major component was cuminaldehyde (24.5 and 26.0%, respectively). GC/MS analysis of NECEO chemical composition exposed 27 components in EOs accounting for 91.5 and 98.9% of CEO and NECEO, respectively. The results showed that solid lipid nanoparticle (SLN) induced the concentration of the main volatile components. Based on the characterization of SLN, the EE percent ranged from 23.00±4.1% to 96.21±5.5%. The loading capacity (LC) of NECEO ranged between 0.00±0.0 to 7.05±0.5%. Moreover, the yield of NECEO was 79.14%. Based on results, Z-average, polydispersity index, and zeta potentials of formulation were 1252±21.4 nm, 0.423±0.03, and 17±0.52, respectively. The IC_{50} value of NECEO (after 48 h) against MCF7, A549, and PC3 (108, 213, and 124 µg/mL, respectively) was significantly lower than CEO (231, 219, and 325 µg/mL, respectively).

Conclusion: The results suggested that NECEO can be regarded as a promising nutrient source. These observations could be used as a basis for future experiments to further evaluate potential nanoparticles and other medicinal plant species.

Keywords: *Breast cancer; Cuminum cyminum; Lung cancer; Prostate cancer; Solid lipid nanoparticle*

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INTRODUCTION

Cuminum cyminum L. is one of the most important aromatic and medicinal plants belongs to Apiaceae family [1]. Cumin is native to Egypt; however India is the main producer and consumer in the world. Cumin used as condiment and ingredient in food industry [2, 3]. The cumin seeds are used in backing bread, biscuit, and rice. It also used in the food flavor processing industries. For example it is used in pickles, cheese, meat, and soups as a flavoring agent [4]. From traditional medicinal points of view, cumin has been used for the treatment of toothache, digestive disorders, wounds, hoarseness, epilepsy, dyspepsia, diarrhea, jaundice, and epilepsy [5]. In this regard, the pharmaceutical studies have also been shown that it is exhibited antioxidant

* *Corresponding author: Email: maryamtatari@yahoo.com*
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activity, anticancer, antimicrobial, antispasmodic, and protective properties against induced colon cancer [3, 6, 7]. Due to valuable constituents in CEO including, cuminaldehyde, ß- pinene, γ- terpinene, p- menthe-1,3- dien-7-al, and p-cymene, it can be used in the pharmaceutical, cosmetic, and perfumery industries [3]. The importance of CEO in the cosmetics industry is due to its potential in improving the volatile compounds in lotions, creams, perfumes, and ointment [4].

The use limitations of EOs in various industries face due to evaporation, poor stability, degradation (in high temperatures and oxidation radiations), and interactions with matrix structures and low solubility in aqueous phase. Encapsulation can enhance the properties of EOs in different industrial processes [8]. In recent years, nanotechnology has been suggested as a great solution to answer this problem. Nano-encapsulation has been used in various pharmaceutical and therapeutic applications such as, drug delivery, antibiotic delivery, vaccination and medical devices. Nano carriers can increase the antimicrobial potential of bioactive compounds by enhancing cellular interactions between them and the pathogens as results of very small size that improves the cellular uptake [9]. Current study expected to determine (1) the chemical composition of CEO and NECEO (2) the effects of SLN on encapsulation efficiency (EE), loading capacity (LC), and (3) biological activities of CEO and NECEO against A549, MCF7, and PC3 cell lines.

MATERIALS AND METHODS

Chemicals

Penicillin/Streptomycin, and (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) were purchased from Sigma-Aldrich (Steinheim, Germany). Dimethyl sulfoxide (DMSO) and methanol (MeOH) (97% purity) were supplied by Merck (Darmstadt, Germany). Fetal bovine serum (FBS) was procured Gibco, USA. RPMI1640 and Trypsin-EDTA were purchased from Bioidea Company, Iran and Bon Yakhteh, Iran, respectively.

Plant material

Cumin seeds were collected from retail market of Khorasan-Razavi province, Iran, in 2021. The seeds were identified at Ferdowsi University of Mashhad, Iran.

Extraction of CEO

CEO was obtained from cumin seed by hydrodistilaze technique using the Clevenger-type apparatus (up to 3 hr). CEO was dehydrated by Na_2SO_4 and incubated in the sealed vials at 4°C for coming tests [10].

GC/MS analysis

GC/MS analysis was done using an Agilent 5975 apparatus for both CEO and NECEO, separately. EOs were dissolved in *n*-hexane. Other analytical set had the following specifications (Table 1) [11]. Chemical composition detection of CEO and NECEO, retention indices (RI) of samples and their mass spectra were compared with those earlier reported. The relative amount of each component was calculated using the area under curve percentage without considering the calibration factor [12].

Preparation of SLN

CEO-loaded SLN was prepared by ultrasound

Table 1. Operating condition for capillary GC/MS

Parameter	Setting
Column	HP-5 MS column (30 m × 0.25 mm i.d., 0.25 µm film thicknesses)
	interfaced with a quadruple mass detector
Oven temperature	50°C (For 5 minutes)
	50–250 °C (Increasing 3°C/ minute)
	250°C (For 10 minutes)
Injector temperature	250°C
Injector volume	0.1 µL
Split ratio	1:50
Carrier gas	Helium
Gas flow rate	1.1 ml/minute
Ionization potential	70 eV
Ionization current	150 µA
Mass range	35-465 mui

method at high-shear homogenization. The lipid phase, including GMS, precirol, and stearic acid (5% and 3%) was liquefied through heating around 70°C. Tween 80 and poloxamer 188 (2.5% and 1.25%) were used as surfactants. The hot aqueous phases and molten lipid were mixed and homogenized with T 25 Ultra Turrax at 20,500 rpm for 5 min. The emulsion was ultrasonicated via probe sonicator. The probe sonication was passed over the six cycles with 30 sec of sonication separated (the intervals were 15 sec). The last formulations were chilled at room temperature. Owing the hydrophobic properties of NECEO, it was melted in the lipid phase [13].

Characterization of SLN

The dynamic light scattering (DLS) method (ZetaSizer Nano-ZS) was used for determination the size of nanoparticle. The Zetasizer was performed at 25°C and the scattering angle of 17ºC by determining the electro mobility with laser Doppler velocimetry [14]. The morphological properties of the nanoparticle formulations were determined by transmission electron microscopy (TEM; Zeiss, Germany) method. Then, the samples were scanned under nitrogen atmosphere at flow rate 20 ml/min, from 0 °C to 200 (5°C/min). Based on the differential scanning calorimetery (DSC), the bulk lipids were warmed to 100 °C and the cooled. The characteristics of SLN including clarity, particle size, zeta potential, as well as physical stability were screening for 3 months (at 2–8 °C) [15].

Encapsulation efficiency (EE), loading capacity (LC) and yield of NECEO

GC was used for determination the EE of NECEO formulations. Table 2 summerizes the operating condition [13].

To purify the SLN- NECEO, the SLN (500 µl) was transferred to the ultra-filter. Then, they were centrifuged at 10,000 rpm/ for 30 min. The filtrate was diluted with methanol/chloroform (2:1) and analyzed using a GC method. The EE (%) and LC (%) of NECEO was assessed using the equations 1 and 2:

(Equation 1)

$$\text{EE}\ (\%) = \frac{Total\ concentration\ of\ NECEO\ after\ purification}{Initial\ concentration\ of\ NECEO} \times 100$$

(Equation 2)

$$LC\ (\%) = \frac{Total\ concentration\ of\ NECEO\ SLN - loaded}{Weight\ of\ nanoemulsion} \times 100$$

The percentage of encapsulated NECEO nanoparticle yield was determined by Equation 3 [16].

(Equation 3)

$$Encapsulated\ NECEO\ nanoparticle\ yield\ (\%) = \frac{W1}{W2} \times 100$$

W1: The amount of lyophilized nanoparticle
W2: The total amount of all the individual components

Cytotoxicity assay

Cell culture

A549, MCF7, and PC3 cell lines were maintained in RPMI-1640 medium supplemented with 10% (v/v) FBS, streptomycin (100 U/mL), and penicillin (100 U/mL). The cultures were incubated at 37 °C, in a humidified atmosphere with CO_2 (5%). The fresh culture medium was replaced every 3 days [17].

Cytotoxicity assay

Cytotoxicity of various EOs against three cancer

Table 2. Operating condition for GC/MS

Parameter	Setting
Column	HP 5973 gas chromatograph column (60 m × 0.32 mm i.d., 0.25 mm film thicknesses) interfaced with FID detector using a DB-23 fused silica column
Column temperature	220°C
Injector temperature	270°C
Detector temperature	250°C
Injector volume	2 µL
Carrier gas	Nitrogen (100 kPa)
Gas flow rate	20 ml/minute
Ionization potential	70 eV
Ionization current	150 µA
Mass range	35-465 mui

cell lines was examined using MTT assay. The cells were seeded in a plate cells (96-well, 5×103 per well), each well supplemented with 100 µl of RPMI with 10% (v/v) FBS. After 24 hr, various dilutions of the different EOs were added (n=3). After 24 and 48 hr, the cells were incubated with MTT (10 µL) at 37 °C for 2 hr. The medium was removed, and then 200 µL DMSO was used for dissolving the formazan crystals as well as it was used to determine the cell death. Cytotoxicity of the extracts was presented as the cell viability (%) (Equation 4). The absorbance was assessed at 545 nm and 630 nm. Doxorubicin (Dox) was used as a positive control [18].

(Equation 4)

$$\text{Cell viability (\%)} = \frac{\text{Atc}- \text{Ab}}{\text{Ac}- \text{Ab}} \times 100$$

Atc: the absorbance of the treated cells
Ab: the absorbance of the blank
Ac: the absorbance of the control

Statistical analysis

All the tests were conducted in triplicate and data was presented as mean ± SD. One way analysis of variance (ANOVA) and Tukey- Kramer test were completed to determine statistical differences among the treatments (P<0.05) by GraphPad Prism 6.0 (GraphPad software, San Diego, CA, USA). The IC_{50} was also calculated by respective software.

RESULTS

Chemical composition in CEO and NECEO

In CEO and NECEO samples, the major components were cuminaldehyde (24.5 and 26.0%, respectively) and γ-terpinen-7-al (22.1 and 23.5%, respectively). GC/MS analysis of EOs chemical composition exposed 27 components in EOs accounting for 91.5 and 98.9% of CEO and NECEO, respectively (Table 3). The results showed that SLN induced the concentration of the main volatile components (Table 3).

Table 3. Chemical composition of CEO and NECEO

No	Compounds	KI	Peak area (%)	KI	Peak area (%)
			CEO (control)		NECEO
1	α-Pinene	927	0.5	930	1.0
2	α-thujene	936	1.1	940	1.2
3	Sabinene	971	1.1	975	1.2
4	Myrcene	973	2.1	976	2.2
5	β-Pinene	981	3.0	985	3.1
6	α-Phellandrene	990	3.1	995	3.3
7	ρ-Cymene	1001	7.3	1010	7.4
8	Limonene	1015	4.1	1025	4.3
9	1,8-Cineol	1023	0.1	1034	0.3
10	**γ-terpinene**	**1040**	**20.1**	**1055**	**21.1**
11	Terpinolene	1062	0.1	1088	0.2
12	Linalool	1098	0.1	1100	0.2
13	Endo- Fenchol	1113	0.1	1118	0.1
14	α-Campholenal	1120	tr	1122	Tr
15	*Trans*- sabinene hydrate	1135	0.1	1137	0.2
16	Terpinen-4-ol	1140	0.2	1145	0.2
17	Pinocarvone	1155	0.2	1160	0.2
18	Borneol	1165	0.2	1166	0.3
19	Terpinen-4-ol	1171	0.1	1178	0.1
20	Cryptone	1182	0.5	1184	1.0
21	α-terpineol	1188	0.1	1190	0.3
22	Myrtenal	1193	0.5	1199	1.2
23	**Cuminaldehyde**	**1233**	**24.5**	**1235**	**26.0**
24	α-guaiene	1330	0.1	1340	0.1
25	**γ-terpinen-7-al**	**1400**	**22.1**	**1421**	**23.5**
26	Bicyclogermacrene	1493	tr	1498	tr
27	*Trans*-calamenene	1530	0.1	1533	0.2
			91.5		98.9

KI: Kovats Index (Measured relative to n-alkanes)
CEO: Cumin essential oil; NECEO: Nano-encapsulation cumin essential oil

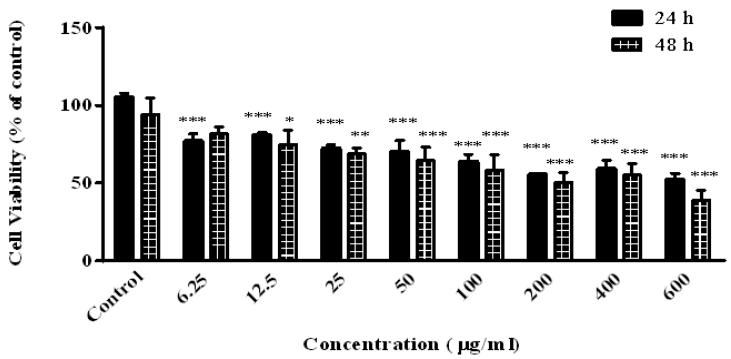

Fig. 1. Cell viability (%) of CEO against A549 cell line (*:*P*<0.05; **: *P*<0.01; ***:*P*<0.001)

SLN characterization

The EE and LC percent of all the various ratios was shown in Figure 1. The EE percent ranged from 23.00±4.1 to 96.21±5.5%. The LC of NECEO ranged between 0.00±0.0 to 7.05±0.5%. The yield of NECEO was 79.14% (Fig. 1). Based on results, Z-average, polydispersity index, and zeta potentials of formulation were 1252±21.4 nm, 0.423±0.03, and 17±0.52, respectively. It seems that, by addition of the surfactant, particle size was decreased. Moreover, it was shown that by increasing the lipid content, the particles with larger size and broader distribution were obtained. The DSC was presented that the melting peak of the lipid cores of the SLN were at low temperature.

Cytotoxic effects of CEO and NECEO against A549

The values of cell viability were determined for CEO and NECEO against A549. Biological evaluations showed that CEO had significant cytotoxic activity against the cell line after 24 and 48 h with comparison to control. The results of CEO (after 24 hr) exhibited that all the concentrations had significant effect in the cell viability percentage (Fig. 2). As shown in Figure 3, all of the concentrations of NECEO exhibited a significant percent of cell viability after 24 and 48 hr.

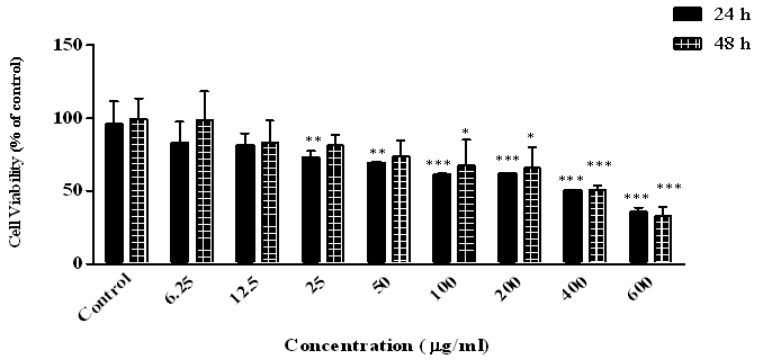

Fig. 2. Cell viability (%) of CEO against MCF7 cell line (*: *P*<0.05; **: *P*<0.01; ***: *P*<0.001)

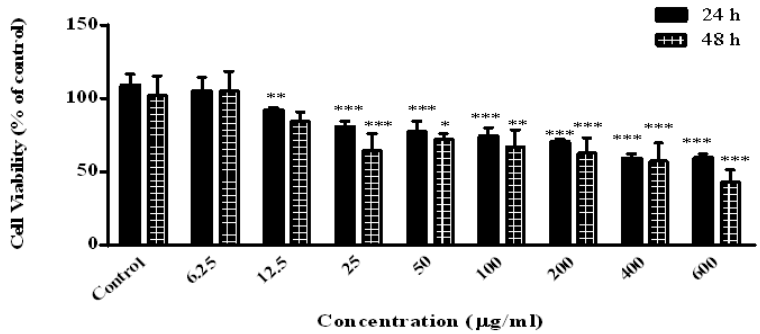

Fig. 3. Cell viability (%) of CEO against PC3 cell line (*: *P*<0.05; **:*P* <0.01; ***:*P*<0.001)

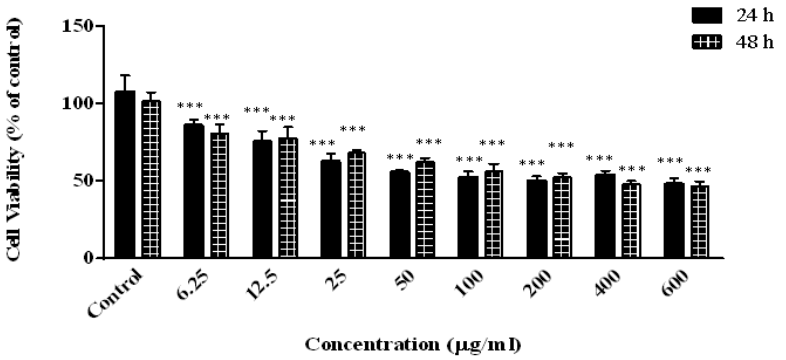

Fig. 4. Cell viability (%) of NECEO against A549 cell line (*: *P*<0.05; **: *P*<0.01; ***: *P* <0.001)

The cell viability (%) values were significantly varied in the range of concentrations 12.6-600 µg/mL after 24 h. Statistically differences were observed among the concentrations of 100, 200, 400, and 600 µg/mL after 48 hr (Fig. 3).

Cytotoxic effects of CEO and NECEO against MCF7

The MTT results of CEO showed that the growth of the cell line was significantly inhibited by the range of 25-600 µg/mL after 24 hr. No significant effects were observed in the levels of 6.25, 12.5, and 25 µg/mL after 48 (Fig. 4). As shown in Fig. 5, all of the concentrations of NECEO exhibited a significant percent of cell viability after 24 and 48 hr. Following utilization of NECEO, cell viability (%) values were significantly varied in the concentrations of 400 and 600 µg/mL after 24 hr. The statistically differences were observed among the levels of 200-600 µg/mL after 48 hr (Fig. 5).

Cytotoxic effects of CEO and NECEO against PC3

Based on our results, the concentrations of 200 and 600 µg/mL CEO (after 24 hr) showed significant cytotoxic activity. Similar results were obtained in the concentration range of 12.5-600 µg/mL after 48 hr. Cell viability (%) determined in the MTT assay revealed the significant differences among the various levels after 24 hr. The percentage of

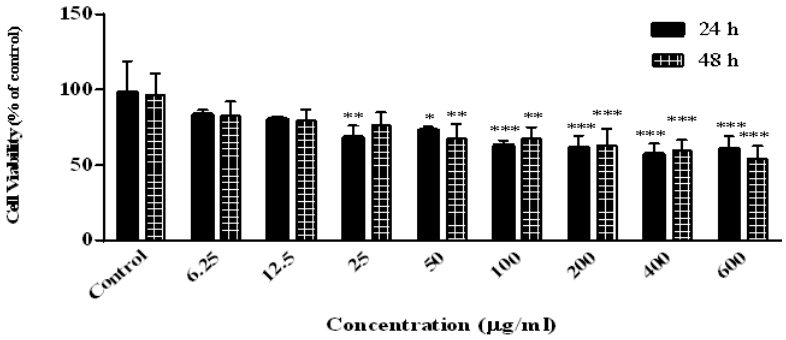

Fig. 5. Cell viability (%) of NECEO against MCF7 cell line (*: *P*<0.05; **: *P*<0.01; ***: *P*<0.001)

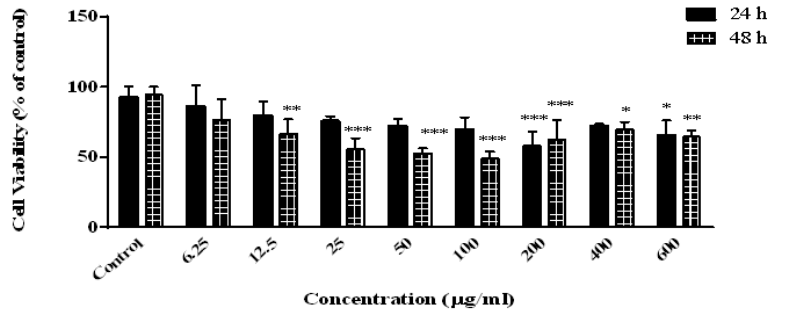

Fig. 6. Cell viability (%) of NECEO against PC3 cell line (*: *P*<0.05; **: *P*<0.01; ***: *P*<0.001)

Table 4. Cytotoxic activity (IC_{50} µg/mL) of CEO and NECEO

Cell line	NECEO		CEO		Dox
	24 h	48 h	24 h	48 h	48 h
A549	278.0±4.0	213.0±30.0	444.0±50.0	219.0±6.0	3.0±0.2
MCF7	312.0±91.0	108.0±65.0	334.0±14.0	231.0±39.0	2.0±0.3
PC3	140.0±3.0	124.0±31.0	967.0±129.0	325.0±18.0	2.0±0.3

CEO: Cumin essential oil; NECEO: Nano-encapsulation cumin essential oil

cell viability was similar for another group of the treatment after 48 hr (Fig. 6). Fig. 7 showed that the NECEO had significant cytotoxic activity against the cell line after 24 and 48 hr. The results of NECEO exhibited that the concentrations of 400 and 600 µg/mL had significant effect on the cell viability percentage after 24 hr, while the range levels of 100-600 µg/mL confirmed the significant effect after 48 hr (Fig. 7).

IC_{50} values of CEO and NECEO

After 24 and 48 hr of the exposure, IC_{50} values were determined for CEO and NECEO against different cancerous cell lines. Dox was used as a positive control and showed the IC_{50} values of 3.0±0.2, 2.0±0.3, and 2.0±0.3 µg/mL against A549, MCF7, and PC3, respectively. Comparison made between the two different EOs showed that IC_{50} values of the NECEO after 48 hr was significantly lower than those of the CEO against A549 and MCF7 cell lines used. The IC_{50} values of the NECEO against PC3 cell line were significantly lower than those of the CEO (Table 4).

EE, LC, and yield of NECEO

The EE and LC percent of all the various ratios was shown in Fig. 7. The EE percent ranged from 23.00±4.1 to 96.21±5.5%. The LC of NECEO ranged between 0.00±0.0 to 7.05±0.5%. The yield of NECEO was 79.14% (Fig. 7).

DISCUSSION

Cancer is one of the major public health problems. Natural components are used under clinical trials against various cancer cells. Despite advances in anticancer drugs, chemotherapy has revealed different side effects in clinical supervision; therefore, it can be noted to use the effective therapeutic approaches with less toxic effects [19]. CEO is the natural source of bioactive compounds. Several researches have previously expressed that CEO could be successfully

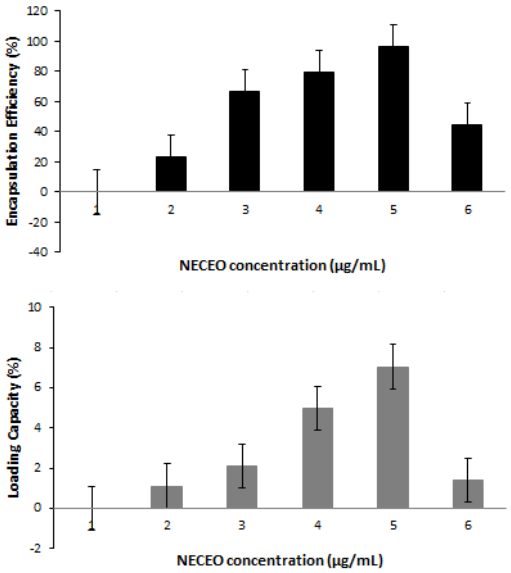

Fig. 7. Encapsulation Efficiency (%) and Loading Capacity (%) of NECEO

incorporated into nutritional and pharmaceutical products. Thus, nutritional enrichment with CEO could be a good attitude to improve the dietary value of foods or feeds [10].

Our results showed significant differences between two CEO, NECEO samples, and respective controls. Several reports have shown that the biological traits could be influenced by nano-encapsulation [20-22]. For example, encapsulation of EO may act as an agent and preserve the abundance of bioactive compounds. It was reported that treatment with nanoparticles influence EOs yield and/or chemical components [23]. Pharmacological activities of NECEO have been attributed to its anticancer potential. Our findings showed that in CEO and NECEO samples, the major components were cuminaldehyde (24.5 and 26.0%, respectively) and γ-terpinen-7-al (22.1 and 23.5%, respectively). GC/MS analysis of NECEO chemical composition exposed 27 components in EOs accounting for 91.5 and 98.9%

of CEO and NECEO, respectively. Our results showed that stimulation of CEO by nanoparticles, markedly improved cytotoxicity potential of CEO. Probably, NECEO potential in preventing free radicles was related to the presence of high levels of major components in the NECEO. Nano-capsulation can activate various signaling pathways against cancer cells. In the current experiment, cytotoxic activity of NECEO was significantly increased after stimulation with nanoparticles as a bio-efficacy factor [24]. Furthermore, an increase in CEO cytotoxicity after nano-capsulation against A549, MCF7, and PC3 cell lines was observed. The IC_{50} values of NECEO against three cell lines were significantly lower than those of the CEO. It seems that several parameters like preserve the amount of major component such as cuminaldehyde as well as type and dose of nanoparticles may influence EO traits. Thus, the results provided that SLN could be used as a delivery agent of EOs based cytotoxic agents in the food system with elevated bio efficacy.

CONCLUSION

In this study, nano-encapsulation was investigated as a method to improve the biological activities of CEO. Our findings showed that in CEO and NECEO samples, the major components were cuminaldehyde and γ-terpinen-7-al. GC/MS analysis of NECEO chemical composition exposed 27 components in EOs accounting for 91.5 and 98.9% of CEO and NECEO, respectively. Phytoconstituents can protect humans against cancers. Cumin may be observed as natural sources with cytotoxic activity. Comparison made between two different EOs (CEO and NECEO) showed that IC_{50} values of NECEO after 24 and 48 hr was significantly lower than those of the CEO against three cell lines used. SLN can be markedly enhanced cytotoxic activity of NECEO. It has great potential as a solution for inhibiting A549, MCF7, and PC3 cell lines.

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CONFLICT OF INTREST

The authors declare that they have no conflict of interest.

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