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ORIGINAL PAPER

Antioxidative, anticancer, and antibacterial activities of a nanoemulsion-based gel containing *Myrtus communis* L. essential oil

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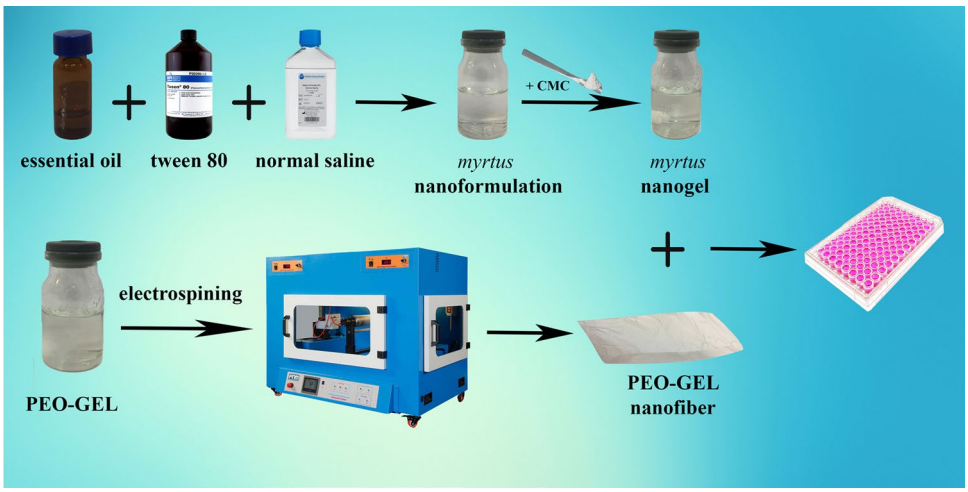
Abstract

Myrtus communis L. is a common medicinal plant with a wide range of biological properties. In this study, an attempt was made to improve its essential oil's antioxidant, anticancer, and antibacterial activities by preparing a nanogel dosage form. Alpha-pinene (29.7%), 1,8-cineole (25.8%), linalool (9.1%), linalool acetate (5.9%), and geranyl acetate (3.4%) were identified as five major compounds in the essential oil using GC–MS analysis. Optimum nanoemulsion with a droplet size of 179 ± 7 nm and a narrow droplet size distribution (SPAN 0.96) was gelified by the addition of carboxymethyl cellulose (3 w/v %). The rheometry analysis at shear rates of 0.1–100 1/s showed the viscosity was fully fitted with the Carreau-Yasuda model. The nanogel with IC_{50} 132.6 µg/mL was 4 folds more potent than the bulk essential oil (IC_{50}: 580.8 µg/mL) against A-375 melanoma cells. Besides, after treatment of *Escherichia coli* and *Staphylococcus aureus* with 1000 µg/mL of the nanogel and the bulk essential oil, their growths were observed at 37.5 and 59.1% as well as 21.4 and 40.6%. Besides, antioxidant activity was investigated using DPPH assay; the nanogel was significantly more potent ($P < 0.001$) than that of bulk essential oil at all examined concentrations (62.6–1000 µg/mL). Furthermore, polyethylene oxide-gelatin electrospun nanofibers (diameter of 359 ± 36 nm) with no effects on the cancer cell and the bacterial growth were proposed as lesion dressing after-treatment with the nanogel. Therefore, the stained nanofiber with the nanogel could be considered a natural potent anticancer and antibacterial agent in vivo study.

Graphical abstract



Keywords Antimicrobials · Electrospinning · Nanotechnology · Nanogel · Antitumor

Extended author information available on the last page of the article

Introduction

Cancers were responsible for about 10 million deaths in 2020 (WHO 2021). Among them, skin cancers are by far on the list of prevalent cancers; they are categorized in non-melanoma and melanoma (Hardy et al. 2010). Melanoma is the most aggressive and deadliest type of skin cancer (Domingues et al. 2018). There are 42 melanoma cell lines on the surface of transcriptomes (Vincent and Postovit 2017). Studies indicate that the A-375 cell line is more aggressive with low sensitivity to chemotherapy treatment (Maksimović-Ivanić et al. 2019; Massi et al. 2014).

Staphylococcus aureus is a gram-positive bacteria that cause various diseases in humans such as wound, skin, urinary and respiratory tract infections, inflammations (i.e., endocarditis, osteomyelitis), toxic shock and scalded skin syndromes, and necrosis (i.e., necrotizing fasciitis and necrotizing pneumonia) (Francis et al. 2005; Miller et al. 2005). *Escherichia coli* is a gram-negative bacterium that is one of the most common causes of urinary tract infections, neonatal meningitis, acute enteritis, traveler's diarrhea, dysentery-like disease, and hemorrhagic colitis (Percival and Williams 2014; Wanke and Sears 2008).

Drug resistance in cancers or microorganisms has become a health challenge in two past decades (Housman et al. 2014; Longley and Johnston 2005). The development of natural drugs has thus been received more attention. For instance, *Myrtus communis* L. (myrtle) in the *Myrtaceae* family is traditionally used in cough, gastrointestinal disorders (i.e., peptic ulcers, diarrhea, and hemorrhoids), urinary diseases (i.e., urethritis), and skin ailments (i.e., reddened skin), inactivation microorganisms and for the wound healing (Alipour et al. 2014; Messaoud et al. 2005). Furthermore, anticancer, antibacterial, antiviral, antifungal, anti-parasitic, and anti-inflammatory properties of *M. communis* essential oil (EO) are due to flavonoids and polyphenols compounds (Aleksic and Knezevic 2014; Alipour et al. 2014).

Nanotechnology is defined as targeted materials in the nanoscale (1–200 nm) to obtain size-dependent properties; preparation of nanostructures containing EOs is a promising approach for improving their efficacy (Esmaili et al. 2021; Osanloo et al. 2020b; Zhou et al. 2021). Hydrogels are 3D structures of swelled polymers in an aqueous media; biodegradability, biocompatibility, and high drug loading capacity are some of their advantages (Guo et al. 2020; Zhang and Khademhosseini 2017). Furthermore, if a nanoemulsion is gelified (nanoemulsion based gel), advantages of nanosystems (improving efficacy, better cellular penetration, loading of hydrophobic materials) are also achievable (Hussain et al. 2016; Qasemi et al. 2021).

In this study, chemical composition of *M. communis* EO was first investigated. An attempt was then made to improve its biological activities (antioxidant, anticancer, and antibacterial) by preparing nanoemulsion-based gel. Moreover, a polyethylene oxide-gelatin (PEO-Gel) electrospun nanofibers mat was prepared as a dressing after treatment.

Materials and methods

Materials

Melanoma cell line A-375 (ATCC CRL-1619), *S. aureus* (ATCC 25923), and *E. coli* (ATCC 25922), provided by the Pasteur Institute of Iran. *M. communis* EO (99.99%) was purchased from Tabib Daru Co., Iran. Merck Co., Germany, provided PEO, Gel, and tween 20 and 80. Penicillin–streptomycin, Dimethyl Sulfoxide (DMSO), and RPMI cell culture medium were supplied by Shellmax (China).

Method

GS-MS analysis

Myrtus communis EO was analyzed as described in our previous study using a gas chromatography device (Agilent 6890, HP-5MS column, USA) connected to a mass spectrometer (Agilent 5973, USA) (Ghanbariasad et al. 2021). Briefly, the initial column temperature was set at 40 °C and fixed for 1 min. The final temperature was set at 250 °C, and the injection port and detector temperature were fixed at 250 and 230 °C. Helium (He) gas 99.999% was applied as the carrier gas. Mass spectra were taken at 70 eV ionization energy, and full scan mode (50–350 m/z) was performed. *Myrtus communis* EO' compounds were identified by combining the temperature programmed retention indices and mass spectra of ADAMS and NIST 17 (Adams 2007, Standards & Technology 2008).

Preparation and characterization of fifteen nanoemulsions

The spontaneous approach (without external energy such as ultra-sonication and homogenizer) was used to prepare nanoemulsion to prevent evaporation of EO components (Abedinpour et al. 2021). A defined amount of the EO (0.2% w/v) with different amounts of emulsifiers (tween 20 or tween 80; $n = 15$) was first stirred at 2000 rpm for 5 min. Then, phosphate-buffered saline (PBS) or normal saline as aqueous phase was added dropwise and stirred for 40 min. The mean droplet size of the samples and droplet size distribution (SPAN) were measured by dynamic light scattering (DLS) using a Scatteroscope (K-one Ltd. Korea). The mean size of droplets has been considered as D_{50} and SPAN calculated by Eq. (1). Where D stands for diameter, and 10, 50,

and 90 are percentages of particles with smaller sizes than mentioned ones.

$$\text{SPAN} = D90 - D10/D50 \quad (1)$$

Preparation and characterization of a nanoemulsion-based gel

Amongst the prepared nanoemulsions, a sample with a mean droplet size < 200 nm and SPAN value < 1 was selected as the optimum nanoemulsion. Then, the nanoemulsion was gelified by adding carboxymethylcellulose (3% w/v); the mixture was stirred overnight at 2000 rpm. Moreover, the blank gel was also prepared similarly, only without the EO. Finally, the viscosity of the nanogel was investigated in different shear rates of 0.1 to 100 S^{-1} utilizing a rheometer machine (MCR-302, Anton Paar-Austria).

Preparation and characterization of an electrospun nanofiber

The electrospinning technique was used to prepare nanofibers; PEO-Gel (10–8% w/v) were first dissolved in distilled water (2000 rpm, overnight, room temperature). The prepared sample was then filled in a 10 mL syringe that connected to a blunted needle (gauge 22) and situated in the syringe pump in an electrospinning device (Fanavaran Nano-Meghyas (FNM), Iran). Instrumental parameters were set: injection rate 0.3 mL/h, the distance between needle and collector 100 mm, collector speed 100 rpm, and voltage 15 kV.

A scanning electronic microscopy (SEM) instrument (Vega 3, TESCAN Co., Czech Republic) was used to investigate the size and morphology of nanofibers 0.5 cm^2, which was sputter-coated by gold vapor (Quorum Technologies, Q150R-ES). The contact angle measurement equipment (CA-500 Å model, Sharif Solar Co., Iran) was used to investigate nanofibers' surface; 7 µL deionized water was injected, and its contact angle (θ) with the surface was recorded immediately. Fourier Transform Infrared (FTIR) spectroscopy was used to assess the functional groups of the PEO, GEL, and PEO-GEL nanofibers (Tensor II model—Bruker–Germany) in a wavenumber of 400–4000 cm^{-1}. Noteworthy, nanofibers 0.5 cm^2 were used as an independent sample for investigating its antioxidant, antibacterial, and anticancer activities, as described in the next sections.

Investigation of antioxidant activity

DPPH assay was used to investigate the antioxidant activities of the EO and nanogel in a concentration range of 62.5–1000 µg/mL. DPPH powder (394.32 g/mole), the EO, and nanogel were first dissolved in absolute ethanol containing 0.5% DMSO to reach 0.3 mM and the mentioned serial dilutions. After that, DPPH solution (150 µL/well) and serial dilutions (50 µL/well) were added to a 96 well plate and incubated 30 min away from light for completing the reaction. Six well/plate was considered a control group (150 µL DPPH solution and 50 µL ethanol), i.e., was not treated with samples. Eventually, each well's optical density (OD) was read at 517 nm using a plate reader device (Synergy HTX Multi-Mode Reader, USA). The antioxidant activities were calculated by Eq. (2). In addition, the antioxidant activities of blank gel, nanofiber, and ascorbic acid (1000 µg/mL, as positive control) were also investigated.

$$\text{Antioxidant effect} = (\text{OD control} - \text{OD sample})/(\text{OD control}) \times 100 \quad (2)$$

Investigation of the cytotoxicity activity

The EO and nanogel were dissolved in normal saline containing 0.5% DMSO. The anticancer effect of the EO and nanogel (in a concentration range of 62.5–1000 µg/mL) was investigated against A-375 cells using MTT assay as described in our previous research (Valizadeh et al. 2021). Cultured cell in RPMI (containing 10% FBS and 1% antibiotics) was added to 96-well plate (100 µL/well) and incubated at 37 °C, and CO2 (5%) to attach the cells to the bottom reached 80% confluence. After that, the liquids of wells were replaced with 50 µL of fresh culture medium, and serial dilutions were added, 50 µL/well. In each plate, 6 was considered the control group filled with 50 µL fresh culture medium and normal saline containing 0.5% DMSO. Treated plates were incubated for 24 h, and the liquid content was replaced with 50 µL mtt solution (0.5 mg/mL) and then incubated for 4 h. To dissolve the created formazan crystals, 50 µL/well DMSO was added, and OD of wells was read at 570 nm. The cell viability at each concentration was determined using Eq. (3). In addition, the cytotoxic activities of blank gel and nanofiber were also investigated.

$$\text{Cell viability} = (\text{mean } A \text{ sample})/(\text{mean } A \text{ control}) \times 100 \quad (3)$$

Investigation of antibacterial activity

Antibacterial activity of the EO and nanogel in a concentration range of (62.5–1000 µg/mL) against *E. coli* and *S. aureus* was investigated using the 96-well plate microdilution method (Osanloo et al. 2020a). The EO and nanogel were dissolved in Muller Hinton broth containing 0.5% w/v DMSO. Bacterial colonies were also dispersed in the Muller Hinton broth medium to reach 0.5 McFarland turbidity (1.5×10^8 CFU/mL); 20 µL/well of the bacterial suspension was added to a 96-wells plate. After that, 30 and 50 µL/

well Muller Hinton broth and as-prepared serial dilutions were added, and the plates were incubated for 24 h at 37 °C. In each plate, control wells ($n=6$) were filled with 20 and 80 µL of the bacteria suspension and the Muller Hinton Broth culture medium containing 0.5% w/v DMSO. The OD of each well was read at 630 nm using the plate reader, and bacteria growth was measured using Eq. (4). In addition, the antibacterial activities of blank gel and nanofiber were also investigated.

$$\text{Growth} = (\text{OD sample})/(\text{OD control}) \times 100 \quad (4)$$

Statistical analysis

All experiments were carried out in triplicates, and data were expressed as Mean ± standard deviation. An independent sample *T*-test was used to determine statistically significant differences between groups. All analyses were conducted using computer-based statistical software (STATA v. 16); a P value < 0.05 was accepted as statistically significant. CalcuSyn software (Free version, BIOSOFT, UK) was used to calculate half-maximal inhibitory concentration (IC_{50}); no overlap between the upper and lower limit of IC_{50} was considered significant.

Results

Identified compounds in the EO

Identified compounds with a higher portion than 1% in the EO are listed in Table 1. Five major compounds are α-pinene (29.7%), 1,8-cineole (25.8%), linalool (9.1%), linalool acetate (5.9%), and geranyl acetate (3.4%).

Prepared nanoemulsions and viscosity of the nanogel

Ingredients and size analyses of the prepared nanoemulsion are listed in Table 2. One sample (My11) only possesses proper size characteristics (droplet size 179 ± 7 nm and SPAN 0.96); its DLS profile is depicted in Fig. 1. The viscosity profile of the nanogel containing *M. communis* followed the Carreau-Yasuda regression model (Fig. 2). It is a well-known empirical equation that has been used for non-Newtonian fluids such as biopolymer and polymeric solutions, emulsions, and protein solutions (Avazmohammadi and Castañeda 2015; Bird et al. 1987). The viscosity of non-Newtonian decreases with increasing the shear rates, while, Newtonian substances have constant viscosity at all shear rates (Kwack and Masud 2014; Zare et al. 2019).

Physicochemical properties of the nanofibers

The SEM image of smooth, randomly oriented, and beadles PEO-GEL electrospun nanofibers are depicted in Fig. 3. Besides, the contact angle (θ) of water with the nanofibers was assessed to determine the degree of hydrophilicity of the surface. In the current study, θ was $< 90°$ (i.e., 19° ± 2), the hydrophilic surface is confirmed (data not shown).

Furthermore, FTIR analysis was carried out to receive the exact information about the functional groups and molecular interactions in electrospun PEO-Gel nanofibers (Kuppan et al. 2013). In Fig. 4, the spectra of the pure components (PEO and Gel powder) are compared with the prepared nanofibers. In the FTIR spectrum of pure PEO, a strong peak at 2878 cm^{-1} is associated with the asymmetric stretching of CH_2 in PEO. Notably, a characteristic triplet (1144, 1094, and 1059 cm^{-1}) was observed with a maximum at 1094 cm^{-1}, which is

Table 1 Identified components (> 1%) in *M. communis* essential oil by GC–MS analysis

Retention Time	Compound	Area	%	Retention Index
9.2	α-thujene	36,284,215	1	930
9.6	α-pinene	1,070,412,470	29.7	932
12.7	Carene	93,299,232	2.5	1002
13.8	3-carene	39,910,111	1.1	1008
15.0	1,8-cineole	928,574,270	25.8	1026
16.4	ɣ-terpinene	41,028,327	1.1	1059
17.2	Terpinolene	47,197,833	1.3	1088
21.3	Linalool	329,363,333	9.1	1095
24.3	α-terpineol	112,704,608	3.1	1188
28.3	Linalool acetate	213,802,170	5.9	1257
29.8	Geranyl acetate	122,346,095	3.4	1381
31.2	*trans*-caryophyllene	44,454,783	1.2	1419
32.6	α-humulene	41,052,490	1.1	1438
37.8	Viridiflorol	58,136,977	1.6	1592

Table 2 Ingredients and size analyses of the prepared nanoemulsions (EO is fixed at 0.2% w/v)

NO	Tween 20 (%)	Tween 80 (%)	PBS[a] (%)	Normal Saline (%)	Droplet size (nm)	SPAN[b]
My1	0.2	–	99.6	–	324	15.2
My2	0.3	–	99.5	–	163	44.4
My3	0.4	–	99.4	–	367	24.5
My4	0.5	–	99.3	–	624	12.9
My5	0.6	–	99.2	–	277	10.3
My6	0.2	–	–	99.6	211	26.7
My7	0.3	–	–	99.5	641	13.6
My8	0.4	–	–	99.4	303	22.1
My9	0.5	–	–	99.3	181	1.6
My10	0.6	–	–	99.2	377	22.7
My11	–	0.2	–	99.6	179	0.96
My12	–	0.3	–	99.5	241	1.5
My13	–	0.4	–	99.4	174	1.7
My14	–	0.5	–	99.3	219	7.4
My15	–	0.6	–	99.2	155	11.8

[a]Phosphate-buffered saline

[b]Particle size distribution

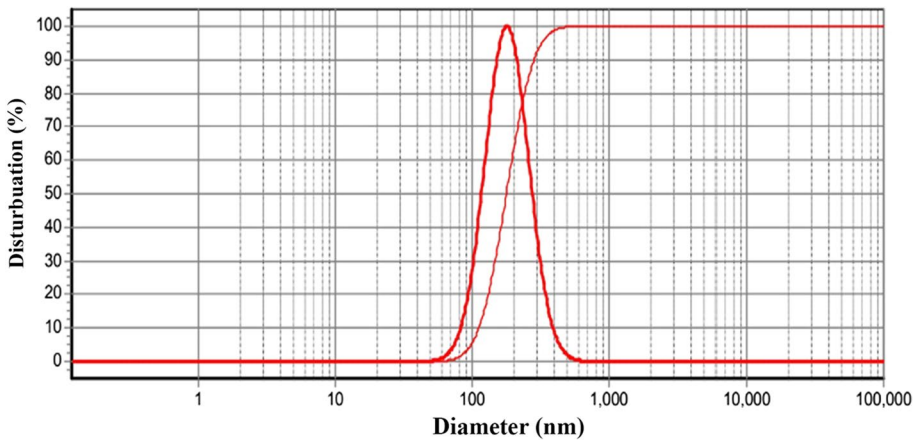


Fig. 1 DLS analysis of the optimum nanoemulsion with a droplet size 179 ± 7 nm and SPAN 0.96

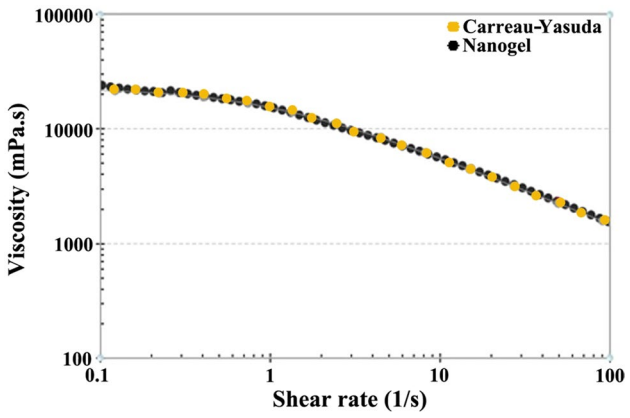
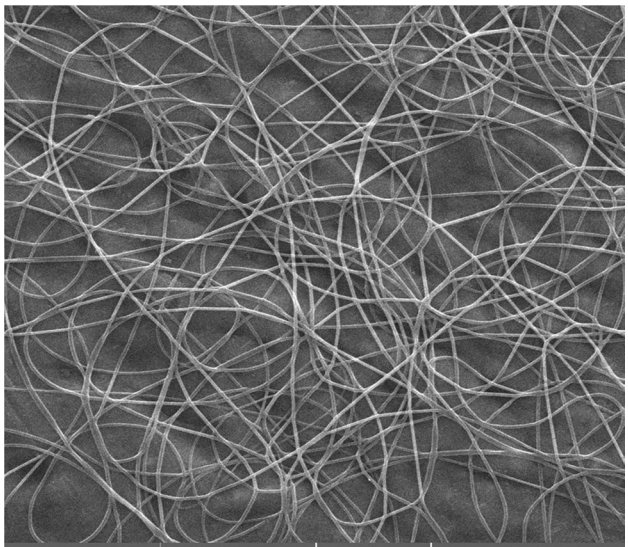


Fig. 2 The nanogel viscosity is fitted with the Carreau-Yasuda regression model

assigned to C–O–C vibration in the structure of PEO. The presence of this triplet in the spectrum of PEO greatly reveals the high crystallinity of PEO. The peaks observed at 960 and 841 cm^{-1} can be ascribed to the $–CH_2–CH_2$ rocking and CH_2 rocking bending, respectively. Moreover, the absorption bands of PEO appeared at 1466, and 1359 cm^{-1} are attributed to CH2 groups (Anilkumar et al. 2017; Zhou et al. 2014). In the spectrum of pure Gel, a broad peak at 3271 cm^{-1} and a weak band at 2945 cm^{-1} are attributed to the hydroxyl (O–H) and CH_2 groups, respectively. The main characteristic peaks of Gel were located at 1628 cm^{-1} (amide I), 1523 cm^{-1} (amide II), and 1238 cm^{-1} (amide III), respectively (Cai et al. 2019; Merk et al. 2021). In the FTIR spectrum of electrospun PEO-GEL, peaks for PEO and Gel are evidence for a perfect



Tool	Measure	n	Mean	SD	Min	Max
Length	Length	10	359/40	36/23	308/89	423/19

Fig. 3 SEM image of polyethylene oxide-gelatin (PEO-Gel) electrospun nanofibers

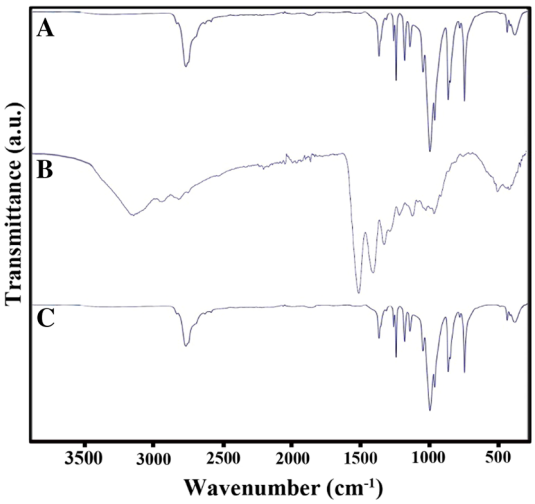


Fig. 4 FTIR spectra of A: polyethylene oxide (PEO) powder, B: gelatin powder (Gel), and C: PEO-Gel nanofiber

combination of these two polymers. Although, the position and intensity of some peaks were slightly changed due to molecular interactions between Gel and PEO. For instance, the peaks at 1628, 1523, and 1238 cm^{-1} for Gel shifted to 1632, 1556, and 1240 cm^{-1} in the PEO/Gel nanofibers spectrum, respectively.

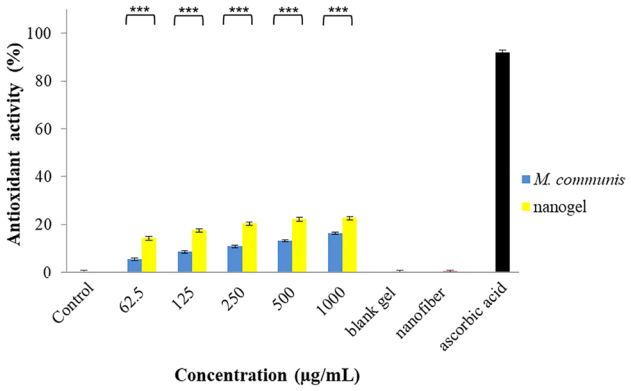


Fig. 5 Antioxidant activity of *M. communis* EO and its nanogel. *** *P* value < 0.001

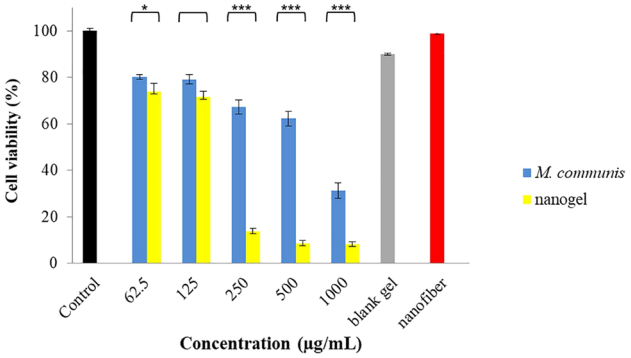


Fig. 6 Cytotoxicity activity of *M. communis* essential oil and its nanogel on A-375 cell line. * *P* value < 0.05, *** *P* value < 0.001, and no star present for *P* value > 0.05

Antioxidant activities of *M. communis* EO and nanogel

Antioxidant activities of the EO and the nanogel are shown in Fig. 5. The nanogel was significantly more potent than the EO at all examined concentrations. *P* value for all concentrations is < 0.001. Ascorbic acid 1000 µg/mL (positive control) showed 92% antioxidant effect. Besides, blank gel and nanofibers did not show antioxidant effects.

Cytotoxic activities of *M. communis* EO and nanogel

The viability of A-375 cells after treatment with different concentrations of the EO and nanogel is shown in Fig. 6. The efficacy of the nanogel was significantly more than the EO at concentrations of 62.5, 250, 500, and 1000 µg/mL (*P* value 0.0421, 0.0000, 0.0000, and 0.0003). The IC_{50} of the nanogel and the EO were obtained as 132.6 (74–237) µg/mL and 580.8 (328–1026) µg/mL (Table 3). Besides, the viability of cells after treatment with blank

Table 3 IC_{50} values of *M. communis* EO and its nanogel against A-375 melanoma cells and two standard bacteria

Species	Nanogel			*M. communis* EO		
	Lower limit 95%	IC_{50}	Upper limit 95%	Lower limit 95%	IC_{50}	Upper limit 95%
A-375	73.9	132.6	237.8	328.6	580.8	1026.6
E. coli	437.5	583.1	777.1	2574.3	4547.9	8034.7
S. aureus	282.6	548.5	1064.4	153.2	394.0	1013.1

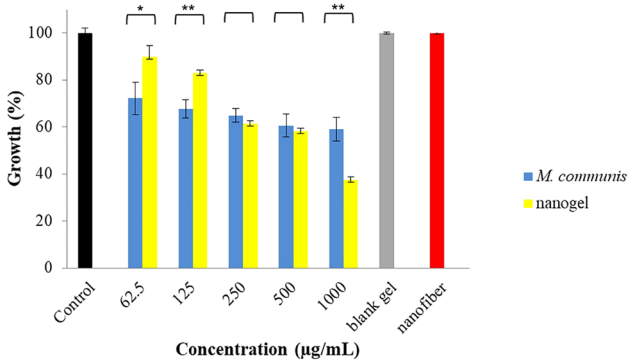


Fig. 7 Antibacterial activity of *M. communis* EO and its nanogel on *E. coli*. *P value < 0.05, **P value < 0.01, and no star present for P value > 0.05

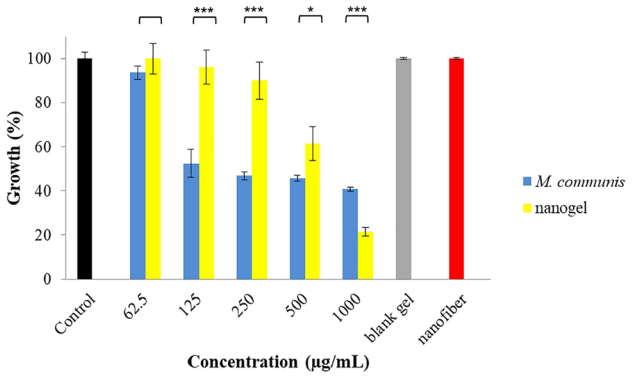


Fig. 8 Antibacterial activity of *M. communis* EO and its nanogel on *S. aureus*. * P value < 0.05, *** P value < 0.001, and no star present for P value > 0.05

gel was only reduced by less than 10%. Moreover, the nanofibers did not significantly affect the viability of cells.

Antibacterial activities of *M. communis* EO and nanogel

The antibacterial activities of the EO and nanogel on *E. coli* are illustrated in Fig. 7. At the highest concentration (1000 µg/mL) efficacy of the nanogel was significantly more potent than the EO (P value 0.0020). However, the potency of the EO at concentrations of 62.5 and 125 µg/mL was significantly more potent than the nanogel (P value 0.0208 and 0.0031). Besides, IC_{50} values of the nanogel and the EO was observed as 583 (437–777) µg/mL and 4547 (2574–8034) µg/mL (Table 3).

The antibacterial activities of the EO and nanogel on *S. aureus* are shown in Fig. 8. The potency of nanogel at 1000 µg/mL was significantly more potent than the EO (P value 0.0001). However, the potency of the EO was significantly more potent than nanogel at concentrations of 125, 250, and 500 µg/mL (P value 0.0016, 0.0010, and 0.0241). IC_{50} values of the nanogel and the EO were 584 (282–1046) µg/mL and 394 (153–1033) µg/mL. Moreover, the blank gel and nanofibers did not significantly affect the growth of *E. coli* and *S. aureus*.

Discussions

In the current study, *M. communis* EO was used as the active agent. Its chemical compositions were investigated using GC–MS analysis with fine major compounds α-pinene, 1,8-cineole, linalool, linalool acetate, and geranyl acetate. Alpha-pinene is identified as the major compound; this finding (30%) is consistent with the literature (30–36) (Hsouna et al. 2019; Sen et al. 2020). Alpha-pinene is a bicyclic monoterpene $(C_5H_8)_n$ with a wide range of biological activities, including bronchodilator, antioxidant, and antimicrobial activities (Aydin et al. 2013; Nissen et al. 2010).

EOs as natural medicine have advantages such as biocompatibility and biodegradability. However, there are some challenges about the EOs, such as low water solubility and high volatility, and their efficacy should be improved (Herman and Herman 2015; Langeveld et al. 2014). Preparing nanostructures containing EO (nanoemulsions, nanofibers, polymeric nanoparticles, and lipidic nanocarriers) is a promising approach to meet the challenges (Bilia et al. 2014). In this study, an oil in water nanoemulsion (droplet size 179 ± 7 nm) containing the EO was first prepared. Nanoemulsions are a dispersion of two immiscible liquids by using amphiphilic material called surfactant (Jiang et al. 2013; Mason et al. 2006). After that, the nanoemulsion was gelified by adding a

semi-synthetic thickening agent (carboxymethylcellulose). The topical application of nanogels is easier than nanoemulsions, and the EO remains stable for a longer period. To the authors' best knowledge, *M. communis* EO or its nanogel delivery system has not been investigated yet on the A-375 cell line. The current study investigated the anticancer and antioxidant activities of the EO and nanogel. Interestingly, the potency of the nanogel against A-375 cells was fourth-folds more than the EO. Besides, significant antioxidant activities of nanogel compared to the EO were also observed. Antioxidants are substances that inhibit free radicals from raising the chance of several diseases (cancer, cardiovascular disease, and aging) (Antolovich et al. 2002). Cancer cells have downregulated gap junctions and become ready for metastasis. Besides, their membrane pores are wider than normal cells for obtaining nutrients molecules (Leithe et al. 2006; Ruch 2020). Weak lymphatic system with large gaps are two important factors in inactive nano drug delivery systems; nanostructures easily enter to cancer cell and do not allowed to exit from cells, so their efficacy are improved (Adepu and Ramakrishna 2021; Zhang and Lu 2014).

Large amounts of EO droplets could be loaded into one nanostructure; they prepare nanotanks containing EO (de Matos et al. 2019; Nazzaro et al. 2013). On the other hand, the entry of nanotanks into bacteria cell walls and membranes is difficult due to a regular cell wall structure (Ploux et al. 2010; Zhang and Rock 2008). However, the entry of dissolved EO in the culture media is easier than nanotanks. In the current study, the potency of the EO at four concentrations (62.5, 125, 250, and 500 µg/mL) is more than the nanogel. While at the 1000 µg/mL concentration (i.e., no-diluted nanogel), the potency of the nanogel is more than the EO. In addition, the role of the bacterial outer membrane in the gram-negative strains (*E.* coli) was significant; the EOs' obtained IC_{50} (4547 µg/mL) 11 folds more than *S. aureus* (394 µg/mL). Moreover, the IC_{50} value of the nanogel against both strains was the same; however, the final efficacy (reduced growth) of the nanogel against *S. aureus* (~80%) was more than *E.* coli (62%).

As mentioned above, we prepared a natural nanogel with topical delivery (anti-melanoma and antibacterial). However, lesions must be covered after treatment; thus, PEO-Gel nanofibers is proposed as a dressing. Furthermore, an appropriate dressing should be biocompatible, biodegradable, and prevent entry from environmental pathogens (Barnea et al. 2010; Kamoun et al. 2017). Electrospun nanofibers with various polymers as precursors, controllable pore size, and excessive surface-to-volume ratio have been widely used in protective clothing, tissue engineering, functional materials, and energy storage (Abrigo et al. 2014; Xue et al. 2019). Gelatin is a natural hydrophilic polymer that is generally extracted from collagen. It has been widely used in the pharmacology industry due to its excellent biocompatibility, low immunological activity, and controllable physical parameters (Dille et al. 2015). However, it is impossible to be electrospun alone (Huang et al. 2004; Ki et al. 2005). PEO was thus added to Gel in the current study to meet this challenge. It is a nonionic hydrophilic polymer with high molecular weight and proper physical strength (Chiappetta and Sosnik 2007; Pinto et al. 2003). As results shows, the prepared PEO-Gel fibers were at the nanometer scale and therefore can prevent the entry of environmental pathogens. Besides, they did not affect cell viability and bacterial growth, so as a neutral dressing could be used. In the current study, a comprehensive comparsion was performed amongst biological activities of the *M. communis* EO and its nanogel dosage form. However, the comparison could also be made with alpha pinene (the main compound) and its nanogel dosage form.

Conclusions

The study evaluated the antioxidant, anticancer, and antibacterial activities of *M. communis* EO and its nanogel dosage form; the efficacy of nanogel was significantly more potent than the EO. Moreover, PEO-Gel electrospun nanofibers without any effect on A-375 melanoma cells, *E. coli,* and *S. aureus* were proposed as dressing after treatment for in vivo studies.

Authors' contributions GhR prepared the nanogel and nanofibers and performed MTT and DPPH assays. GhR and YY wrote the MS. AA performed the antibacterial tests. MS interpreted ATR-FTIR. FR contributed to the preparation of nanogel and MTT assay. MO design of the study, statistical analysis, and revised the MS. All authors contributed to drafting MS and approved the final version.

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Data availability Ok.

Declarations

Conflict of interest Researchers have no conflict of interest in this study.

Ethical approval This study has been ethically approved by the Fasa University of Medical Sciences' ethical committee, IR.FUMS.REC.1400.129.

Inform consent This research did not involve human study; thus, no constant form was used.

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