

Development and characterization of an active biodegradable Tara gum film incorporated with *Lavandula angustifolia* essential oil

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Abstract

This study aimed to develop Tara gum (TG)-based films incorporated with Lavender essential oil (LEO) at different concentrations (5%-20% (w/w)) to enhance the properties of the films. GC-MS analysis confirmed the presence of linalool and linalyl acetate as the major compounds in LEO. SEM micrographs showed the highest homogeneity in the film containing 5% (w/w) LEO, and CLSM images revealed the even distribution of LEO within the films. FTIR spectra demonstrated intermolecular interactions between TG and LEO, as evidenced by a new peak at 1458 cm^{-1} in the LEO-loaded films. The addition of LEO resulted in a significant decrease in water content (WC) and water solubility (WS) of the films, while thickness, contact angle (CA), opacity, water vapor permeability (WVP), and oxygen permeability (OP) increased. The TG-LEO films exhibited higher elongation at break (EB) and lower Young's modulus (YM). Additionally, LEO positively affected the antioxidant and antimicrobial properties of the films. Overall, the results authenticated the hypothesis of this study and indicated that TG-LEO films have great potential as biodegradable films with promising functionalities for food packaging applications.

1. Introduction

Nowadays, due to the disadvantages of petroleum-derived traditional plastics, the demand for the use of biodegradable and renewable packaging has increased. Indeed, biodegradable films are a suitable alternative to non-degradable packaging due to the availability and abundance of resources, the ability to become compost, and compatibility with the environment and food (Nur Hanani, Roos, & Kerry, 2014). So far a lot of research has been done on the production of biodegradable films and coatings based on three main biopolymers, carbohydrates (Bertsch, Etter, & Fischer, 2021; Hosseini, 2021; Huang, Zou, & Wang, 2023; Singh, Gu, Castellarin, Kitts, & Pratap-Singh, 2020), proteins (Arciello et al., 2021; Malm, Narsimhan, & Kokini, 2019), and lipids (Chen et al., 2021; Jo et al., 2014). Among carbohydrates, hydrocolloids are one of the available sources.

Tara gum (TG), a neutral and commercial hydrocolloid extracted from the seed's endosperm of the *Caesalpinia spinosa* tree, is widely used in the food industry because of its low cost (Ma et al., 2016; Pizato, Cortez-Vega, de Souza, Prentice-Hernández, & Borges, 2013). It is a high molecular weight galactomannan ($M_w = 1519 \times 10^3\text{ g/mol}$) consisting of a linear main chain of (1–4)- β -D-mannopyranose units linked with branched chains of (1–6)- α -D-galactopyranose (Ma, Du, Yang, & Wang, 2017; Mukherjee et al., 2022), with mannose/galactose ratio (M/G) of 3 (Wu, Ding, & He, 2018). It has also film-forming properties, indicating they can be used in food packaging. However, pure TG films have disadvantages such as high affinity to water and poor mechanical properties, which limits their use in food packaging (Ma et al., 2016). Therefore, it necessitates a green approach to address this problem. Recently, to improve the properties of films, incorporating hydrophobic compounds into films with hydrophilic nature has been suggested (Dissanayake et al., 2023; Zhou, Wu, Chen, & He, 2021). Some research has demonstrated the utilization of essential oils (EOs) such as oregano EO (Martins, Bagatini, & Martins, 2021), cinnamon EO (Fan et al., 2023), lemon EO (Song, Zuo, & Chen, 2018), cumin EO (Alizadeh-Sani, Rhim, Azizi-Lalabadi, Hemmati-Dinarvand, & Ehsani, 2020), and clove EO (Xu et al., 2020) can affect

the characteristics of biodegradable films such as water permeability and mechanical properties. In addition, EOs can increase shelf life and improve the functional properties of biodegradable films due to their antioxidant and antimicrobial characteristics (Atarés & Chiralt, 2016).

Lavender essential oil (LEO) has strong antioxidant activity and extensive activity against bacteria. Different studies have been conducted on the impact of LEO on the properties of the films which are based on polysaccharides (Jamróz, Juszczak, & Kucharek, 2018, Sun et al., 2021). However, no studies have focused on the enhanced mechanical properties of TG-based films incorporated with LEO, as well as their antibacterial and antioxidant activities. This study hypothesized that the incorporation of LEO into Tara gum-based films will significantly improve their mechanical, antioxidant, and microbial properties. Therefore, this study was intended to investigate the effects of various concentrations of LEO on the mechanical, biological, and physicochemical properties, as well as the optical and barrier properties of the TG films.

2. Materials and methods

2.1. Materials

Tara gum powder extracted from *Caesalpinia spinosa* was purchased from the Silva team (Italy) and used without further purification. Lavender essential oil (LEO) derived from *Lavandula angustifolia* was bought from Adonis Co., Ltd (Tehran, Iran). Tween 80 and Glycerol were supplied by Shanghai Chemex Group Ltd. (Shanghai, China).

2.2. Preparation of film-forming solutions and films

First, having prepared an aqueous solution (0.8%, w/v) of TG, it was mixed with 30% (w/w, TG basis) glycerol, and then the mixture was stirred at 45°C for 20 min. 0.2% (v/v, LEO basis) tween 80 was used in the solutions to dissolve LEO more easily. Afterward, LEO at a specified concentration (5%, 10%, 15%, and 20%, w/w, TG basis) was added, and then the solutions were homogenized (IKA T25 basic, Staufen, Germany) at 12000 rpm for 3 min. The film solutions were cast into petri dish templates (130 mm diameter) and dried at 50°C for 24 h. The final samples were kept in desiccators with a temperature of 25°C and relative humidity of 50% for 48 h. The LEO incorporated into the TG films titled TG-LEO5, TG-LEO10, TG-LEO15, and TG-LEO20, which showed the LEO content in the TG films were 5%, 10%, 15%, and 20% (w/w), on TG basis, respectively.

2.3. Gas Chromatography-Mass Spectrometer (GC-MS) analysis of LEO

The chemical components of LEO were analyzed based on the procedure used by Karabagias, Badeka, and Kontominas (2011). For the quantitative analysis of each of LEO constituents, a Hewlett-Packard 6890 series gas chromatograph (Perkin Elmer Auto System XL, USA), coupled to a Hewlett-Packard, model 5973, and an HP- 5MS capillary column (60 m × 0.320 mm i.d., film thickness 1 µm) were used.

The program of the oven's temperature was the following: 100°C held for 5 min, before increasing temperature from 100°C to 130°C at 10°C/min, then rose to 270°C (7°C/min), and remained for 3 min. For operating the mass spectrometer, the EI ionization, temperature source, scan range, and quadrupole temperature were considered 70 eV, 230°C, 30–330 m/z , and 150°C, respectively. The carrier gas (helium) flow rate was 1.5 mL/min. Injections were made at a temperature of 270°C using the split mode of 20:1. The chemical composition was achieved based on the chromatogram peak area with the Wiley 275 MS database.

2.4. Scanning Electron Microscopy (SEM)

The surface morphology of the TG-based films was observed using EM-3200 scanning electron microscopy (KYKY, China) at an accelerating voltage of 20 kV. Before observation, the films were fixed on the holder using aluminum tape, then sputtered with gold in BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein).

2.5. Confocal laser scanning microscopy (CLSM)

The distribution of oil droplets in the film samples was evaluated by confocal laser scanning microscopy (CLSM), employing a ZEISS LSM 780 confocal microscopy (Carl Zeiss Microscopy GmbH, Jena, Germany). Based on the procedure used by Xu et al. (2018), 10 μ L of Nile red solution was used to dye the square films (1 $\times$ 1 cm). Having been covered with slides, the samples were observed with proper fluorescence intensity and focal length.

2.6. Fourier transforms infrared (FTIR) spectroscopy

The FTIR spectra of all samples were scanned using a Bruker Tensor 27 FTIR spectrometer (Bruker Optic GmbH, Germany). FTIR analysis was performed in a range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1} .

2.7. Water content (WC), water solubility (WS), Contact angle (CA), and thickness measurement

2.7.1. Thickness

The measurements of the films' thickness were carried out through a digital micrometer (Labthink Instruments. Co. Ltd, Shandong, China). The average thickness of each film at ten different random positions with an accuracy of 0.1 μm was calculated. The experiments were determined in triplicate.

2.7.2. Water content

The water content of the film samples (with the dimensions of 3 $\times$ 3 cm) was measured gravimetrically. An oven was used to dry the samples at 105°C for 24 h. The experiment was determined in triplicate.

2.7.3. Water solubility

To determine the water solubility (WS) of the films, each sample was dried separately in an oven for 24 h at 105°C to reach a constant weight (W_i). Subsequently, each film was immersed in 50 mL distilled water (25°C / 24 h) with gradual stirring. In the next step, the undissolved substances of the film samples were dried at 105°C for 24 h and weighed (W_f) (Singh, Chatli, & Sahoo, 2015). The WS value was measured using Eq. (1).

$$Watersolubility(\%) = \frac{W_i - W_f}{W_i} \times 100$$

1

2.7.4. Contact angle measurement

The films' contact angles were determined using a contact angle analyzer (OCA25, Dataphysics, Germany). 10 µL of deionized water was deposited on the films' surface, before taking photos immediately. To contact angle measurement, the angles between the drop and the surface of the films were measured.

2.8. Water vapor permeability (WVP) and oxygen permeability (OP)

2.8.1. Water vapor permeability (WVP)

The water vapor permeability (WVP) of the films was measured according to the method described by Adjouman et al. (2017). The film samples were used to seal up tubes with a diameter of 20 mm and a height of 60 mm, containing 5.0 g of anhydrous calcium chloride (0% RH). The weight of the sealed tubes was measured, after which they were kept in a desiccator containing sodium chloride solution (75% RH) at 25°C. The desiccator was placed in an incubator at 25°C and the weight of the tubes was measured every few hours for seven days. Changes in the tubes' weight were plotted against time and the line slope was calculated using linear regression ($R^2 > 0.99$). The water vapor transmission rate (WVTR) was expressed by the curve slope and the area of the exposed film surface (m^2). The water vapor transmission rate (WVTR) and water vapor permeability (WVP) were calculated from the following equations:

$$WVTR = \frac{\Delta W}{\Delta t} \times A$$

2

$$WVP = \left[\frac{WVTR}{S} (R_2 - R_1) \right] \times T$$

3

where $\Delta W/\Delta t$ is the changes in the tubes' weight as a function of time (g/s), A is the area of the exposed film surface (mm^2), T is the film thickness (mm), S is the saturation vapor pressure of water (Pa) at 25°C , R_1 and R_2 are the relative humidity in the permeation cell and desiccator, respectively.

2.8.2. Oxygen permeability (OP)

Oxygen permeability (OP) was evaluated by the deoxidizer absorption method. The film samples were used to seal up the top of the vials containing 5 g deoxidizer (reduced iron powder: activated carbon: sodium chloride, 1:2:3). The vials were weighed initially, and then placed in a desiccator which contained a saturated solution of barium chloride (90% RH) at 25°C for 48 h (Zhang, Zhao, & Shi, 2016). Changes in the weight of the vials were recorded and OP ($\text{g}\cdot\text{m}^{-2}\text{s}^{-1}$) was calculated from the following equation:

$$OP = \frac{m_f - m_i}{t \times A}$$

4

where m_f and m_i are the final and initial weight of the vials, respectively, t is equilibrated time (s), and A is the area of the exposed film surface (mm^2).

2.9. Color, transmittance, and opacity measurement

2.9.1. Color

The measurement of color values in the system of CIE0 (L^* , a^* , and b^*) was carried out using a CR-400 Minolta colorimeter (Minolta Co., Osaka, Japan). The instrument calibration was carried out using a standard white plate ($L^* = 97.24$, $a^* = -0.45$, $b^* = -0.8$), followed by measurements. In addition, the total color difference value (ΔE) and whiteness (WI) of the films were calculated by equations (5) and (6), respectively.

$$\Delta E = [(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2]^{0.5}$$

5

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{0.5}$$

6

2.9.2. Transmission and opacity

The UV-visible spectra of the films were determined by a UV spectrophotometer (Shimadzu- UV-1800, Japan). Based on the method of Socaciu et al. (2020), the film samples were cut equally, then placed in the spectrophotometer test cell. The air was used as reference and the light transmission of the films was recorded in the wavelength range of 200–800 nm. The opacity of the films was calculated using Eq. (7).

$$Opacity = A_{600}/X$$

7

where A_{600} is the absorbance at 600 nm and X is the thickness (mm) of the film.

2.10. Mechanical properties

Based on the standard method D882-12 (ASTM, 2012), mechanical properties were determined by a TA.TX Plus Texture Analyzer (Stable Micro Systems, Surrey, UK, England). Prior to the measurement, the films were cut into strips (15 × 80 mm), which were mounted between tensile grips. The initial grip separation was set to 50 mm and a strain rate of 1 mm/s was applied. By Texture Exponent 32 software, the tensile strength (TS), elongation at break (EB), and Young's modulus (YM) were calculated. At least seven replications were carried out for each sample.

2.11. Total phenol content

The total phenol content (TPC) of the films was determined based on the method described by Ruiz-Navajas, Viuda-Martos, Sendra, Perez-Alvarez, and Fernández-López (2013). 25 mg of each film sample was dissolved in 3 ml of ethanol for 3 h. In the next step, 0.3 ml of each film solution was mixed with 2.5 ml of Folin-Ciocalteu (10%) reagent and 2 ml sodium carbonate (7.5% w/v). The mixture was gradually stirred and incubated at 40°C for 10 min. After that, the absorbance of samples was recorded at a wavelength of 760 nm employing a UV-vis spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan). The results of total phenol content were expressed as mg gallic acid equivalents (GAE) per gram of the dried film according to the following equation:

$$T = (C \times V)/M$$

8

where T is the total content of phenolics compounds (mg/g dried film, in GAE), C is the concentration of gallic acid obtained from the calibration curve (mg/mL), V is the volume of the film extract (mL) and M is the weight of the dried film (g).

2.12. Determination of antioxidant capacity

The antioxidant capacity of the films was assessed based on the procedure used by Siripatrawan and Harte (2010). The mixture of each film sample (25 mg) and distilled water (5 ml) was prepared, following which the film extract (0.1 ml) was mixed with 3.9 ml of 0.1 mM prepared DPPH' in methanol. The incubation of the mixture was done in the dark at room temperature. The absorbance was measured at 517 nm using a spectrophotometer (Shimadzu UV-vis 1601, Japan). The DPPH' scavenging activity was measured using Eq. (9).

$$DPPH^{\bullet} \text{ scavenging activity } (\%) = 100 \times \left(1 - \frac{A_{\text{sample}}}{A_{DPPH}} \right)$$

where A_{sample} and A_{DPPH} are the sample solution's absorbance and DPPH solution's absorbance (control), respectively. All measurements were performed in triplicate.

2.13. Determination of antibacterial activities

The antibacterial activity of the film samples was assessed using the agar diffusion method. The tests were performed against: *E. coli*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, *B. cereus*, *S. typhimurium*, *S. putrefaciens*, *P. aeruginosa*, and *Y. enterocolitica*. BHI broth was used to grow the mentioned bacteria (Merck, Germany) for 24 h at 37°C. The film samples were trimmed (6 mm diameter of the disks), and then placed on BHI agar plates covered by a mixture of bacterial cultures (10^8 CFU/mL). Then, the incubation of the plates was carried out at 37°C for 24 h (Seydim & Sarikus, 2006). The inhibition zone (mm^2) around the film disks was recorded in three independent experiments.

2.14. Statistical analysis

The data analysis was performed using SPSS software (version 21.0, Statistical Package for the Social Sciences Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to measure statistical differences. The significant differences among mean values of the film properties were determined using Duncan's multiple range test ($p < 0.05$).

3. Result and discussion

3.1. The chemical composition of LEO

The main components of LEO are presented in Table 1. The major constituents were linalool (38.47%) and linalyl acetate (27.19%) which, according to several studies, possess strong antimicrobial effects (Blazekovic et al., 2011; Soković et al., 2010). Linalool and linalyl acetate were the dominant compounds, consistent with Xu et al. (2023) and Danila et al. (2021). However, Giovannini et al. (2016) reported different major constituents, which can arise from environmental conditions, genetic factors, geographical location, plant nutritional status, and collection time. In order to produce suitable packaging for food, the materials used in the packaging should not have an offensive smell. Lavandulyl acetate (5.30%) was the third major component that can assist linalyl acetate in a more floral aroma (Guo and Wang, 2020). However, other significant LEO components such as eucalyptol (4.34%), camphor (3.05%), terpinen-4-ol (1.84%), and cis-linalool oxide (1.72%) can negatively impact aroma which is not noticeable. Table 1, illustrates oxygenated monoterpenes and oxygenated sesquiterpenes as the major and minor substances, respectively. Oxygenated monoterpenes, represented by linalool and linalyl acetate, were the dominant compounds in LEO. The major constituents, linalool and linalyl acetate, are reported to possess strong antimicrobial effects, indicating a potential for developing active food packaging from LEO-loaded films.

Table 1
Main compounds in lavender essential oil (LEO)

No	Compound	LEO (%)	Monoterpenes (%)	
1	Linalool	38.47	Hydrocarbons	Oxygenated
2	Linalyl acetate	27.19	11.24	74.81
3	Lavandulyl acetate	5.30		
4	Eucalyptol	4.34		
5	Camphor	3.05	Sesquiterpenes (%)	
6	Terpinen-4-ol	1.84		
7	<i>Cis</i> -linalool oxide	1.72	Hydrocarbons	Oxygenated
8	Beta-pinene	0.63	4.38	0.92
9	Geranyl acetate	0.38		
10	Beta-ocimene	0.19		
*Expressed as a percentage of the total peak area of the chromatograms.				

Table 1.

3.2. Morphological properties

The morphology of the TG films was investigated using SEM and CLSM micrographs (Fig. 1). The control TG sample showed a uniform surface without pores or irregularities. However, with increasing LEO content, film surface homogeneity decreased. Similar findings were reported by Noshirvani et al. (2017) and Evangelho et al. (2019). The TG film with 5% LEO showed less agglomeration, followed by the good distribution of LEO droplets within the film matrix and emulsion stability during casting. However, higher LEO concentrations in the films showed greater agglomeration, possibly due to the subsequent coalescence of lipid molecules (Fabra et al., 2011). As seen in Fig. 1, the TG-LEO15 and TG-LEO20 films showed higher heterogeneity and irregularity on their surfaces, probably related to the weakening of the polysaccharide matrix by the placement of LEO molecules between them. During film drying, coalescence, flocculation, and creaming phenomena likely occurred, leading to the migration of oil droplets to the film surface where the EOs evaporated.

The CLSM images of the TG-based samples are shown in Fig. 2. The micrographs indicate an even distribution of oil droplets (red) within the film's matrix, regardless of the oil content incorporated. However, the dispersibility of LEO in the matrix of the other samples was clearly visible, indicating an oil-in-water emulsion was formed. As the LEO concentration increased, a higher number of oil droplets was observed in CLSM images and lipid droplet aggregation occurred. Compared to the other films, larger oil droplets appeared in the TG films with 15% and 20% LEO. These findings indicate that LEO

concentrations greatly influence droplet distribution in the TG films. The CLSM results follow the SEM micrographs, which showed agglomeration of EO droplets in the TG matrix with 15% and 20% LEO. Agarwal et al. (2020) and Zhang et al. (2021) reported that the combination of higher percentages of geraniol and thyme essential oil in the film formulation resulted in more and larger oil droplets.

Figure 1.

Figure 2.

3.3. Fourier-transform infrared spectroscopy (FTIR) of films

Fourier-transform infrared (FTIR) analysis was conducted to investigate the interaction between TG and LEO. The FTIR spectra of the TG control sample and TG-LEO films are shown in Fig. 3. A broad band between 3000 to 3650 cm^{-1} , which may be attributed to the stretching vibration of inter-molecular or intra-molecular hydrogen bonds in the film-forming matrix of TG, was observed in all TG-based film samples (Santos, Carvalho, & Rojas, 2021). The FTIR spectra of the pure TG film showed a hydroxyl group absorption peak at 3324 cm^{-1} . With increasing LEO content, the absorption peaks shifted to lower frequencies at 3322 cm^{-1} , 3318 cm^{-1} , 3316 cm^{-1} , and 3314 cm^{-1} in TG-LEO5, TG-LEO10, TG-LEO15, and TG-LEO20, respectively. The increase in peak intensities with increasing LEO content indicated significant interactions between TG and LEO molecules (Sun et al., 2021). The broadband from 3000 to 2800 cm^{-1} was attributed to ν_{asym} (C–H) and ν_{sym} (C–H) stretching from the CH_3 group and ν_{sym} (C–H) stretching from the CH_2 group (Bogdan et al., 2021; Zahabi et al., 2021). The observed peak around 1738 cm^{-1} in the spectra indicated the C = O stretching vibration of the carbonyl group from aliphatic esters, which are significant functional groups in EOs (Alizade-Sani et al., 2020; Tongnuanchan, Benjakul, Prodpran, Pisuchpen, & Osako, 2016). This peak confirmed the presence of linalool and linalyl acetate, which are major constituents in LEO (Jamróz et al., 2018; Velmurugan, Ganeshan, Fathima, & Rao, 2017). The FTIR spectrum also revealed a peak at 1634 cm^{-1} attributed to amide I (Sun et al., 2021). The intensity of this peak increased with increasing LEO content, indicating the presence of interactions between TG and LEO. Moreover, the amide I peak shifted slightly from 1634 to 1638 cm^{-1} , which was assigned to hydrogen-bonding changes of the C = O (Bigi, Haghighi, Siesler, Licciardello, & Pulvirenti, 2021). The FTIR spectra showed a new peak at 1458 cm^{-1} in the TG-LEO samples, which suggests that the polymer matrix was affected by the molecular incorporation of the LEO alkane group and TG. Similar results were reported by Jamróz et al. (2018) and Sun et al. (2021). The strong peak observed around 1024 cm^{-1} was associated with the stretching vibration of C–O, which is characteristic of polysaccharide compounds and glycerol (Atef, Rezaei, & Behrooz, 2014; Jamróz et al., 2018). In summary, the FTIR analysis confirmed that the addition of LEO influenced the position, width, and intensity of the spectra. The incorporation of LEO and TG also resulted in changes in molecular interactions and the structure of the matrix. The findings suggest that the development of new functional materials with enhanced properties could be achieved by utilizing LEO in TG-based films.

Figure 3.

3.4. Physical properties of films

3.4.1. Thickness

The film thickness plays an important role in the water vapor barrier and tensile properties. As shown in Table 2, the film thickness ranged from 43.2 μm to 51.7 μm . The TG-LEO20 film had the greatest thickness at 51.7 μm while the control TG film had the lowest at 43.2 μm . There were no significant differences ($p > 0.05$) between the control sample and films containing LEO except TG-LEO20. A similar trend was reported by Haghighi et al. (2019) and Zhang et al. (2021), who found that incorporating EOs increased the thickness of chitosan-gelatin blended films and gellan/chitosan films, respectively. Increasing film thickness with higher EO content may be due to changes in the film matrix structure and interactions caused by EO incorporation, as indicated by FTIR and morphological analyses. The larger LEO droplets present at higher EO concentrations could alter chain mobility and packing during film formation, impacting film thickness.

Table 2
Thickness, water content (WC), water solubility (WS), water vapor permeability (WVP), oxygen permeability (OP), and contact angle of TG-based films.

Sample	Thickness (μm)	WC (%)	WS (%)	WVP ($10^{-11} \text{ g.s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$) 1)	OP ($10^{-3} \text{ g.s}^{-1} \text{ m}^{-2}$) 2)
TG	43.2 $\pm$ 2.2 ^b	18.67 $\pm$ 0.93 ^a	85.22 $\pm$ 1.59 ^a	5.31 $\pm$ 0.19 ^d	6.20 $\pm$ 0.26 ^c
TG-LEO5	46.8 $\pm$ 4.1 ^{ab}	17.21 $\pm$ 0.86 ^a	67.38 $\pm$ 1.42 ^b	5.82 $\pm$ 0.28 ^c	6.63 $\pm$ 0.37 ^c
TG-LEO10	47.2 $\pm$ 3.8 ^{ab}	14.93 $\pm$ 0.71 ^b	58.79 $\pm$ 1.04 ^c	6.29 $\pm$ 0.32 ^b	7.38 $\pm$ 0.41 ^b
TG-LEO15	47.4 $\pm$ 5.0 ^{ab}	13.24 $\pm$ 0.82 ^c	51.43 $\pm$ 1.29 ^d	6.98 $\pm$ 0.24 ^a	7.91 $\pm$ 0.18 ^{ab}
TG-LEO20	51.7 $\pm$ 4.6 ^a	11.03 $\pm$ 0.69 ^d	47.32 $\pm$ 1.08 ^e	7.41 $\pm$ 0.17 ^a	8.32 $\pm$ 0.29 ^a
*Values are means $\pm$ standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).					

3.4.2. Water content (WC)

The moisture contents of the TG film samples are shown in Table 2. The film with 5% LEO showed an insignificant decrease in water content from 18.67% (control) to 17.21% ($p > 0.05$). By increasing the LEO concentration from 10–20%, the moisture content of the films significantly decreased ($p < 0.05$) with TG-LEO20 having the lowest. This can be attributed to the addition of hydrophobic LEO and interactions

between LEO functional groups and the hydrocolloid, limiting the availability of hydrogen groups to form hydrophilic bonds with water molecules and thus decreasing water content (Song et al., 2018). Simsek et al. (2020) also reported decreasing the moisture content of carboxymethyl cellulose-based films by adding *Santolina chamaecyparissus*, *Schinus molle*, and *Eucalyptus globulus* essential oils.

3.4.3. Water solubility (WS)

Water solubility is a crucial parameter for packaging high humidity products. Film sensitivity to water greatly influences the shelf life of packaged foods. High water solubility can limit using films for packaging high water content foods (Haghighi et al., 2020). Table 2, shows film solubility at different LEO concentrations. As LEO concentration increased from 5–20%, water solubility decreased from 85.22–47.32%. Decreased solubility relates to interactions between TG and LEO functional groups. Film structure is a determinant factor in solubility and it can extend the duration of separation and dispersion of particles within the solvent (Hosseini & Razavi, 2022). LEO constituent can reduce the probability of interplay among TG hydroxyl groups and H₂O molecules, thus decreasing film affinity for water solubility (Dashipour et al., 2015; Hafsa et al., 2016). Similar results were observed adding tea tree essential oil (Song et al., 2022) and *Artemisia sieberi* and *Achillea santolina* essential oils (Hashemi & Khodaie, 2021) to bio-based films. Overall, incorporating LEO into the TG films significantly decreased their water solubility compared to the control film, especially at higher LEO concentrations. Decreased solubility likely correlates with interactions between LEO and the TG film matrix that reduce bonding sites for water, as well as the hydrophobic nature of LEO.

3.4.4. Contact angle (CA)

The contact angle (surface hydrophobicity) was measured to evaluate barrier properties against liquid moisture (Phan et al., 2005) and the surface hydrophobicity of the films (Hashemi & Khodaie, 2021). The contact angle (CA) depends on cohesive and adhesive molecular forces between the film surface and water. Figure 4 shows CA measurements of the TG-based films. The CA for pure TG and LEO-containing films ranged from 42.28° to 64.38°. As LEO content increased, the CA of the films significantly increased ($p < 0.05$). This can be attributed to LEO covering hydrophilic and functional groups of the gum, reducing film hydrophilicity and thus increasing CA. Li et al. (2020) and Hashemi and Khanegah (2017) reported that loading thyme essential oil into gelatin films and oregano essential oil into basil seed gum films, respectively, significantly increased CA. This trend suggests that LEO decreases the number of hydrophilic sites on the film surface, particularly at higher concentrations.

Figure 4.

3.5. Barrier properties of films

3.5.1. Water vapor permeability (WVP)

Water vapor permeability (WVP) is one of the physical properties of biodegradable films which indicates the mechanism of water molecules transfer from the film surface or possible interactions between the

polymer and essential oil in the food packaging film (Peighambardoust, Peighambardoust, Pournasir, & Mohammadzadeh Pakdel, 2019). WVP of the pure TG film and the films loaded with LEO are depicted in Table 2. The WVP was $5.31 \times 10^{-11} \text{ g.s}^{-1}\text{m}^{-1}\text{Pa}^{-1}$ for the pure TG film, which increased significantly to $6.98 \times 10^{-11} \text{ g.s}^{-1}\text{m}^{-1}\text{Pa}^{-1}$ when the LEO content increased from 0–15% ($p < 0.05$). However, there was no significant difference between the WVP value of the TG-LEO20 film ($7.41 \times 10^{-11} \text{ g.s}^{-1}\text{m}^{-1}\text{Pa}^{-1}$) and TG-LEO15 ($p > 0.05$). Generally, adding LEO into the films, increased the WVP value. It can be due to the hydrophobic characteristics of the LEO, which affects the interaction between TG molecules by reducing the availability of hydrogen bonds between TG molecular chains, leading to the formation of discontinuities in the polymer matrix (Roy, Shankar, & Rhim, 2019; Simsek et al., 2020). In addition, the pores in the film structure can be created by LEO evaporation during the drying process, as a result, the number of channels for water transfer increases (Chen & Liu, 2016; Evangelho et al., 2019). Zhang et al. (2021) also observed that by the addition of TEO to gellan-chitosan films, the WVP significantly increased compared to the control sample.

Water vapor permeability (WVP) indicates the mechanism of water molecule transfer from the film surface or possible interactions between the polymer and essential oil in food packaging films (Peighambardoust et al., 2019). Table 2, shows WVP of the pure TG film and films loaded with LEO. WVP was $5.31 \times 10^{-11} \text{ g.s}^{-1}\text{m}^{-1}\text{Pa}^{-1}$ for the pure TG film, significantly increasing to $6.98 \times 10^{-11} \text{ g.s}^{-1}\text{m}^{-1}\text{Pa}^{-1}$ when LEO content increased to 15% ($p < 0.05$). However, there was no significant difference between the WVP of TG-LEO20 ($7.41 \times 10^{-11} \text{ g.s}^{-1}\text{m}^{-1}\text{Pa}^{-1}$) and TG-LEO15 ($p > 0.05$). Generally, adding LEO into the films increased WVP which can be due to the hydrophobic characteristics of LEO, affecting TG molecule interactions by reducing hydrogen bonds between TG molecular chains, and forming discontinuities in the polymer matrix (Roy et al., 2019; Simsek et al., 2020). Additionally, pores in the film structure can form during LEO evaporation, increasing channels for water transfer (Chen & Liu, 2016; Evangelho et al., 2019). Zhang et al. (2021) also observed that adding thyme essential oil to gellan-chitosan films significantly increased WVP compared to the control. In summary, incorporating LEO into the TG films significantly increased their WVP compared to the control film, likely due to the hydrophobicity of LEO disrupting the TG network and forming pores during film formation. However, WVP did not significantly differ between TG-LEO15 and TG-LEO20 films.

3.5.2. Oxygen permeability (OP)

Oxygen permeability is a main criterion for selecting food product packaging, revealing the oxygen transfer capacity of films. The oxygen permeability of film samples is summarized in Table 2. The pure TG film's oxygen permeability (OP) was $6.20 \times 10^{-3} \text{ g.s}^{-1}\text{m}^{-2}$. Increasing LEO content to 5% showed no significant difference in OP ($p > 0.05$). At higher LEO concentrations, OP values incremented to $7.38 \times 10^{-3} \text{ g.s}^{-1}\text{m}^{-2}$ (TG-LEO10), $7.91 \times 10^{-3} \text{ g.s}^{-1}\text{m}^{-2}$ (TG-LEO15), and $8.32 \times 10^{-3} \text{ g.s}^{-1}\text{m}^{-2}$ (TG-LEO20). However, there was no considerable difference between TG-LEO15 and TG-LEO20 film OP ($p > 0.05$). Overall, increasing the LEO content in TG films enhanced the OP value due to the porous texture formed in the EO-containing film structures during LEO evaporation. Additionally, LEO's hydrophobicity could be the other

mechanism to increase oxygen solubility in biodegradable films and facilitate oxygen transport (Perdones et al., 2014). In this regard, Zhou et al. (2021) also observed that OP values significantly increased with increasing cinnamon EO concentration in cassava starch-based films.

Table 2.

3.6. Optical properties and light transmission of films

Appearance and optical properties are important for food packaging commercial acceptance and application. Table 3, shows color values (L^* , a^* , b^* and ΔE , WI) and opacity parameters of TG films. Increasing LEO concentration from 5–20% decreased lightness (L^*) and whiteness (WI) from 98.37 to 94.74 and 97.53 to 93.27, respectively. There were no significant differences between TG-LEO5 and TG-LEO10 or TG-LEO15 and TG-LEO20 for these parameters ($p > 0.05$). Liu et al. (2022) stated that incorporating LEO into soluble soybean polysaccharide/carboxymethyl chitosan coatings reduced L^* and WI, making coatings slightly darker. Index a^* represents the color greenness or redness; negative values indicate green and positive values indicate red (Hosseini & Pazhouhandeh, 2023). This index decreased from -0.51 to -0.84 in films with 0–20% LEO, respectively. Parameter ΔE significantly increased from 2.83 to 5.52 with increasing LEO content ($p < 0.05$). Films with LEO also had increased b^* values from 1.79 to 4.11 as LEO content increased, likely related to LEO phenolic compounds (Liu et al., 2022). Similar trends were observed by Cai and Wang (2021) and Sun et al. (2021). However, Jamróz et al. (2018) found L^* , a^* , and b^* parameters unaffected by LEO concentration ($p > 0.05$), possibly due to LEO extraction methods and plant growth factors. Film transmittance and opacity are perceived as important parameters affecting product appearance and consumer choice. As LEO concentration increased in films, UV transmittance (Fig. 5) decreased at all wavelengths (200–800 nm). According to Table 3, film opacity ranged from 8.64–13.79, significantly enhanced by increasing LEO content ($p < 0.05$). Increased opacity may relate to LEO migration to film surfaces during drying and film thickness (Cai & Wang, 2021). These trends correlate with changes in film composition and structure due to LEO incorporation.

Table 3
Color parameters (L, a, b, ΔE , and WI) and opacity of the TG films loaded with different concentrations of LEO

Sample	Color parameters					Opacity (600 nm)
	L*	a*	b*	ΔE	WI	
TG	98.37 $\pm$ 0.31 ^a	-0.51 $\pm$ 0.09 ^a	1.79 $\pm$ 0.05 ^e	2.83 $\pm$ 0.17 ^e	97.53 $\pm$ 0.52 ^a	7.53 $\pm$ 0.21 ^e
TG-LEO5	97.71 $\pm$ 0.54 ^{ab}	-0.58 $\pm$ 0.06 ^{ab}	2.25 $\pm$ 0.02 ^d	3.09 $\pm$ 0.10 ^d	96.74 $\pm$ 0.64 ^{ab}	8.64 $\pm$ 0.42 ^d
TG-LEO10	96.85 $\pm$ 0.73 ^b	-0.65 $\pm$ 0.09 ^{bc}	2.87 $\pm$ 0.11 ^c	3.70 $\pm$ 0.03 ^c	95.69 $\pm$ 0.78 ^b	11.16 $\pm$ 0.39 ^c
TG-LEO15	95.42 $\pm$ 0.36 ^c	-0.73 $\pm$ 0.03 ^{cd}	3.56 $\pm$ 0.04 ^b	4.73 $\pm$ 0.10 ^b	94.15 $\pm$ 0.69 ^c	13.17 $\pm$ 0.45 ^b
TG-LEO20	94.74 $\pm$ 0.47 ^c	-0.84 $\pm$ 0.08 ^d	4.11 $\pm$ 0.06 ^a	5.52 $\pm$ 0.16 ^a	93.27 $\pm$ 0.87 ^c	13.79 $\pm$ 0.51 ^a
*Values are expressed as mean $\pm$ standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).						

Table 3.

Figure 5.

3.7. Mechanical properties (TS, EB, and YM) of films

Mechanical strength and extensibility are required for packaging to tolerate external stresses during packaging, transportation, and storage. Tensile strength (TS), elongation at break (EB), and Young's modulus (YM) of TG-based films were evaluated (Fig. 6). Increasing LEO concentration from 5–20% significantly decreased TS from 33.94 to 21.52 MPa ($p < 0.05$). Decreased TS may result from weak interactions between LEO hydrophobic molecules and the TG matrix, caused by insufficient emulsification of the Tween 80 emulsifier in film-forming solutions (Sun et al., 2021). In addition, as shown in SEM images (Fig. 1), the occurrence of heterogeneity in the surface structures of the TG-LEO films during the drying process following the increase in LEO concentration can play a dominant role in the TS reduction. In this regard, Ma et al. (2016) and Cai and wang (2021) revealed that TS was reduced significantly by increasing the content of oleic acid in the TG films and ginger EO in fish sarcoplasmic protein/chitosan films, respectively. TG-LEO20 and pure TG films showed the highest (10.84%) and lowest (6.88%) EB values which indicate the flexibility and stretchability of the films. The placement of LEO among TG macro-molecules facilitates the mobility of the polymer chains under stress by creating free volumes, which leads to the enhancement of their flexibility. Noshirvani et al. (2017) and Zhang et al. (2021) reported that TS decreased while EB significantly increased as cinnamon/ginger EO and thyme EO content increased in their films. According to Fig. 6, the films with LEO had lower YM compared to pure

TG film ($p < 0.05$). LEO reduced intrinsic stiffness from 352.4 MPa (pure TG film) to 119.4 MPa (TG-LEO20), indicating more flexibility and less rigidity. It could be due to the EOs' plasticizing role in the matrix of the films and hindering polymer molecule interactions. These results agree with incorporating oregano EO into hydroxypropyl methylcellulose-based films (Lee et al., 2019) and apricot kernel EO into chitosan-based films (Priyadarshi et al., 2018).

Figure 6.

3.8. Total phenol content (TPC) and antioxidant capacity of films

The total phenolic content of the film samples is presented in Fig. 7A. As can be seen, the TPC in the pure TG film was 1.87 mg/g as the lowest amount, whereas the highest content was observed in the TG-LEO20 film with 6.28 mg/g. In addition, as the incorporation of LEO increased from 5–20%, the TPC of the films also considerably increased ($p < .05$). The same results were also obtained for the radical-scavenging activity of the TG films in the absence and presence of LEO (Fig. 7B). According to the data, the pure TG film exhibited little inhibition activity (17.34%), while the films incorporated with the LEO showed a higher level of radical scavenging activity with values of 20.76%, 22.34%, 25.11%, and 29.62% for 5%, 10%, 15%, and 20% of LEO-containing films, respectively. These results can be attributed to the main compounds of LEO, in the high concentration of linalool (Table 1). Hamad et al. (2013) also reported that the high share of linalool in LEO was followed by the antioxidant activity of LEO. It has also been revealed that linalool as a non-phenolic compound can be responsible for antioxidant activity (Ahmed et al., 2019). Moreover, the presence of phenolic compounds in plants can play a prominent role in the radical scavenging and antioxidant capacity (Hashemi & Jafarpour, 2020; Hashemi et al., 2016). In this regard, Nurzyńska-Wierdak and Zawiślak (2016) expressed that there was a positive correlation between total phenol contents and the ability to reduce DPPH, indicating that phenolic compounds resulted in the antioxidant activity of the medicinal lavender plant. Therefore, an increase in the DPPH radical scavenging activity of the TG-LEO films is associated with an increase in the concentration of total phenolic content in the films.

Figure 7.

3.9. Antimicrobial activity of films

The antimicrobial activity of the films was evaluated to determine the resistance of the films against bacterial growth using film disks. The size of the clear zone reflects the antibacterial ability of different films against bacteria. The results of the antibacterial activities of the TG and TG-LEO film samples are shown in Table 4. The pure TG film had no inhibitory activity on bacterial growth. However, with the addition of 5% LEO, the zones of inhibition were observed against *S. putrefaciens* (24.57 mm²) and *Y. enterocolitica* (23.19 mm²). The TG-LEO10 films had a significant antibacterial effect against all the studied bacteria ($p < 0.05$). In addition, the lowest and highest inhibition zones were observed against *E. coli* O157:H7 (22.82 mm²) and *S. putrefaciens* (41.63 mm²), respectively, compared to the other bacteria.

In the case of TG-LEO15, the diameters of the clear zones increased significantly ($p < 0.05$) against all nine pathogen bacteria. In contrast with TG-LEO10, the lowest and highest inhibition zones by applying TG-LEO15 were created around *P. aeruginosa* bacteria (32.58 mm^2) and *Y. enterocolitica* (69.47 mm^2), respectively. The proportions of LEO had significant contributions to antimicrobial activities. As expected, the TG films loaded with 20% LEO showed higher antimicrobial activities than other films ($p < 0.05$). Moreover, the superior antibacterial action of the TG-LEO20 film was observed on *S. putrefaciens* (101.53 mm^2), *Y. enterocolitica* (97.58 mm^2), and *L. monocytogenes* (89.12 mm^2), respectively. Among the examined bacteria, *S. typhimurium* and *S. putrefaciens* demonstrated the highest and lowest persistence against the LEO-loaded films, respectively. By correlating the FTIR spectral data with those obtained from the GC-MS analysis (Table 1), it can be seen that the major constituents of LEO were the linalool (38.47%) and linalyl acetate (27.19%), which have been detected as strong antimicrobial agents (Herman, Tambor, & Herman, 2016; Hossain et al., 2017). Furthermore, the interaction between these main compounds and minor components of EOs can be effective (Chouhan, Sharma, & Guleria, 2017). The antimicrobial activity of EOs is not related to a single mechanism because of the molecules' diversity in EOs. There are many mechanisms at different sites of the bacterial cell's inner and outer components, cytoplasm leakage, ion exchange, disruption of the enzyme system, and inhibition of respiration can cause bacterial cell death (Nisar et al., 2018). Bogdan et al. (2021) stated that the antibacterial mechanism of the main components of LEO (linalyl acetate, linalool, and terpinen-4-ol) was based on damaging the lipid layer of the cell membrane, which led to bacterial cell leakage. In summary, by increasing the LEO concentration in the film matrixes due to the increase of antibacterial compounds, the inhibitory properties of the disks were enhanced and larger clear areas were observed.

Table 4
Antimicrobial activities of TG-based films incorporated with different concentrations of LEO

Bacteria	Inhibition zone diameters (mm ²)				
	TG	TG-LEO5	TG-LEO10	TG-LEO15	TG-LEO20
<i>E. coli</i>	ND ^d	ND ^d	24.51 ± 4.98 ^c	41.12 ± 3.75 ^b	67.62 ± 5.16 ^a
<i>E. coli</i> O157:H7	ND ^d	ND ^d	22.82 ± 3.42 ^c	36.31 ± 4.29 ^b	66.14 ± 6.01 ^a
<i>L. monocytogenes</i>	ND ^d	ND ^d	31.48 ± 5.91 ^c	52.69 ± 6.88 ^b	89.12 ± 7.96 ^a
<i>S. aureus</i>	ND ^d	ND ^d	29.13 ± 4.37 ^c	48.72 ± 4.03 ^b	84.61 ± 8.37 ^a
<i>B. cereus</i>	ND ^d	ND ^d	23.75 ± 2.94 ^c	35.89 ± 3.52 ^b	65.28 ± 7.93 ^a
<i>S. typhimurium</i>	ND ^d	ND ^d	22.89 ± 2.41 ^c	32.71 ± 3.12 ^b	47.69 ± 4.78 ^a
<i>S. putrefaciens</i>	ND ^e	24.57 ± 3.45 ^d	41.63 ± 5.28 ^c	68.70 ± 6.13 ^b	101.53 ± 9.71 ^a
<i>P. aeruginosa</i>	ND ^d	ND ^d	23.92 ± 2.74 ^c	32.58 ± 4.73 ^b	61.77 ± 6.82 ^a
<i>Y. enterocolitica</i>	ND ^e	23.19 ± 3.12 ^d	38.27 ± 4.98 ^c	69.47 ± 7.18 ^b	97.58 ± 9.02 ^a
*Values are means ± standard deviation. Different letters in the same row indicate significant differences (p < 0.05).					

Table 4.

4. Conclusion

This study successfully developed biodegradable Tara gum-based films incorporated with various concentrations of LEO. The results indicated that increasing LEO concentration led to a decrease in water content and water solubility while increasing the thickness and heterogeneity of the films. The uniform distribution of LEO within the film matrix was confirmed by CLSM and SEM analyses, and FTIR spectra revealed the interaction between Tara gum and LEO. Additionally, the color and optical properties of the films were influenced by LEO concentration, with higher LEO concentrations resulting in lower L*, a*, and whiteness index values, but higher b*, ΔE, and opacity values. The barrier and mechanical properties were found to be affected by LEO concentration, with increased oxygen permeability and water vapor permeability values, but reduced tensile strength and Young's modulus at higher LEO concentrations. Importantly, the addition of LEO enhanced the flexibility, antimicrobial, and antioxidant activities of the films. However, further improvements in water vapor and oxygen resistance are necessary for optimal performance in food packaging applications. Overall, the incorporation of LEO into Tara gum-based films significantly improved their mechanical, antioxidant, and microbial properties, highlighting the potential of TG-LEO films for use in food packaging applications.

Declarations

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Declaration of competing interest

The authors declare no conflict of interest and no ethical issues. Moreover, we declare that this manuscript has not been submitted or published elsewhere.

Author contributions

Ahmad Reza Hosseini: Conceptualized and designed the experiments, performed data analysis, and prepared figures. Additionally, contributed significantly to the writing of the manuscript. Nafiseh Zahabi: Contributed to data analysis and interpretation. Also, made substantial contributions to the writing of the manuscript. Fahimeh Pazhouhandeh: Contributed to the writing of the manuscript. Farzaneh Sharif: Contributed to the writing of the manuscript.

Data availability

Data will be available on request.

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Figures

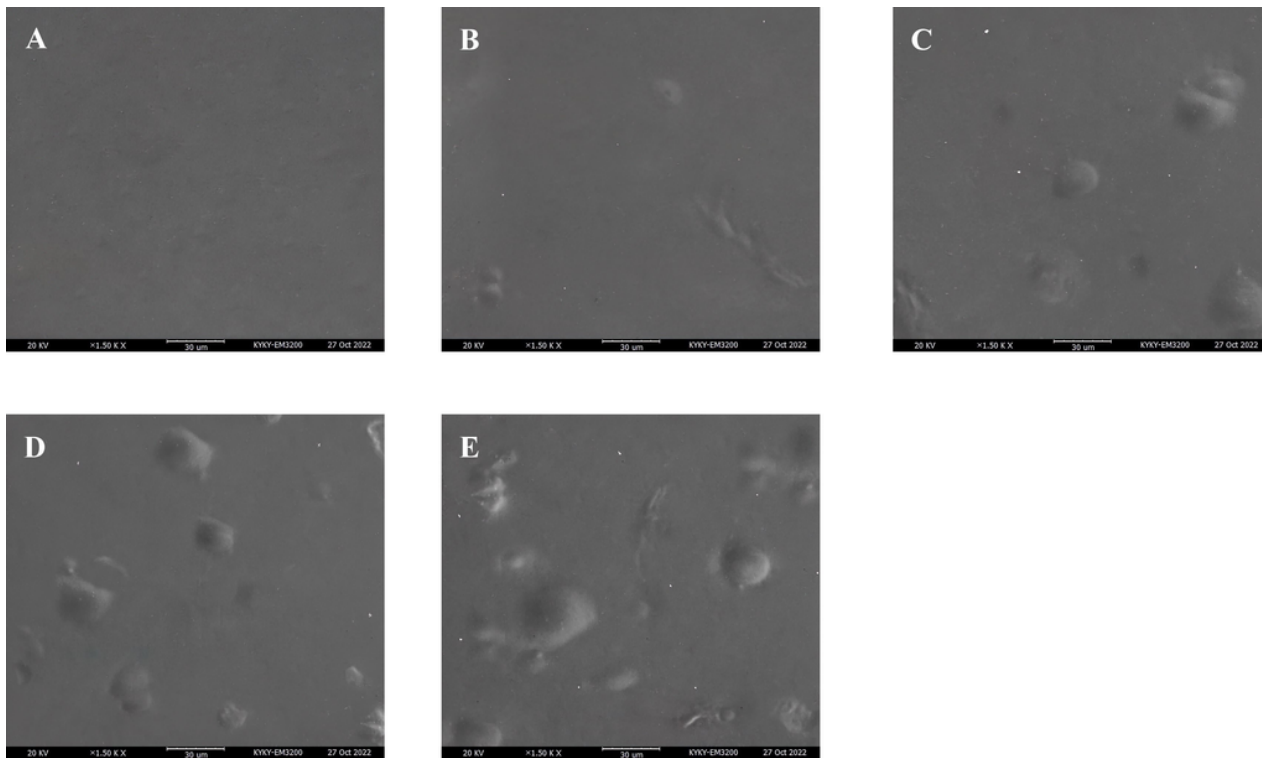


Figure 1

SEM micrographs of the surface of TG films loaded with different concentrations of LEO: pure TG (A), TG-LEO5 (B), TG-LEO10 (C), TG-LEO15 (D), and TG-LEO20 (E).

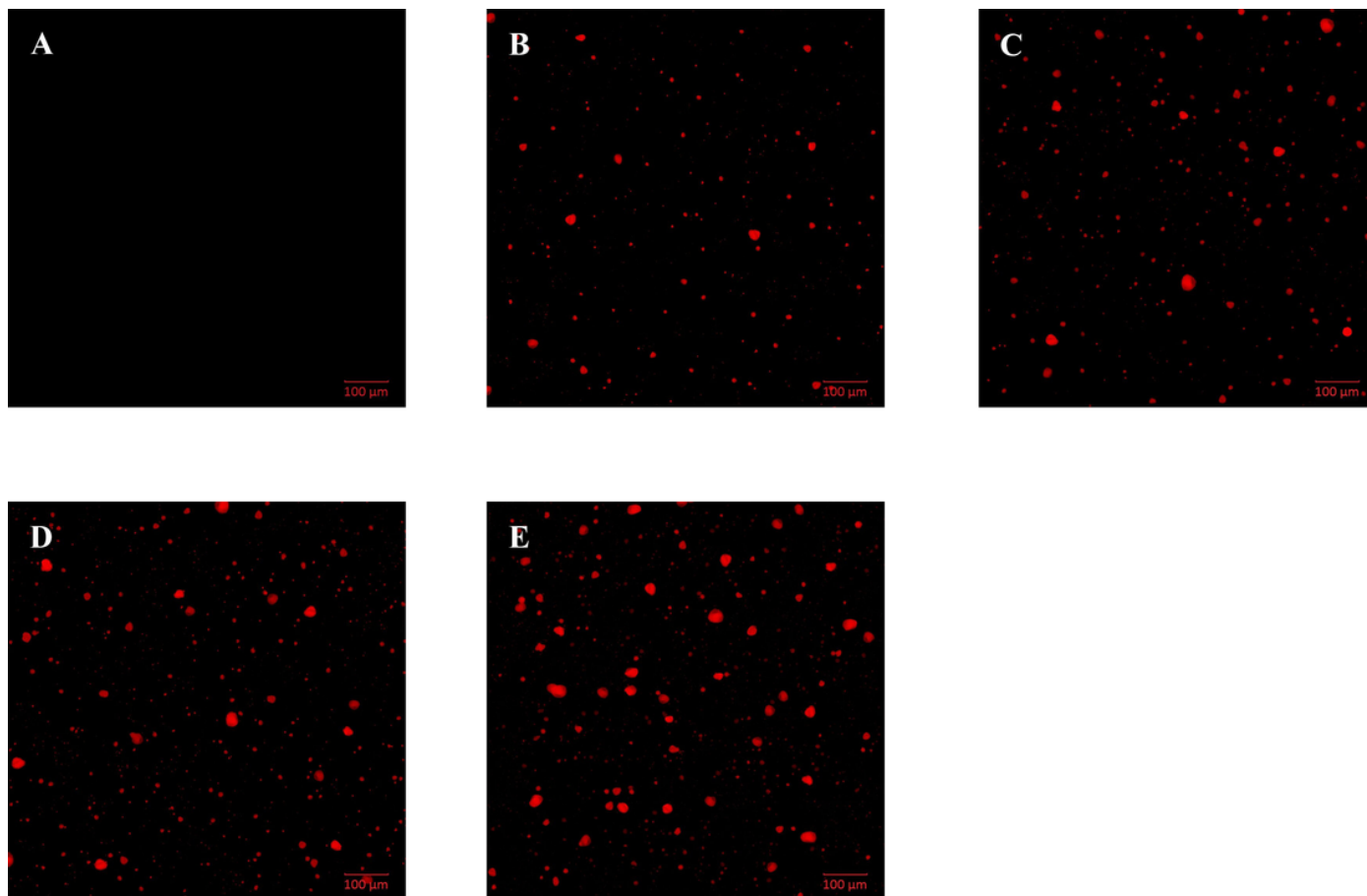


Figure 2

CLSM images of pure TG (A), TG-LEO5 (B), TG-LEO10 (C), TG-LEO15 (D), and TG-LEO20 (E).

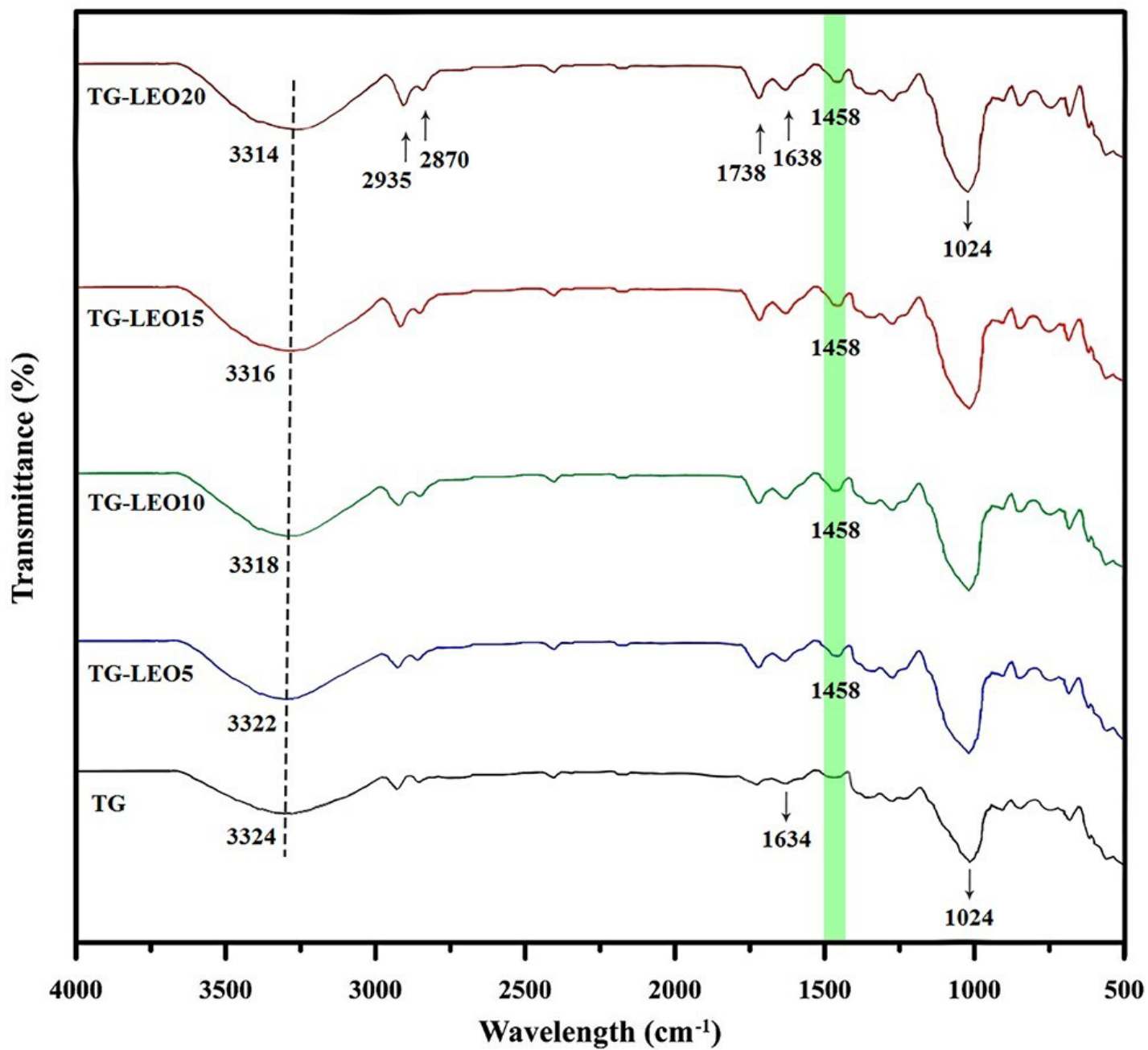


Figure 3

FTIR spectra of TG films loaded with different concentrations of LEO (0 %, 5%, 10%, 15%, and 20%).

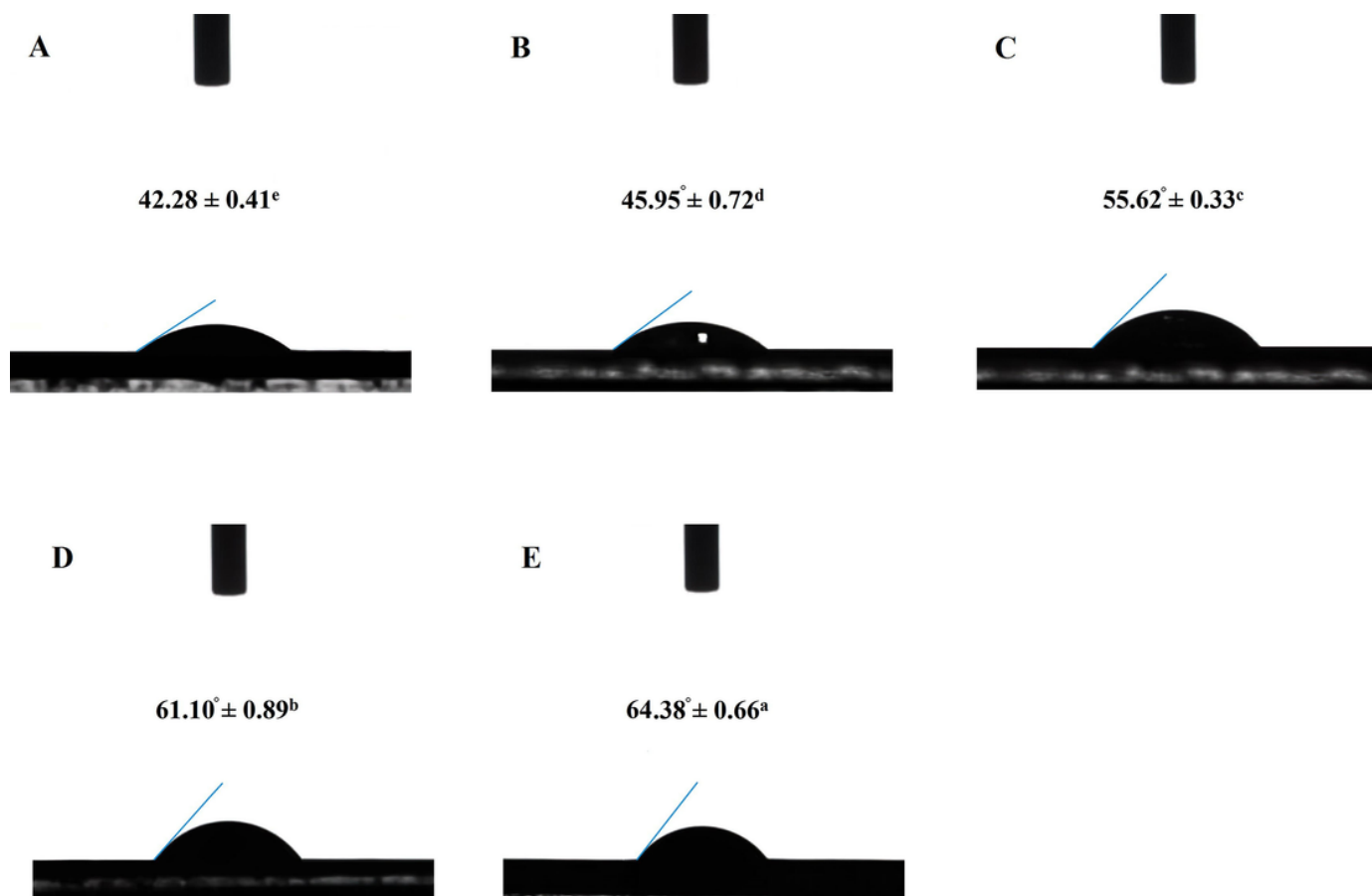


Figure 4

Contact angle images of pure TG (A), TG-LE05 (B), TG-LE010 (C), TG-LE015 (D), and TG-LE020 (E).

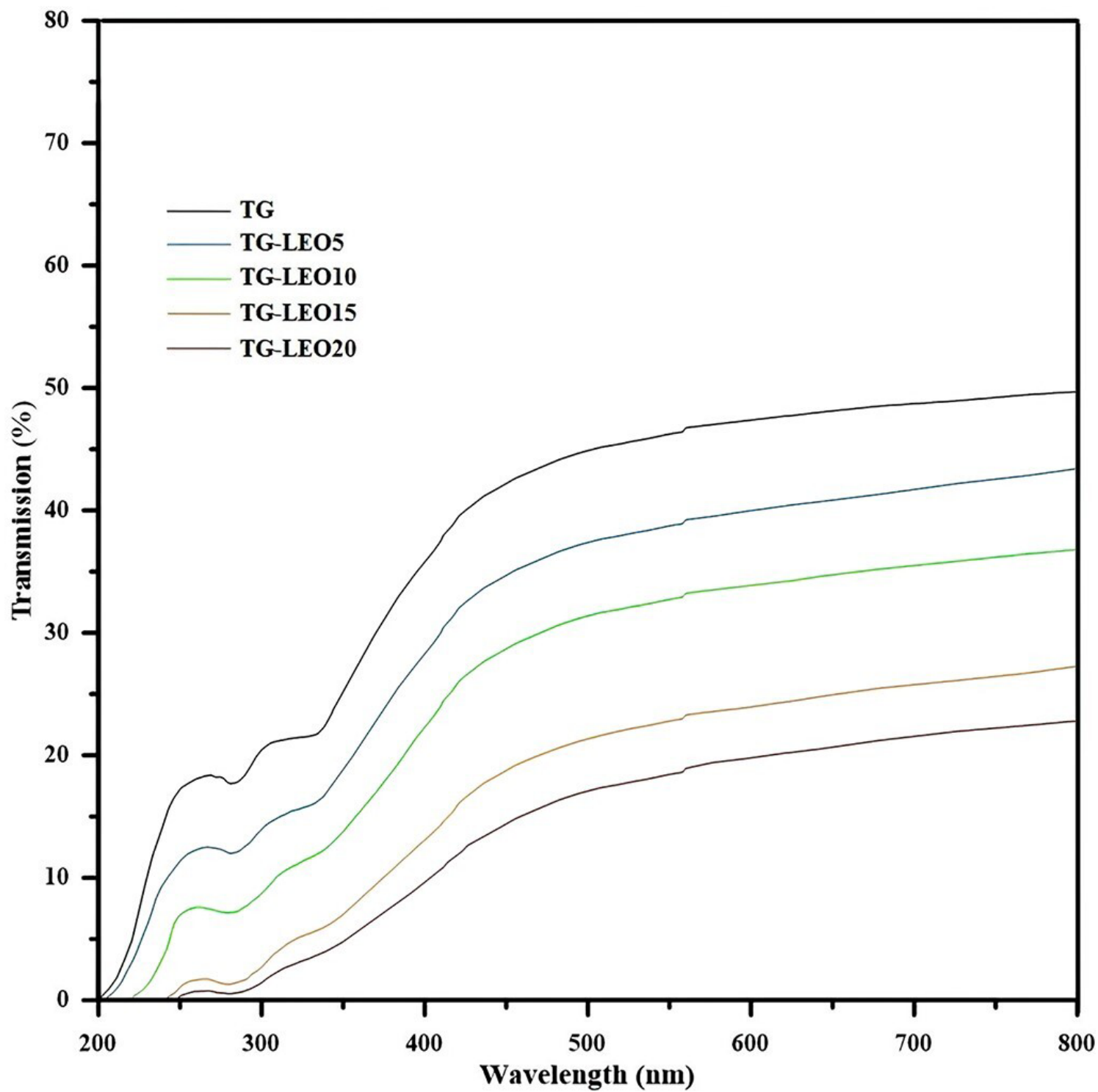


Figure 5

UV transmittance of the TG-based film with different concentrations of LEO.

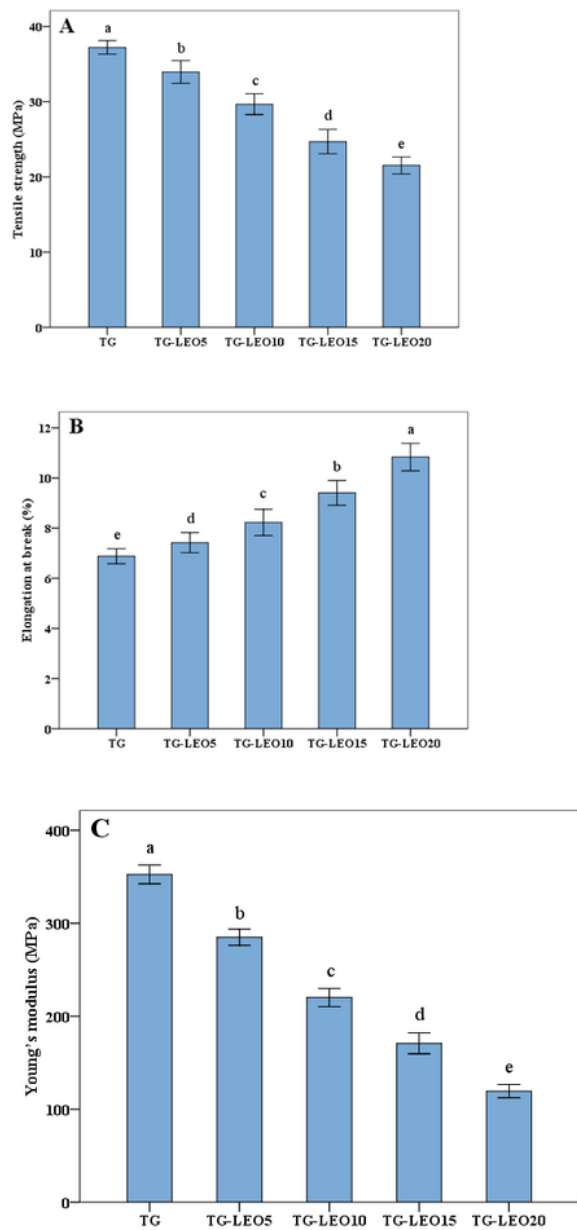


Figure 6

Mechanical properties of the films incorporated with different concentrations of LEO; Tensile strength (A), Elongation at break (B), and Young's modulus (C).

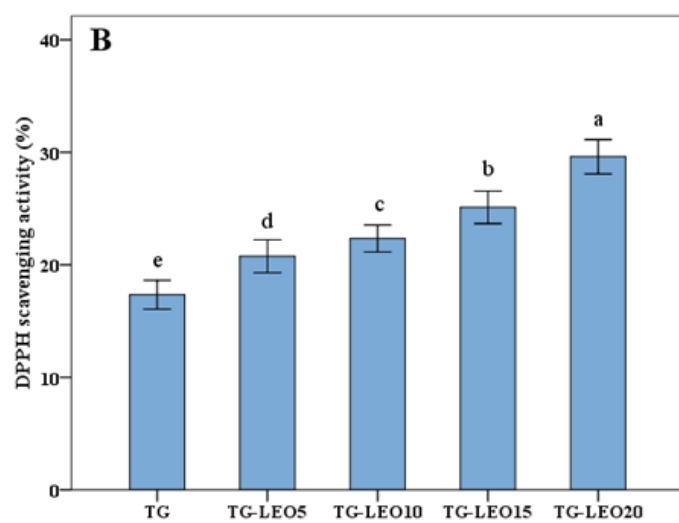
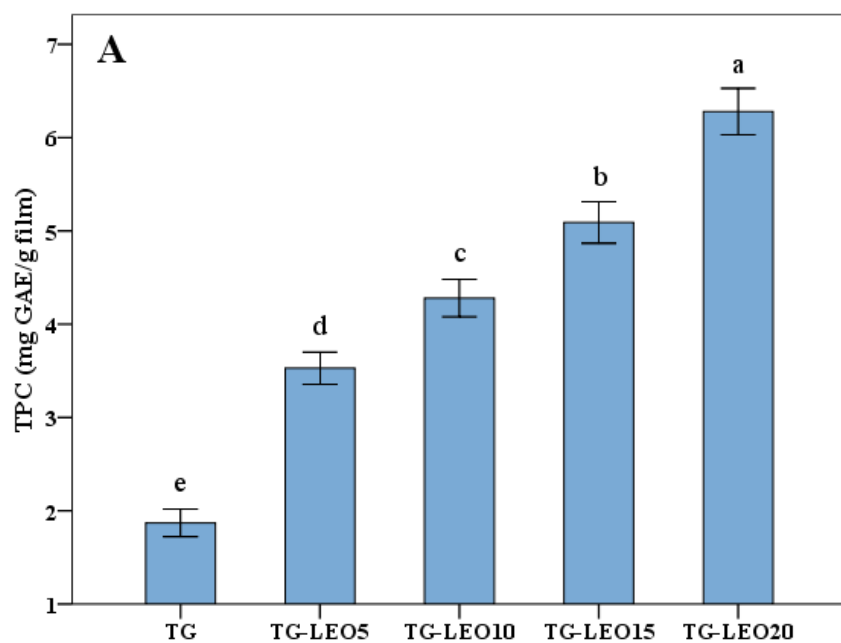


Figure 7

Total phenolic content (A) and DPPH radical-scavenging activity (B) of TG films loaded with different concentrations of LEO.