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# The comparison of antimicrobial and antioxidant activity of essential oil of *Oliveria decumbens* and its nanoemulsion preparation to apply in food industry

Leila Nikravan[1], Siavash Maktabi[2*], Maryam Ghaderi Ghahfarrokhi[3] and Mohammad Mahmoodi Sourestani[4]

[1] PhD Graduated from Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Associate Professor, Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3] Assistant Professor, Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[4] Associate Professor, Department of Horticultural Science, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran

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## Abstract

The aim of this study was to compare the antibacterial and antioxidant effects of essential oil (*Od*-EO) and nanoemulsion (*Od*-NEO) of *Oliveria decumbens* for practical use in food industry. The plant was collected from the North-East of Khuzestan province and essential oil was extracted by Clevenger device. The components of *Od*-EO were identified by GC–MS analysis. The *Od*-NEO was prepared by stirring tween 80, distilled water and *Od*-EO and then using a sonicator with a power of 200 W and a piezoelectric crystal with a probe diameter of 15 mm. The antibacterial effects of *Od*-EO and *Od*-NEO were evaluated on *Escherichia coli O157:H7*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Listeria monocytogenes* by disk diffusion agar and microdilution methods. Antioxidant effect was also evaluated using DPPH and ABTS scavenging methods. Data showed that thymol (53.4%), γ-terpinene (20.48%), p-cymene (18.02%) and myristicin (2.7%) were the most predominant compounds of *Od*-EO. The particle size of *Od*-NEO was 45.71nanometer and the Zeta potential was -36.3 mV. The value of $IC_{50}$ in the DPPH test for BHT, *Od*-EO and *Od*-NEO were 18.57, 1456.95 and 757.29 (µg/ml), respectively. In ABTS method, the $IC_{50}$ rates were 12.32, 565.83 and 507.89 (µg/ml). The MIC of the *Od*-EO and *Od*-NEO ranged between 0.312 to 20 mg/ml. The lowest MIC value was obtained for *S. aureus* and highest value was obtained for *P. aeruginosa*. Data showed that the antioxidant activity of *Od*-NEO was significantly higher than *Od*-EO ($p<0.05$). Also, *Od*-NEO had a greater inhibitory effect on the studied bacteria than *Od*-EO and gram positive bacteria showed more sensitivity. Due to higher antioxidant and antimicrobial properties of *Od*-NEO, need for increased attention to this issue and the *Od*-NEO could potentially be used in the food industry.

**Keywords**: Antimicrobial, Antioxidant, Essential oil, Nanoemulsion, *Oliveria decumbens*

## Introduction

In recent decades, a great deal of attention has been paid to the use of natural preservation and flavorings in food to the extent the shelf life. Consumers are less willing to use foods that contain artificial preservatives or synthetic additives due to their harmful effects (Hamedo and Abdelmigid, 2009; Settanni et al., 2012).

* **Corresponding Author**: Siavash Maktabi, Associate Professor, Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran E-mail: s.maktabi@scu.ac.ir

Essential oils (EOs) are natural compounds which extracted from different parts of plants such as flower, bud, seed, leaf, bark, wood, fruit and root (Jayasena and Jo, 2013). EOs have antioxidant, antimicrobial, antiviral and antifungal properties and they have also been widely used in food, medicine and perfume industries (Burt, 2004). The chemical composition of plant essential oils is variable under the influence of genetic and environmental factors such as climate and seasonal changes and growth stage (Djenane, 2015).

Today, the use of nanoencapsulation of essential oil in the food industry instead of free EOs has increased for various reasons such as unpleasant organoleptic effects, poor chemical stability, low solubility in water and toxic effects in the use of high concentration of EOs (Li et al., 2015). Several new applications of nanotechnology such as micelles, liposomes and nanoemulsion have been considered, including the use of nanocomponents (Sozer and Kokini, 2009). The antimicrobial properties of these fine particles may grow due to better penetration into the phospholipid membrane of the microorganism. Antimicrobial properties of nanoemulsions are directly related to the method of its formation (Shahbazi et al., 2017). Particle diameter in nanoemulsion is commonly ranged between 10-100 nanometers. These nano-sized emulsions have physicochemical properties; they are more transparent, more stable and less effective on organoleptic properties of food (Rao and McClements, 2011).

*Oliveria decumbens* belongs to Umbelliferae family and grows in warm regions in countries such as south and west of Iran, Iraq, Syria and Turkey. This plant is widely used in traditional medicine, in treating indigestion, diarrhea, abdominal pain and fever. The aerial parts of the plant contain a significant amount of essential oil which contains oxygenated monoterpenes (Amin et al., 2005). The plant has antimicrobial and antioxidant compounds such as thymol and carvacrol that can be used in the food and pharmaceutical industries (Esmaeili et al., 2018).

So far, various effects of essential oil of *Oliveria decumbens* (*Od*-EO) including antimicrobial effects (Tabatabaei Yazdi et al., 2018), antioxidant properties (Khosravinezhad et al., 2017) and the effect on skin wound healing in mice (Mahboubi et al., 2016) have been reported, but, there is no data on the biological properties of *Oliveria decumbens* nanoemulsion (*Od*-NEO). The aim of this study was to compare the antibacterial and antioxidant effects of *Od*-EO and *Od*-NEO for possible application in the food industry.

## Materials and Methods

### Collected and preparation of the plant

*Oliveria decumbens* fresh plant was collected from the North-East of Khuzestan province and in the full flowering stage. The collected plant was identified and approved by the faculty of Agriculture of Shahid Chamran University of Ahvaz. After being washed with distilled water, the plant was kept in a dark place at room temperature (25˚C) for a week to get dry.

### Extraction of essential oil

*Od*-EO was extracted by hydro-distillation method using Clevenger device for three hours. The essential oil was then dried under anhydrous sodium sulphate, and stored in dark-colored container in the dark place, at 4°C, until used.

### Chemical analysis of essential oil

Chemical analysis of *Od*-EO was carried by analytical gas chromatograph coupled with mass spectrometer detector (Agilent 5977B, USA) (GC-MS). The capillary column was HP-5MS (5% phenyl methyl silicone and 95% dimethylpolysiloxane), (length: 30m; internal diameter: 0.25mm and 0.25µm film thickness). Column temperature program was set as follows: The oven temperature was kept at 60 °C for 1 min and next changed from 60 to 250 °C

at 5 °C/min, then was kept at 250 °C for 2 min. Injection volume was 0.2 µL and The temperature of the injector was 250°C. The carrier gas was helium with purity 99.99%, constant flow rate 1.1ml per min, and a split ratio equal to 1:100. . The procedure was operated in the electron impact mode at 70 eV. The GC-MS analysis was done in triplicate. The individual compounds were identified by comparing their retention indices with those of normal alkanes under the same condition and their mass spectra with internal reference available from the library (Adams, 2007).

**Nanoemulsion preparation**

*Od*-NEO was formulated according to the method of Noori et al. (2018). Briefly, *Od*-EO (2% v/v) and tween 80 (30% essential oil weight) were added to distilled water and stirred at 3000 rpm for 10 min. The resulting emulsion was then transferred to a sonicator (Hielscher, UP200Ht, Germany) with a power of 200 W and a piezoelectric crystal with a probe diameter of 15 mm. The sonication process was carried out for 5 min. During this step, the temperature was controlled by the ice around the sample. Then particle size, the polydispersity index (PDI) of nanoemulsion and zeta potential were measured by dynamic light scattering (DLS) method using a Zetasizer Nano-ZS (Malvern instruments, Worcestershire, UK) according Hosseinnia et al., 2017.

**Bacterial strains**

*Escherichia coli O157:H7* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 9027), as gram-negative-bacteria, and *Staphylococcus aureus* (ATCC6538) and *Listeria monocytogenes* (ATCC 19118) as gram-positive bacteria were used in this experiment. All strains were obtained from culture collection of the Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz and confirmed by biochemical tests and PCR assay.

**Agar disc diffusion assay (DDA)**

To investigate the antibacterial activity of *Od*-EO or *Od*-NEO, agar disk diffusion method was used according to CLSI, 2012. The studied bacteria were cultured (initial inoculum at $1x10^8$ CFU/ml) in BHI agar medium using sterile swab. Then, sterile paper discs (Whatman No. 1) containing 20 µl of *Od*-EO or *Od*-NEO was placed on the surface of BHI agar. The plates were incubated for 24 hours at 37°C and the diameter of the inhibition zone was measured. Negative control (DMSO) and positive controls includes ampicillin and gentamicin (10µg/disk) also was used.

**Determination of MIC and MBC**

MIC and MBC values were assessed by microdilution method in broth culture medium according to NCCLS (2000). Briefly, dimethyl sulfoxide (DMSO) 5% (v/v) as an emulsifier and agar-agar 0.05% (w/v) as a stabilizer were dissolved in BHI broth and then a serial dilutions (0.312, 0.625, 1.25, 2.5, 5, 10 and 20 mg/ml) of *Od*-EO or *Od*-NEO was made. Subsequently, 180µl of the BHI containing specified concentrations of *Od*-EO or *Od*-NEO were added to each well of a 96- microplate with u-shaped bottom and 20µl of bacterial suspension was transferred to each well. Positive control includes BHI medium contains DMSO and bacterial suspension and negative control includes BHI medium contains DMSO and *Od*-EO or *Od*-NEO. The microplate was incubated for 24-hours at 37 °C.

Bacterial growth was determined by measuring absorbance at 600 nm using a microplate reader (Biotek, USA). In order to evaluate MBC, 20 µL of each well without any significant growth in the MIC determination assay was cultured on BHI agar and incubated at 37 °C for 24 h. The concentration of *Od*-EO or *Od*-NEO in those plates with no visible colonies was considered to be the MBC.

**Determination of DPPH radical scavenging activity**

Scavenging activity against DPPH radical was determined by a method according to Lee and Yen (2006) with some modifications. The DPPH radical was dissolved in ethanol with a final concentration of 0.1 mmol $L^{-1}$. 0.3 ml of various concentration of ethanolic solution of *Od*-EO or *Od*-NEO (between 312.5 to 5000 µg/ml) with 2.7 ml. DPPH was put in a dark place for 30-minutes at 25°C, and its absorption was then measured at 517nm against ethanol as the blank reference. 2.7 ml DPPH, 0.3 ml ethanol is considered as control. The DPPH scavenging activity was measured and compared with butylated hydroxytoluene (BHT) and expressed according to the following equation:

DPPH scavenging activity (%) = (AC - AS)/AC * 100

Where AC is the absorbance of the negative control, and AS is the absorbance of control. All samples were analyzed in triplicates. The scavenging activity was expressed as the 50% inhibitory concentration ($IC_{50}$), which was defined as the sample concentration necessary to inhibit DPPH radical activity by 50% after incubation.

**ABTS Radical-Scavenging Assay**

ABTS radical-scavenging assay also was used to evaluate of antioxidant effects of *Od*-EO or *Od*-NEO. For the preparation of ABTS cation, the reaction solution of 7 mM ABTS and 2.45 mM potassium persulfate was used. The solution was prepared 12 to 16 hours before being used and it was stored in a dark place at room temperature. The final solution was diluted with methanol until that its absorbance reached to 0.7±0.02 nm at the wavelength of 734 nm. A suitable amount of sample was added to the diluted ABTS solution and its absorbance was measured at 734 nm. BHT synthetic antioxidant was used as standard (Srinivasan et al., 2007).

**Statistical Analysis**

All experiments were performed in triplicate. Results were analyzed by one-way analysis of variance using SPSS (version 16; SPSS Inc., Chicago, USA). The significance levels are expressed at 95% confidence level ($p < 0.05$) throughout.

## Results

**Essential oil composition**

The components of *Od*-EO were determined by GC- mass. The Major chemical compositions of *Od*-EO are presented in Table 1. Thymol (53.4%), γ-terpinene (20.48%), p-cymene (18.02%) and myristicin (2.7%) were the most predominant compounds of *Od-EO*.

**Physical properties of nanoemulsion**

The average size of the *Od*-NEO particles diameter and the Zeta potential are shown in Figure 1. The particle size and Zeta potential of *Od*-NEO were 45.71 nm and -36.3 mV respectively.

**Antimicrobial activity on bacteria**

The summarized results of MIC and MBC of *Od*-EO or *Od*-NEO against the studied bacteria can be seen in Table 2. The results showed that both *Od*-EO and *Od*-NEO have antimicrobial effects against the studied microorganisms. Generally, Gram positive bacteria compared to Gram negative bacteria were more sensitive to both *Od*-EO and *Od*-NEO. The MIC of the *Od*-EO or *Od*-NEO ranged between 0.312 to 20 mg/ml. The lowest MIC value was obtained for *S. aureus* and highest value was obtained for *P. aeruginosa*. MIC and MBC values for *Od*-NEO were clearly lower than the values for *Od*-EO.

The results of antibacterial activity of *Od*-EO and *Od*-NEO by disc diffusion method in agar can be seen in Table 3. Again, gram positive bacteria were more sensitive to the *Od*-NEO and the inhibitory effects of *Od*-NEO were significantly ($P<0.05$) more than the *Od*-EO.

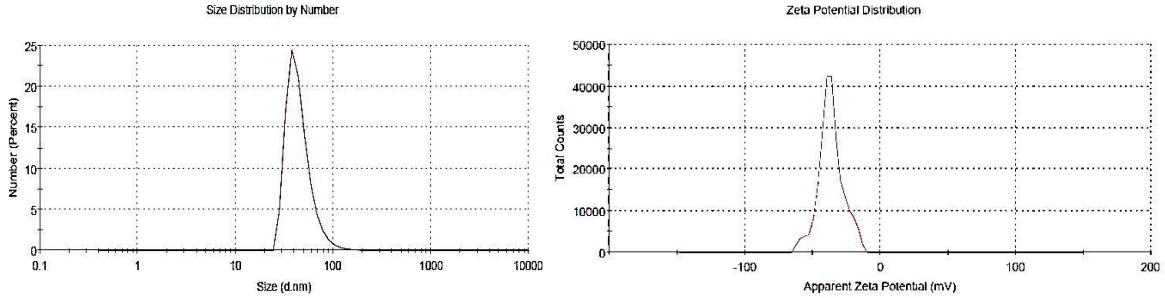


**Figure 1. Particle size and zeta potential of *Od*-NEO**

**Table 1. Chemical composition of *Od-EO***

| No | RT | RI | Compounds | % |
|---|---|---|---|---|
| 1 | 5.45 | 924 | Alpha-thujene | 0.24 |
| 2 | 5.633 | 932 | Alpha-pinene | 0.15 |
| 3 | 6.823 | 974 | Beta-pinene | 1.16 |
| 4 | 7.23 | 988 | Beta.-myrcene | 0.4 |
| 5 | 8.071 | 1014 | Alpha- Terpinene | 0.12 |
| 6 | 8.368 | 1020 | Cymene | 18.02 |
| 7 | 8.488 | 1024 | Limonene | 1.5 |
| 8 | 8.569 | 1026 | 1,8-Cineole | 0.04 |
| 9 | 9.604 | 1054 | Gamma-Terpinene | 20.48 |
| 10 | 10.669 | 1086 | Terpinolene | 0.05 |
| 11 | 14.228 | 1174 | Terpinene-4-ol | 0.6 |
| 12 | 14.806 | 1186 | Alpha. terpineol | 0.04 |
| 13 | 18.988 | 1289 | Thymol | 53.4 |
| 14 | 19.337 | 1298 | Carvacrol | 0.66 |
| 15 | 28.458 | 1517 | Myristicin | 2.7 |
| | | Total | | 99.56 |

RT: Retention Time
RI: Retention index

**Table 2: MIC and MBC (mg/ml) of *Od*-EO and *Od*-NEO against bacteria**

| Microorganism | *Od*-EO | | *Od*-NEO | |
|---|---|---|---|---|
| | MIC | MBC | MIC | MBC |
| *S. aureus* | 0.312 | 0.625 | 0.312 | 0.312 |
| *L. monocytogenes* | 2.5 | 5 | 1.25 | 2.5 |
| *E. coli O157:H7* | 10 | 10 | 2.5 | 2.5 |
| *P. aeruginosa* | 10 | 20 | 2.5 | 10 |

**Table 3. Antimicrobial effects of *Od*-EO and *Od*-NEO using disk diffusion method**

| Microorganism | Diameter of inhibition zone (mm) | | | | |
|---|---|---|---|---|---|
| | *Od*-EO | *Od*-NEO | Gentamicin | Ampicillin | DMSO |
| *S. aureus* | $30.56\pm1.91^{a}$ | $33.43\pm1.88^{a}$ | $22.66\pm1.24^{c}$ | $26.66\pm0.47^{b}$ | $0.00\pm0.00^{d}$ |
| *L. monocytogenes* | $19.73\pm2.65^{bc}$ | $21.6\pm0.57^{b}$ | $25.7\pm2.47^{ab}$ | $29.56\pm2.18^{a}$ | $0.00\pm0.00^{d}$ |
| *E. coli O157:H7* | $13.86\pm1.32^{c}$ | $16.26\pm0.52^{b}$ | $19.76\pm0.88^{a}$ | $14.53\pm0.72^{bc}$ | $0.00\pm0.00^{d}$ |
| *P. aeruginosa* | $11.43\pm0.49^{c}$ | $14.1\pm2.21^{b}$ | $17.76\pm0.55^{a}$ | $0.00\pm0.00^{d}$ | $0.00\pm0.00^{d}$ |

Mean values of three replicates ± the standard deviation of the mean. Different superscript letters represent the significant differences ($p<0.05$).

**Table 4. Antioxidant capacity of *Od*-EO and *Od*-NEO using DPPH and ABTS$^+$ methods**

| Method | IC50 (µg/mL) | | |
|---|---|---|---|
| | BHT | *Od*-EO | *Od*-NEO |
| *DPPH* | 18.57±3.41$^c$ | 1456.95±109.99$^a$ | 757.29±16.62$^b$ |
| *ABTS* | 12.32±0.37$^c$ | 565.83±25.05$^a$ | 507.89±26.17$^b$ |

Mean values of three replicates ± the standard deviation of the mean. Different superscript letters represent the significant differences ($p<0.05$).

## Antioxidant activity

In this study the antioxidant effect of *Od*-EO and *Od*-NEO was evaluated using two methods, DPPH and ABTS. The results of both methods are shown in Table 4. In the both methods the antioxidant activity of *Od*-EO and *Od*-NEO were clearly less than the standard group (BHT). In general, the antioxidant activity of *Od*-NEO was higher than *Od*-EO and this difference was statistically significant ($P<0.05$).

## Discussion

Various studies have been conducted on the chemical composition and antimicrobial properties of *Od*-EO (Motamedi et al., 2010; Khosravaninezhad et al., 2017; Alizadeh Behbahani et al., 2017). One of the commonalities in such studies is the determination of the effective composition of the *Od*-EO, which can have sixty different components. The main constituents of the *Od*-EO can make up to 85% of the weight of the essential oil, and the minor constituents are only slightly available.

In the current study, thymol (53.4%), γ-terpinene (20.48%), p-cymene (18.02%) and myristicin (2.7%) were the most predominant compounds. The results are more consistent with study conducted by Amin et al. (2005) and, (Khosravinezhad et al., 2017), meanwhile is in contrast with Rahimifard et al. (2010). These differences in the amount of essential oil components could be due to seasonal and climate changes, geographical area, plant age and harvest time, which can affect the antimicrobial and antioxidant activities of the EO plant.

The particle size of nanoemulsion in the present study was 45.71 nanometer, which is consistent with the study of Noori et al. (2018), who prepared a nanoemulsion of ginger essential oil using tween 80 and reported an average particle size of 57.4 nm. In contrast, Severino et al. (2015), reported the particle size of citrus, tangerine and lemon nanoemulsion, prepared using tween 20, between 176.4-133.4 nm. This difference in particle size can be related to the role of tween 80. It seems that to produce oil- in- water emulsion, the utilization of tween 80 is more appropriate than other polysorbates. In light of the fact that it creates a greater balance between hydrophilic and lipophilic compounds (Ghosh et al., 2013). When emulsions are exposed to intense mechanical stresses such as ultrasonic, they may release hydroxyl and carboxyl groups from the chemical compounds of essential oil, which are effective in increasing the negative charge on the droplet surface. Negative Zeta potential contributes to product stability. The presences of similar surface charges on the nanoemulsion cause a relative repulsion and prevent them from sticking together and merging (Changet et al., 2015). In the present study, the Zeta potential was reported to be -36.3 mV. The lower polydispersity index (PDI) of the nanoemulsion is also one of the advantages of using ultrasonic method which causes constant distribution of particles in

nanoemulsion. In this study the recorded figure for *Oliveria decumbens* was 0.420.

In the current study, MIC and MBC results showed that Gram-positive bacteria are more sensitive to both *Od*-EO and *Od*-NEO. Subsequently, disk diffusion assay also supported the finding. The results can be compared with others. For example, Motamedi *et al*. studied the antimicrobial effect of ethanolic and methanolic extracts of *Oliveria decumbens* against some photogenic bacteria. In that study *S. aureus* had the highest sensitivity to the extract and *Salmonella* Thyphi and *P. aeroginosa* showed the highest resistance. Mahboubi et al. (2014), found the antimicrobial effect of *Od-EO* to be more effective on the clinical isolates of *Acinetobacter baumannii* than *Pelargonium graveolens* and *Ziziphora tenuir*. The highest percentage of thymol, carvacrol and *p*-cymene in *Oliveria decumbens* can be the reason for the antimicrobial properties of the plant. Due to the fact that thymol is a nonpolar compound with high dissolution power, it can easily cross the membrane of a bacterial cell wall and damage the cell. In general, the phenolic compounds in EO's cause a change in the permeability of the material transfer channels in the bacterial membrane and by changing the ion gradient; it leads to the cessation and disruption of the basic function of the cell, consequently causing their death (Seow et al., 2014). Similar to the present study, Mahboubi et al. (2008) reported that the antimicrobial properties of *Od-EO* are more effective on gram- positive bacteria than gram- negative bacteria. Also, fungi are more sensitive to *Od-EO* than bacteria. Hydrophobic structure of cell wall in gram negative bacteria is impermeable to essential oil molecules, so they are less sensitive to essential oils than gram positive bacteria which lipophilic molecules easily enter the thick peptidoglycan layer (Nazzarlo et al., 2013). The results of current study also show that gram-positive bacteria are more sensitive to both *Od*-EO and *Od*-NEO than gram-negative bacteria. Statistical analysis shows that *Od*-NEO significantly ($P< 0.05$) has more growth inhibitory effects on bacteria than *Od*-EO.

As shown in Table 4, the average $IC_{50}$ in the DPPH test for BHT, *Od*-EO and *Od*-NEO were 18.57±3.41, 1456.95±109.99, 757.29±16.62 (µg/ml), respectively. In ABTS method, the $IC_{50}$ rates were 12.32±0.37, 565.83±25.05, 507.89±26.17 (µg/ml), that were consistent with the results obtained from the study of Khosravinezhad et al. (2017). Esmaili et al.(2018) studied the chemical composition and antioxidant activity of *Od-EO* in different stages such as vegetative, buds, flower and seed. In vegetative stage, the reported amounts of γ-terpinene, carvacrol and thymol were 33.6%, 16.9% and 16% respectively. As the plant grows to the flowering stage, the amounts of γ-terpinene was significantly reduced and converted to thymol and carvacrol, therefore thymol reached to 37.8% and carvacrol to 29.38%. Also, the reported antioxidant activities in flowering and vegetative stages were 86.1 and 98.5 µg/ml, respectively. To evaluate the precise amounts of antioxidant activity of a particular essential oil, the assessment of its optimum concentration is compulsory. On the contrary, the observed dissimilarities between various methods might, partially, be described by the overall measure of minor compounds in the oils, which can significantly influence the ultimate oil antioxidant effect. The intensity and function of antioxidant activity depends on genotype, maturity stage and sprouting setting.

In general, there is no data regarding antibacterial and antioxidant activity of *Od*-NEO. Our study shows a good antimicrobial and antioxidant activity of the *Od*-NEO. Regarding the fact that the use of essential oil is limited due to low solubility in water, low stability and high volatility of its active ingredients and sever organoleptic properties, the use of nanoemulsion can be recommended (Prakash and Kiran, 2016). Thanks to their small particle size and

increase in surface area compared to their volume, nanoemulsion can reach the surface of cell membranes more easily and have more antimicrobial properties (Salvia-Trujillo et al., 2015a). For instance, Moghimi et al. (2016) reported that nanoemulsion of *Thymus daenensis* essential oil has about 10 times more antibacterial activity than pure essential oil. Bhargava et al. (2015) stated that the use of oregano nanoemulsion is a more effective antimicrobial control approach than pour essential oil. In 2019, Khanzadi et al., also compared the antimicrobial activity of emulsion and nanoemulsion of *Ziziphora* against *E.coli* and reported that the nanoemulsion could be used as a natural preservative in the food industry. In general, the antimicrobial properties of nanoemulsion depend on the components of the essential oil, particle size, and viscosity and also how the nanoemulsion is made (Donsi and Ferrari, 2016). In addition, the electrical properties of the particles, such as the zeta potential, can also play a role in antimicrobial activity.

In conclusion, *Od*-NEO had a greater inhibitory effect on the studied bacteria than *Od*-EO. It also showed a significant increase in its antioxidant properties. Due to the greater stability of the *Od*-NEO and its higher antioxidant and antimicrobial properties than *Od*-EO, more attention needs to be paid to this issue and the nanoemulsion of this plant could potentially be used in the food industries.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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# مقایسه خاصیت ضد میکروبی و آنتی‌اکسیدانی اسانس و نانوامولسیون اسانس گیاه لعل کوهستان *Oliveria decumbens* به منظور کاربرد در صنایع غذایی

لیلا نیکروان[۱]، سیاوش مکتبی[۲*]، مریم قادری‌قهفرخی[۳] و محمد محمودی‌سورستانی[۴]

[۱] دانش‌آموخته دکتری تخصصی بهداشت مواد غذایی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
[۲] دانشیار گروه بهداشت مواد غذایی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
[۳] استادیار گروه بهداشت مواد غذایی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
[۴] دانشیار گروه باغبانی، دانشکده کشاورزی، دانشگاه شهید چمران اهواز، اهواز، ایران

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## چکیده

هدف این مطالعه مقایسه‌ی اثرات ضد باکتریایی و آنتی‌اکسیدانی اسانس (*Od*-EO) و نانو امولسیون (*Od*-NEO) گیاه لعل کوهستان با نام علمی *Oliveria decumbens* برای استفاده عملی در صنایع غذایی بود. این گیاه از شمال شرقی استان خوزستان جمع‌آوری شده و اسانس توسط دستگاه کلونجر استخراج شد. ترکیبات *Od*-EO با استفاده از دستگاه GC-MS تعیین شدند. نانوامولسیون گیاه به وسیله‌ی هم زدن توپین ۸۰، آب مقطر و اسانس و سپس با استفاده از یک دستگاه سونیکاتور با قدرت ۲۰۰ وات و یک کریستال پیزوالکتریک با قطر پروب ۱۵ میلی‌متر انجام شد. اثرات ضد باکتری *Od*-EO و *Od*- NEO بر روی چهار باکتری *اشریشیا کولای* O157:H7، *سودوموناس آئروژینوزا* ، *استافیلوکوکوس اورئوس* و *لیستریا مونوسیتوژنز* با استفاده از روش‌های انتشار در دیسک آگار و میکرودیلوشن مورد بررسی قرار گرفت. اثر آنتی‌اکسیدانی نیز با استفاده از روش‌های مهار DPPH و ABTS بررسی شد. داده‌ها نشان داد که تیمول (۵۳/۴٪)، الفا ترپینن (۲۰/۴۸٪)، پی=سیمن (۱۸/۰۲٪) و میریستیسین (۲/۷٪) غالب‌ترین ترکیبات *Od*-EO بودند. اندازه‌ی ذرات نانوامولسیون ۴۷/۷۱ نانومتر و پتانسیل زتا ۳۶/۳- میلی ولت بود. مقدار $IC_{50}$در آزمون DPPH برای BHT، Od-EO و Od-NEO به ترتیب ۱۸/۵۷، ۱۴۵۶/۹۵ و ۷۵۷/۲۹ (میکروگرم بر میلی‌لیتر) بود. در روش ABTS ، این مقادیر به ترتیب ۱۲/۳۲، ۵۶۵/۸۳ و ۵۰۷/۸۹ (میکروگرم در میلی‌لیتر) بود. مقادیر MIC برای Od-EO و Od-NEO بین ۰/۳۱۲ تا ۲۰ میلی‌گرم در میلی‌لیتر بود. کم‌ترین مقدار MIC برای باکتری *S. aureus* و بیش‌ترین مقدار برای *P. aeruginosa* به دست آمد. داده‌ها نشان داد که فعالیت آنتی‌اکسیدانی *Od*-NEO بیش‌تر از *Od*-EO است و این اختلاف از نظر آماری معنی‌دار بود ($P<0.05$). همچنین، *Od*-NEO اثر مهاری بیش‌تری نسبت به *Od*-EO بر روی باکتری‌های مورد مطالعه داشت و باکتری‌های گرم مثبت حساسیت بیش‌تری نشان دادند. با توجه به بالاتر بودن خاصیت آنتی‌اکسیدانی و ضد میکروبی *Od*-NEO، توجه بیش‌تری به این مسئله لازم است و *Od*-NEO می‌تواند به طور بالقوه در صنایع غذایی مورد استفاده قرار گیرد.

**کلمات کلیدی:** ضد میکروبی، آنتی اکسیدان، اسانس، لعل کوهستان

*نویسنده مسئول : سیاوش مکتبی، دانشیار گروه بهداشت مواد غذایی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

E-mail: s.maktabi@scu.ac.ir