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Investigation of Chemical Compositions and Antimicrobial Properties of Eucalyptus Leaf Essential Oil and Its Application in the Production of Doogh

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ABSTRACT

Using natural preservatives such as plant essential oils is a healthy and environmentally friendly approach that serves as a suitable alternative to chemical additives and helps maintain consumer health. Eucalyptus, or the blue gum plant, with the scientific name *Eucalyptus globulus*, belongs to the Myrtaceae family. This study was conducted with the aim of identifying chemical compounds and evaluating the antibacterial activity of eucalyptus essential oil (EEO) on a number of pathogenic microorganisms in vitro. The chemical compounds of EEO were identified using a GC-MS device. Subsequently, the effects of various concentrations of EEO (0, 2, 4, and 6 µl/l) on some pathogenic bacteria and the microbial characteristics of doogh samples during a 45-day storage period were investigated. The GC-MS analysis results showed that 1,8-cineole (48.6%) and alpha-pinene (23.86%) constituted the highest percentages of EEO compounds. By adding 6 µl/l of EEO to doogh, no mold and yeast growth was observed throughout the entire storage period, and the sample containing 4 µl/l of essential oil also contained an acceptable number of mold and yeast at the end of the storage period. Additionally, EEO caused a reduction in the total microbial count. Based on the results of this study, gram-negative bacteria were more resistant to the plant essential oil than gram-positive bacteria, and *Salmonella typhi* was identified as the most resistant and *Bacillus cereus* as the most sensitive bacteria in the disk method. The results of this research indicated that EEO possesses suitable antimicrobial activity against pathogenic strains. Therefore, by employing this essential oil in the production of doogh, in addition to increasing the product's shelf life, it is possible to produce doogh with appropriate medicinal properties. In any case, to ensure the safe application of EEO in the formulation of food products such as doogh, further studies are essential.

1. Introduction

Despite modern advancements in food production hygiene principles, food safety is an issue that has increasingly gained significant importance. Today, new methods are needed to reduce foodborne pathogens and prevent various diseases. Essential oils possess antimicrobial properties, and this characteristic has been documented for many years. To date, approximately 3000 types of essential oils have been identified, among which 300 types are commercially important, and these essential oils are primarily used for flavoring and aroma purposes [1]. More than 2000 years ago, essential oils were first produced in the East (Egypt, India, and Iran) by distillation and used for medical purposes [2]. Plants such as wild pistachio, rosemary, mountain cypress, clove, nutmeg, fennel, cinnamon, and lavender were utilized. In addition to medical applications, in the 19th and 20th centuries, essential oils were used as flavor and fragrance agents [2]. Essential oils, due to their hydrophobic properties, penetrate the lipid membranes of bacterial cells and lead to the leakage of ions and cellular contents, resulting in cellular dysfunction and ultimately cell death. Low pH, low temperature, and low oxygen levels are the most important physical conditions that enhance the antimicrobial properties of essential oils. In addition to antimicrobial properties, these compounds are used as antiviral, antifungal, insecticidal, antiparasitic, and antioxidant agents [3]. To date, the antimicrobial and antioxidant properties of many plant essential oils have been proven through various investigations [4-6].

Doogh is one of the traditional and popular beverages that has high consumption due to its fresh taste and nutritional properties. Doogh is one of the fermented dairy products produced by mixing yogurt with water and a certain amount of salt. This fermented beverage is widely consumed as a popular drink in Iran and other Middle Eastern countries, especially during warm seasons [7]. Doogh has high digestibility and more vitamins compared to milk. Additionally, consuming doogh leads to increased calcium absorption [8]. However, if hygienic operations during doogh preparation are not properly observed, spoilage-causing microorganisms such as mold and yeast remain viable and proliferate, leading to changes in taste, aroma, and also bloating of the product during storage. This spoilage is considered one of the major drawbacks of doogh, which also reduces its market appeal [8-9]. Flavored doogh has special acceptability among consumers. In Iran, to improve the taste of doogh, essential oils from vegetables such as oregano, mint, and thyme are used [10].

Eucalyptus globulus, with the scientific name *Eucalyptus globulus*, belongs to the Myrtaceae family. Its primary origin is Australia, and it has rapid growth. The antimicrobial effects and other properties of this plant have been noted for a long time. This plant is a rich source of polyphenols and terpenoids, and the main compound in its leaves is eucalyptol or cineole (70 to 80 percent) [11]. Studies conducted on various extracts of eucalyptus have shown that eucalyptus extract inhibits the growth of gram-positive and negative bacteria such as *Salmonella typhimurium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and also *Candida albicans* [12]. The microorganisms *Staphylococcus aureus*, *Shigella dysenteriae*, *Escherichia coli*, *Salmonella paratyphi*, *Candida albicans*, and *Bacillus subtilis* are, in order, from the most sensitive to the most resistant microbes in this regard [13].

The antimicrobial effect of natural preservatives, especially plant essential oils, on pathogenic bacteria in doogh is one of the important aspects of maintaining the quality and safety of this beverage.

Essential oils often, due to their strong antimicrobial compounds, can destroy the cellular structure of pathogenic bacteria walls, inhibit vital enzyme activities, and limit their growth and proliferation [14-17]. Therefore, investigating and applying natural essential oils with antimicrobial properties can be an effective strategy to increase the shelf life of doogh and ensure consumer health. Additionally, this method is a suitable alternative to chemical preservatives that may have side effects. The present research aimed to identify the chemical compounds of eucalyptus essential oil (EEO) and investigate its antimicrobial effect using agar diffusion methods with disk agar, minimum inhibitory concentration, and minimum bactericidal concentration. In this study, the antimicrobial effect of the essential oil on two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two gram-negative bacteria (*Salmonella typhi* and *Escherichia coli*) was examined under laboratory conditions during a 45-day storage period. Additionally, the effect of the essential oil on the microbiological quality of doogh samples (coliform, mold and yeast, and total microbial count) was investigated.

2-Materials and Methods

2-1-Preparation of Raw Materials

Eucalyptus essential oil (EEO) from Zarbond Company, bacterial strains *Bacillus cereus* (PTCC1154), *Staphylococcus aureus* (PTCC1112), and two gram-negative bacterial species *Escherichia coli* (PTCC1329) and *Salmonella typhi* (PTCC1609) in lyophilized form (Iranian Scientific and Industrial Research Organization), culture media Nutrient Agar, PCA, YGC, and VRB (Quelab, USA), Mueller-Hinton Broth (Merck, Germany), dimethyl sulfoxide with 99.9% purity (Sigma-Aldrich, Germany), 0.5 McFarland standard solution, chloramphenicol antibiogram disks and sterile disks (Padtan, Iran), sodium hydroxide and cyclohexane (Merck, Germany) were used in this research.

2-2-Identification of EEO Compounds

Identification of the essential oil constituents was performed using a gas chromatography device model Agilent 6890A equipped with an HP-5 column (30 m length, 250 µm internal diameter, and 0.25 µm stationary phase thickness) and a gas chromatography model Agilent 5975C connected to a mass spectrometer manufactured in the USA (Agilent Technologies). The temperature program for the injection port and detector was 250°C and 300°C, respectively, and helium gas with a flow rate of 1 mL/min was used as the carrier gas. Identification of the essential oil compound types was done using the spectrum of normal alkanes (C8-C24) with an ionization voltage of 70 electron volts, and the percentages of the compounds present in the examined essential oil were calculated using a gas chromatography device model Agilent 6890A equipped with an FID detector and by using the area under the peak curves [18 and 19].

2-3-Preparation of Doogh Containing Essential Oil

The milk used (containing 3.2% fat and acidity of 14.5 Dornic degrees) was obtained from the livestock station of Khuzestan University of Agricultural Sciences and Natural Resources and transferred to the laboratory of the Food Science and Industries Department of Ramin Khuzestan University of Agricultural Sciences and Natural Resources for yogurt production. For yogurt production, it was pasteurized for ten minutes at a temperature of 90 ± 2°C and after cooling to 45°C, 3% yogurt starter containing an equal mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria was

added for inoculation. Then, fermentation was carried out at 43°C for approximately 4 hours until the pH reached 4.5 [20]. To prepare doogh samples, 50% yogurt along with 50% pasteurized water and 0.85% salt was used. EEO was used at 3 different levels (2, 4, and 6 microliters) in the doogh formulation, and a sample without plant essential oil was used as the control sample. Doogh samples were bottled in polyethylene terephthalate (PET) containers and stored for 45 days at 5°C.

2-4-Microbial Evaluation

For microbial evaluation, an initial suspension (1 mL of each doogh sample added to 9 mL of sterile physiological serum) was prepared. From the initial suspension, appropriate dilutions were used for total microbial count on PCA culture medium (Quelab, Canada), for coliform count on specific VRB culture medium (deep culture) at 37°C incubator, and for mold and yeast on YGC culture medium with dilutions of 10^-2 and 10^-3 (surface culture) at 25°C incubator in three replicates. Plates were incubated for 3 days. Then, the number of colonies was determined using a colony counter and reported as Log cfu per cc of sample [21 and 22].

2-5-Antimicrobial Property Evaluation

In this study, three diverse antimicrobial methods were used, including 1- disk diffusion method, 2- determination of minimum inhibitory concentration (MIC), and 3- determination of minimum bactericidal concentration (MBC) to evaluate the antimicrobial activity of *Eucalyptus globulus* essential oil [23].

2-5-1-Activation of Microbial Species

The studied bacteria included gram-positive bacteria *Bacillus cereus*, *Staphylococcus aureus*, and two gram-negative bacterial species *Escherichia coli* and *Salmonella typhi*. Bacterial species were cultured on Nutrient Agar and incubated for 24 hours at 37°C. Then, to prepare the microbial standard, according to the 0.5 McFarland standard at 620 nm wavelength (with absorbance between 0.13-0.18), it was proceeded. In this case, the 0.5 McFarland standard creates turbidity equivalent to a bacterial suspension of 1.5×10^8 cells per milliliter [24].

2-5-2-Disk Diffusion

The disk diffusion method was performed according to the method of Alizadeh et al. [23]. First, EEO was passed through a syringe filter with a 0.22 µm pore diameter and poured into a sterile container, then 20 microliters of EEO was added to sterile blank disks (6 mm diameter). Subsequently, the disks impregnated with EEO were carefully placed on Petri dishes containing Mueller-Hinton Agar culture medium (Merck, Germany) and pathogenic bacteria (according to the 0.5 McFarland standard). The distance of the disks from the Petri dish wall was at least 20 mm and from each other at least 25 mm. Finally, each Petri dish was incubated at the optimal bacterial growth temperature (37°C) for 24 hours, then the diameter of the no-growth halo was precisely measured and recorded using a ruler. Additionally, 30 µg chloramphenicol antibiotic disks were used on separate plates on microbial cultures as positive control.

2-5-3-Determination of Minimum Inhibitory Concentration (MIC)

To determine the inhibitory concentration (MIC) of the essential oil, sterile 96-well microplates were used. The main solution of EEO was prepared at a dilution of 150 mg in 1 ml dimethyl sulfoxide, and different concentrations of essential oils were prepared by diluting the main solutions with Mueller-Hinton Broth culture medium (Merck, Germany).

Bacteria were cultured 24 hours before the experiment at 37°C on Nutrient Agar culture medium. To prepare a microbial suspension of 10^6 cfu/ml, the 0.5 McFarland dilution was used. After filling the wells, microplates were placed in a 37°C incubator for one day and night, and then the wells without turbidity were reported as MIC [23 and 25].

2-5-4-Determination of Minimum Bactericidal Concentration (MBC)

To determine the minimum bactericidal concentration (MBC) of eucalyptus essential oil, 5 microliters from wells where no turbidity was observed were transferred to solid culture medium (Mueller-Hinton Agar) and stored for one night at 37°C. The first concentration where no growth was observed was considered as the minimum bactericidal concentration (MBC) [23].

2-6-Statistical Analysis

The tests were conducted in a completely randomized design with three replications. The obtained results were analyzed using analysis of variance (ANOVA) with SPSS software version 20, mean comparisons using Duncan's multiple range test at probability level ($p>0.05$), and graphs were drawn with EXCEL software (2003).

3-Results and Discussion

3-1- Investigation of EEO Compounds

Based on the results obtained from the analysis of eucalyptus essential oil, 11 compounds were extracted from the eucalyptus essential oil, comprising 99.87% of the essential oil. As seen in Table 1, the two compounds 1,8-cineole at 48.6% and alpha-pinene at 23.86% were identified as the main compounds, followed by alpha-terpineol at 6.55%, trans-pinocarveol at 6.34%, and pinocarvone at 4.53% as the major groups in eucalyptus essential oil, and the lowest amount of compound in the essential oil is spathulenol at 1.02% (Table 1). Therefore, based on the results of this study, cineole is the main substance giving the eucalyptus essential oil, which matched the results of Samate et al. [26]. Ansari et al. [27] also reported that 1,8-cineole at 58.1% and alpha-pinene at 3.3% as the main compounds of eucalyptus essential oil. Additionally, Mohkami et al. [28] stated that the most important compounds of the essential oil are alpha-pinene (20.3%) and 1,8-cineole (17.21%), which were approximately similar to the results of the present study. In all these studies, 1,8-cineole was the main constituent of eucalyptus essential oil. In eucalyptus essential oil, like other plant essential oils, the existing compounds result from ecological differences such as latitude and longitude, temperature, humidity, altitude, climate, and soil, metabolic pathways, and biosynthesis of active substances in these plants, resulting in diverse secondary metabolites biosynthesized under different environmental conditions; hence, differences in percentage and type of constituent compounds in different parts of the world are justifiable [29].

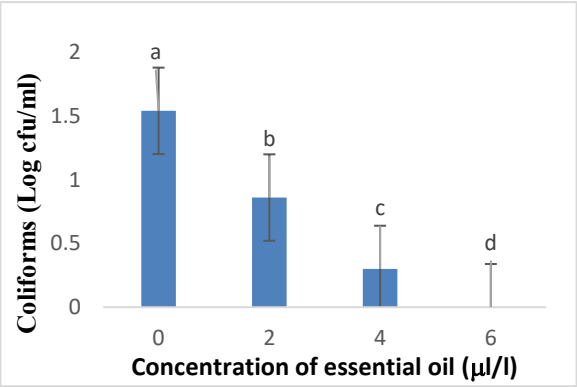
Table 1. Chemical composition of *Eucalyptus globulus* essential oil.

Compound	Percentage
1,8- cineol	48.60
alpha-pinene	23.86
Terpineol alpha	6.55
Trans- pinocarveol	6.34
pinacarvone	4.53
4-terpineol	2.66

pinocarveol	2.42
alpha-terpinyl acetate	1.50
limonene	1.28
guaiene	1.11
spathulenol	1.02

3-2-Coliform

The results showed that in the doogh sample containing 6 microliter per liter of essential oil storage, no coliform was observed, and the highest amount was observed in the control sample with an average of 1.54 Log cfu (Figure 1-a). Additionally, on the first days of storage, no coliform was observed in doogh samples, but on days 15, 30, and 45 of storage, an increase in coliform was observed, such that the highest coliform (2.14 Log cfu/ml) was observed in the 45-day storage sample (Figure 1-b). The results of Mehraban Sang Atash et al. [30] showed that mountain thyme essential oil at the tested concentrations had no significant effect on yogurt starter bacteria, and the effect of mountain thyme extract was only significant at the highest concentration from the seventeenth day onward. Razzaghi et al. [31] examined the antimicrobial properties after enriching doogh with melissa extract and powder. The results of the study showed that adding melissa to doogh prevented coliform growth until the twentieth day of storage. According to Figure 1-c, the highest number of coliform (3.81 Log cfu/ml) was determined in the control sample on the last day of storage. This was while no growth of coliforms was observed in the sample containing 6 microliter of essential oil in all storage periods.



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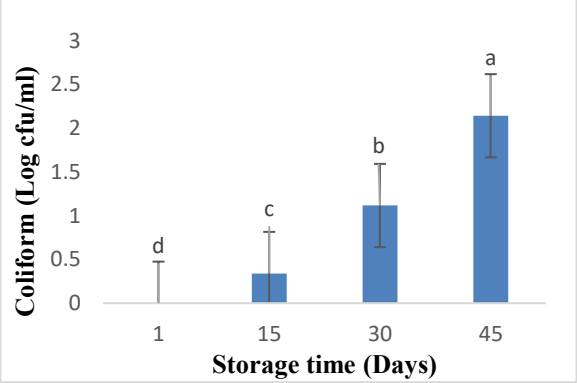

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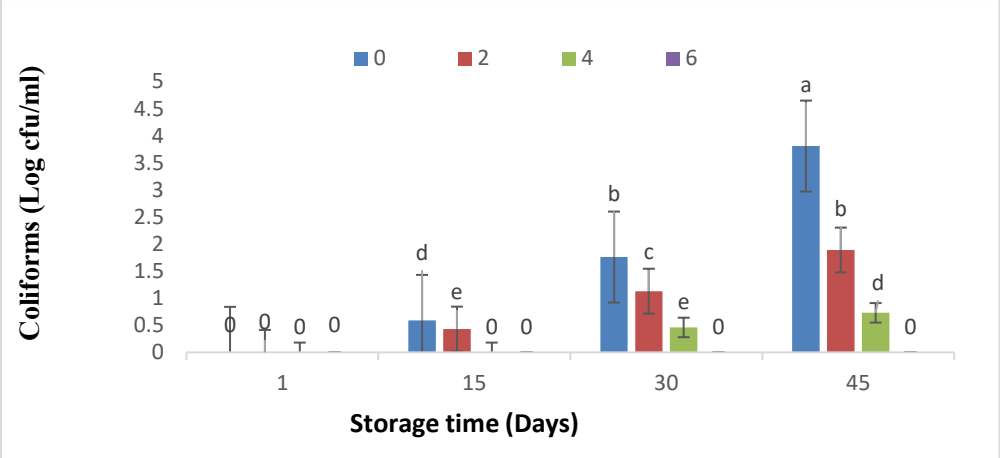

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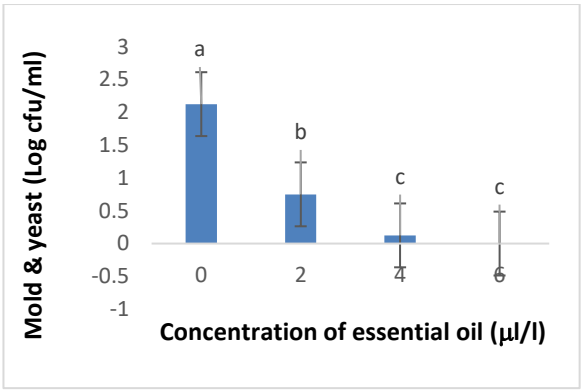
Figure 1. The count of coliforms in Doogh samples containing different concentrations of essential oils (a) during storage period (b) and their intraction effect (c).

3-3-Mold and Yeast

Fungi grow well in relatively dry food materials with low water activity, acidic foods or those containing salt, as well as foods stored in cold conditions. The data obtained from counting mold and yeast in the control sample and samples treated with EEO indicated the power of essential oils in preventing mold and yeast growth compared to control doogh (Figure 2). In such a way that the control doogh sample had more mold and yeast growth compared to other doogh samples containing essential oil ($p>0.05$). Additionally, with increasing storage time, the number of mold and yeast increased, and the highest mold and yeast (2.63 Log cfu/ml) was observed in the 45-day storage treatment, and no significant difference was observed in the other treatments (Figure 2-b). Also, the results of comparing the average interaction effects of essential oil × storage period length showed that doogh treated with 4 and 6 microliters of essential oil remained consumable until day 45 of storage and were free of mold and yeast (Figure 2-c). Among the samples containing essential oil during 45 days of storage, only the sample containing 2 micrograms per liter of essential oil on day 45 of storage contained a notable number (3.00 Log cfu/ml) of mold and yeast. Also, the control doogh on day 30 (1.50 Log cfu/ml) and especially at the end of the storage period (7.00 Log cfu/ml) contained a high number of mold and yeast (Figure 2-c). Numerous factors influence the inhibitory capability of essential oils in food systems, including protein, fat, salt, oxygen amount, food matrix, pH value, and aw. The increase in mold and yeast numbers in doogh samples during storage can be attributed to the favorable growth conditions for mold and yeast due to acid production by *Lactobacillus bulgaricus* bacteria [32]. Ndagijimana et al. [33] reported that the number of *Saccharomyces cerevisiae* colonies in orange juice containing orange essential oil was less than the control sample. Also, the results of Zarali et al. [34] showed that the control power of mold and yeast growth by extracts of Echinophora and mountain tea decreased over time.



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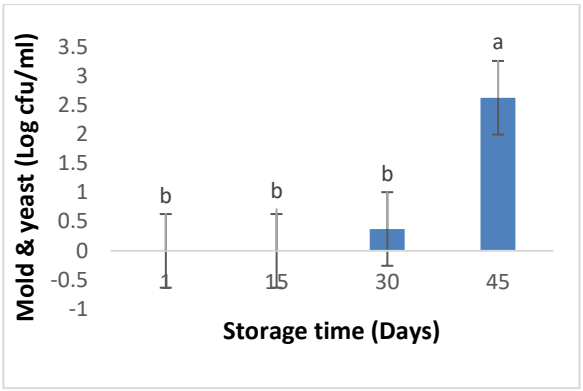

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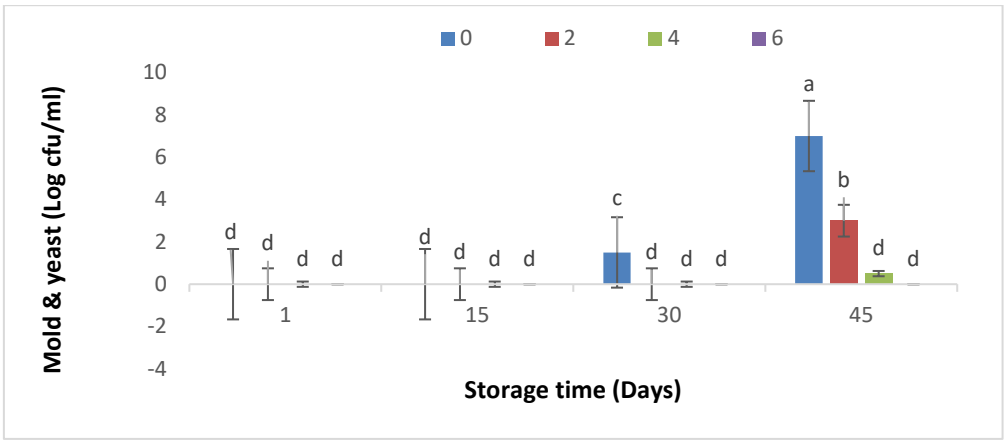


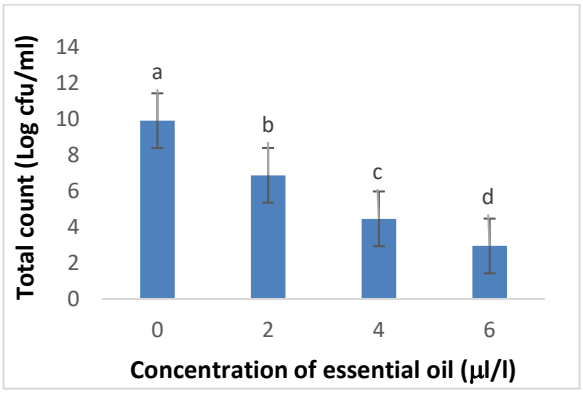
(c)

Figure 2. The count of of mold and yeast in Doogh samples containing different concentrations of essential oils (a) during storage period (b) and their intraction effect (c).

3-4-Total Microbial Count

Based on Figure 3, it was determined that with increasing essential oil concentration, the bacterial population significantly decreased, and the highest microbial population with an average (9.92 log per milliliter) was in doogh without essential oil, and the lowest microbial population with an average (2.95 Log cfu/ml) was observed in the doogh sample containing 6 microliters per liter of essential oil (Figure 3-a). Also, doogh samples during the storage period significantly increased in terms of total microbial count (Figure 3-b). In such a way that the highest microbial population belonged to the sample on day 45 of storage with an average (7.5 Log cfu/ml), and the lowest microbial population belonged to the sample on the first day of storage with an average (3.87 Log cfu/ml), which almost showed that with increasing storage time, the microbial population doubled (Figure 3-b). The results of interaction effects of essential oil concentration during the storage period showed that the highest microbial population (12.47 Log cfu/ml) was in doogh without essential oil at 45 days of storage (Figure 3-c). Therefore, it can be

concluded that plant essential oils caused a reduction in the growth of microorganisms present in doogh samples, and with increasing storage time, the number of bacteria increased exponentially. Zarali et al. (2015) in confirmation of the results of this study reported that doogh samples containing Echinophora mountain tea extract had a lower total count compared to the control sample, and at the end of the storage period, the number of microorganisms increased significantly [34].



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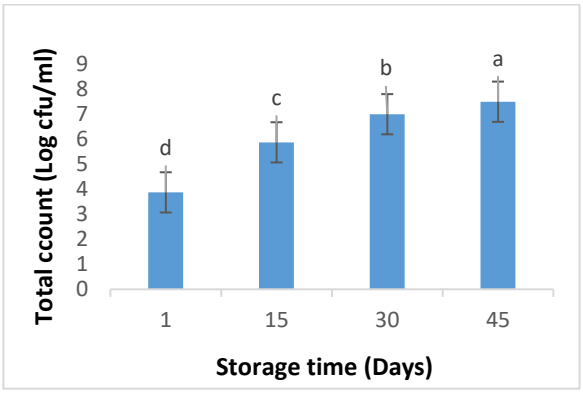

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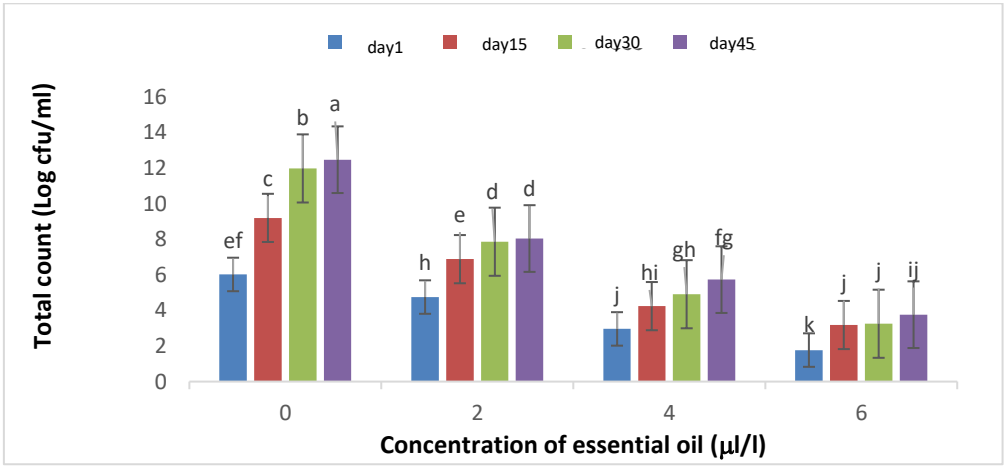

(c)

Figure 3. The count of of total count in Doogh samples containing different concentrations of essential oils (a) during storage period (b) and their intraction effect (c).

3-5-Antimicrobial Effect of Essential Oil

The results of analysis by agar disk diffusion method are shown in Table 2, and the results of minimum inhibitory

concentration and minimum bactericidal concentration are shown in Table 3. According to the results of this study, it was determined that the diameter of the no-growth halo in bacteria increased with increasing essential oil concentration; such that in gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus*, with increasing essential oil concentration, the no-growth halo diameter increased significantly (P>0.05). As can be seen in Table 2, the halo diameter for *Bacillus cereus* and *Staphylococcus aureus* bacteria at 512 mg/ml essential oil concentration was 25.50 and 26.50 mm, respectively, and at 32 mg/ml concentration was 15.50 and 12.50 mm. This result indicates that *Staphylococcus aureus* bacteria have somewhat more resistance. In examining the no-growth halo of gram-negative bacteria *Salmonella typhi* and *Escherichia coli*, it was observed that with increasing essential oil concentration, the no-growth halo diameter increased (P>0.05). The highest halo diameter or inhibitory effect in *Salmonella typhi* and *E. coli* bacteria was observed with 25 and 25 mm at 512 mg/ml essential oil concentration, respectively. The lowest halo diameter was also observed with 11.50 and 14.50 mm at 32 mg/ml concentration. This result indicates that in gram-negative bacteria, *E. coli* bacteria have somewhat more resistance. Many researchers attribute this to differences in the cell wall structure of gram-negative bacteria compared to gram-positive bacteria. Perhaps the greater resistance of gram-negative bacteria can be attributed to the presence of an outer phospholipid membrane that is almost impermeable to lipophilic compounds. In other words, the reason for the greater resistance of gram-negative bacteria to plant extracts can be attributed to the structural differences and greater complexity of the cell membrane of gram-negative bacteria compared to the single-layered membrane of gram-positive bacteria. The findings obtained from the present research are consistent with the results of studies [24, 35, and 36]. Overall, the results showed that at low concentrations (32 mg/ml), in terms of resistance, the bacteria *Salmonella typhi* > *Staphylococcus aureus* > *E. coli* > *Bacillus cereus* showed the highest resistance, respectively. Based on the results of Alizadeh Behbahani et al. [24], the sensitivity of pathogenic microbes to *Eucalyptus globulus* essential oil (disk diffusion) from the most resistant to the most sensitive strain was determined as *Salmonella typhi* > *Escherichia coli* > *Pseudomonas aeruginosa* > *Bacillus subtilis* > *Streptococcus pyogenes* > *Staphylococcus aureus*, respectively.

Table 2. The results of inhibition zone diameters (mm) by agar disk diffusion (ADD) method.

Concentration (mg/ml)	*Bacillus cereus*	*Staphylococcus aureus*	*Salmonella typhi*	*Escherichia coli*
32	15.50 ±0.37^{d}	12.50 ±0.28^{e}	11.50 ±0.33^{e}	14.50 ±0.21^{d}
64	16.5 ±0.29^{c}	13.50 ±0.34^{d}	13.50 ±0.41^{d}	18.50 ±0.53^{c}
128	22.5± 0.61^{b}	18 ±0.48^{c}	15 ±0.29^{c}	20 ±0.76^{b}
256	25± 0.43^{a}	21 ±0.35^{b}	17.5 ±0.38^{b}	20.50 ±0.50^{b}
512	25.5 ±0.58^{a}	26.50 ±0.29^{a}	25 ±0.52^{a}	25 ±0.61^{a}

Values are expressed as mean, n = 3; different letters (a, b, c and d) in each column show significant differences

Table 3. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *Eucalyptus globulus* essential oil on some pathogenic bacteria

Microorganism	MIC (mg/ml)	MBC (mg/ml)
Bacillus cereus	32	128
Staphylococcus aureus	16	128
Escherichia coli	32	256
Salmonella typhi	32	512

The results showed that at the highest inhibitory concentration (512 mg/ml), in terms of resistance, the bacteria *Salmonella typhi* > *Bacillus cereus* > *E. coli* > *Staphylococcus aureus* showed the highest resistance, respectively. According to the results obtained in the disk method, *Salmonella typhi* bacteria was the most resistant bacteria, and *Bacillus cereus* bacteria was the most sensitive bacteria. Overall, gram-negative bacteria are more resistant to plant essential oil than gram-positive bacteria. Numerous researchers have attributed this to differences in the cell wall structure of gram-negative bacteria compared to gram-positive bacteria. Perhaps the greater resistance of gram-negative bacteria can be attributed to the presence of an outer phospholipid membrane that is almost impermeable to lipophilic compounds. The findings obtained from the present research are consistent with the results of studies [20, 31, and 32].

4-Conclusion

The antimicrobial properties of essential oils have been proven for many consecutive years. Various researches have shown that essential oils contain various antimicrobial compounds such as carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde, cineole, and cinnamic acid. The results of the present research also showed that the two compounds 1,8-cineole (48.6%) and alpha-pinene (23.86%) are the main compounds of eucalyptus essential oil. The results of the antimicrobial effect of EEO according to the disk method showed that *Salmonella typhi* bacteria is the most resistant bacteria. Overall, gram-negative bacteria are more resistant to plant essential oil than gram-positive bacteria. Based on the results of this study, by adding 6 microliters per liter of EEO to doogh, no coliform growth was observed in all sample storage periods. Additionally, adding EEO caused a reduction in total microbial growth. This means that the rate of yogurt souring during storage can be delayed by eucalyptus essential oil. In a general conclusion, it is suggested that given the medicinal properties of this plant essential oil, its use as a natural preservative and flavoring in food materials should be considered. In any case, for the commercialization of this essential oil's application in doogh and other food cases, a comprehensive examination of the potential negative effects of the essential oil on human health needs to be studied.

5-Acknowledgments

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مقاله علمی_پژوهشی

بررسی ترکیبات شیمیایی و ویژگی‌های ضدمیکروبی اسانس برگ اکالیپتوس و کاربرد آن در تولید دوغ

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چکیده

استفاده از مواد نگهدارنده طبیعی مانند اسانس‌های گیاهی، رویکردی سالم و سازگار با محیط زیست است که جایگزین مناسبی برای افزودنی‌های شیمیایی محسوب می‌شود و به حفظ سلامت مصرف‌کنندگان کمک می‌کند. اکالیپتوس یا گیاه صمغ آبی با نام علمی *Eucalyptus globulus* متعلق به خانواده میرتاسه است. این مطالعه با هدف شناسایی ترکیبات شیمیایی و ارزیابی فعالیت ضد باکتریایی اسانس اکالیپتوس بر تعدادی از میکروارگانیسم‌های بیماری‌زا در شرایط آزمایشگاهی انجام شد. در این پژوهش ترکیبات شیمیایی اسانس اکالیپتوس با دستگاه GC-MS شناسایی شد. سپس تأثیر غلظت‌های مختلف اسانس (صفر، ۲، ٤ و ٦ میکرولیتر در لیتر) بر برخی باکتری‌های بیماری‌زا و خصوصیات میکروبی نمونه‌های دوغ طی مدت ٤٥ روز نگهداری مورد بررسی قرار گرفت. نتایج آنالیز GC-MS نشان داد که ۱،۸- سینئول (٤٨/٦٪) و آلفا - پینن (٢٣/٨٦٪) بیشترین درصد ترکیبات اسانس را تشکیل می‌دهند. با افزودن ٦ میکرولیتر در لیتر اسانس اکالیپتوس در دوغ، هیچ گونه رشد کپک و مخمر در تمام مدت نگهداری مشاهده نشد و نمونهٔ حاوی ٤ میکرولیتر در لیتر اسانس نیز در پایان مدن نگهداری حاوی تعداد قابل قبول کپک و مخمر بود. همچنین اسانس اکالیپتوس باعث کاهش شمارش کلی میکروارگانیسم‌ها گردید. براساس نتایج این تحقیق، باکتری های گرم منفی نسبت به اسانس گیاهی مقاوم‌تر از باکتری‌های گرم مثبت بودند و *سالمونلا تیفی* در روش دیسک به عنوان مقاوم‌ترین و *باسیلوس سرئوس* به عنوان حساس‌ترین باکتری شناخته شد. نتایج این پژوهش نشان داد اسانس اکالیپتوس دارای فعالیت ضدمیکروبی مناسبی در برابر سویه‌های بیماری‌زا می‌باشد. بنابراین، با به‌کارگیری این اسانس در تولید دوغ، علاوه بر افزایش قابلیت نگهداری محصول، می‌توان دوغی با خواص دارویی مناسب تولید نمود. درهرحال به‌منظور کاربرد مطمئن اسانس اکالیپتوس در فرمولاسیون مواد غذایی نظیر دوغ، انجام مطالعات بیشتر ضروری است.