INTRODUCTION

Food preservation is an essential method to prevent the growth of spoilage or pathogen microorganisms such as bacterial and molds on the food. Nowadays and due to the health issues attributed to the chemical preservatives, there is a high tendency in food industry to replace the chemical preservatives with their natural alternatives (Hossain et al., 2016). Essential oils (EOs) are plants...
based and naturally occurring antimicrobials with a high potential biological activity. The antimicrobial effects of EOs in order to control the food spoilage have been reported in many researches (Hashemi, Niakousari, Saharkhiz, & Eskandari, 2011; Mohammadi & Aminifard, 2013; Olmedo, Nepote, & Grosso, 2013; Sumalan, Alexa, & Poiana, 2013; Tian et al., 2012). Recently, the demand for potential natural food preservatives with a broad spectrum of anti-oxidant and antimicrobial activities to enhance the shelf life of perishable foods is increasing (Fratianni et al., 2010). Marzeh Khuzestani (Satureja Khuzestanica Jamzad) is an aromatic endemic plant growing wildly in the southern-west side of Iran with a wide application as a traditional medicine due to its anti-septic and sedative properties (Hashemi et al., 2012; Siavash Saei-Dehkordi, Fallah, Heidari-Nasirabadi, & Moradi, 2012). Marzeh Khuzestani EO is rich in natural monoterpenoid and carvacrol with diverse biological activities such as antimicrobial, antitumor, analgesic, anti-inflammatory, anti-parasitic, anti-hepatotoxic, and hepatoprotective activities which makes it an excellent antimicrobial agent in food industry (Can Baser, 2008).

Marzeh Bakhtiari (Satureja bachtiriaca Bunge) is another traditional medicine that is widely distributed in the central Zagros mountains, Iran. Antibacterial, antifungal, anti-viral, anti-oxidant, anti-spasmodic, anti-diarrheal, and anti-inflammatory are some of the biological properties attributed to Marzeh Bakhtiari EOs. P-cymene, carvacol, and thymol are the major components of the S. bachtiriaca (Babadi, Ghasemi Pirbalouti, Nourafcan, & Hamedi, 2012).

In the term of food preservation, natural preservatives such as EOs are more suitable than chemical preservatives but the dosage and the costs for natural preservatives limit their application in food industry (Ju et al., 2019). Hence, evaluation of synergistic effect of combined EOs may be an effective way to solve this issue (Ju et al., 2020). Kong et al. (2016) reported a synergistic effect against Fusarium solani from combination thymol and salicylic acid which it was more than two-fold higher than when it used alone. The synergistic effect of thymol and carvacol against Pseudomonas aeruginosa and Staphylococcus aureus has been reported by Lambert, Skandamis, Coote, and Nychas (2001). Ju et al. (2020) observed that the combination of eugenol and citral significantly improved the antifungal effects (3.4-fold) against the main bread spoilage fungi (Penicillium roqueforti and Aspergillus niger) compared to each agent used separately.

Lactic acid bacteria (LABs) are one of the major groups of probiotics and they are widely used in the production of dairy products (Hashemi & Gholamhosseinpoor, 2019). Some species of LABs, such as Lactobacillus plantarum, are commonly isolated from dairy products as well as fruits and vegetables and their role as probiotics in human health has been confirmed (Amin, Jorfi, Khosravi, Sambarafzadeh, & Farajzadeh Sheikh, 2009). In the case of the addition EOs into probiotic food, it is necessary that EOs exhibit a low inhibitory effect on the probiotic culture with an acceptable inhibitory activity on the pathogens. Table or coffee cream is an extremely viscous product contains 18% milk fat and can be fermented by the defined strains of LAB. This cream can be used as condiment along with the snacks or vegetables or also can be used as an ingredient in sauces or dressings (Tamime, 2009).

Therefore, the aim of the current research was to assess in vitro inhibitory potential of Marzeh khuzestani and Marzeh baktiertai EOs alone or in combination against the growth of Lactobacillus plantarum LU5 as a probiotic culture or Shigella flexneri, and Escherichia coli as the pathogenic bacteria and the effectiveness of these EOs as natural preservatives for table cream were evaluated.

## 2 | MATERIALS AND METHODS

### 2.1 | Bacterial strains

L. plantarum LU5 was obtained from Department of Food Science and Technology, Fasa University, Fasa, Iran. The probiotic potential of this strain was proved by Hashemi, Shahidi, Mortazavi, Milani, and Eshaghi (2014b). L. plantarum LU5 was reactivated in the MRS broth (Oxoid) at 37°C for 48 hr. E. coli PTCC 1399 and S. flexneri PTCC 1865 were purchased from Iranian Research Organization for Science and Technology, Tehran, Iran. The pathogenic bacteria were reactivated in nutrient broth (HiMedia) at 37°C for 24 hr.

### 2.2 | Plant materials and essential oil extraction

Marzeh khuzestani (Satureja khuzestanica) and Marzeh bakhtiari (Satureja bachtiriaca Bunge) with the moisture content of 13% (dry basis) were purchased from a local market in Kohgiluyeh and Boyer-Ahmad province, Iran and kept at 4°C prior extraction. For essential oil extraction, hydrodistillation of 35 g of each dried plant in 500 ml distilled water was applied for about 3.5 hr by an all-glass Clevenger-type apparatus (British Pharmacopoeia, 1980).

### 2.3 | Essential oil analysis

Gas Chromatography-Mass equipment (GC-MS; 6890N, Agilent Technologies) was used for determination of essential oils components. HP-5MS capillary column (30-m length; 0.25-mm internal diameter; 0.25-μm film thickness) was applied at a split ratio of 1:30, and the speed of helium was controlled at 1.3 ml/min. The oven temperature was adjusted at 60°C for 6 min, consequently enhanced to 280°C at 3°C/min (Hashemi, Niakousari, Saharkhiz, & Eskandari, 2014a; Hashemi et al., 2017).

### 2.4 | Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC)

Briefly, innoculums were prepared at 10⁶ CFU/ml and concentration range of each essential oil was adjusted at 0.8-100 μg/ml (dimethyl sulfoxide as solvent). Consequently, 95 μl of nutrient broth
or MRS broth, 5 µl of each microbial inoculum, and 100 µl of each essential oil concentration were added to each well of the 96-well plates. Afterward, incubation was performed at 37°C for 24 hr. For measurement of MBC, 100 µl of the microbial suspensions from MIC was cultured in nutrient agar and the lowest concentration with no growth was determined as MBC (Clemente, Aznar, & Nerín, 2019).

2.5 | Fractional inhibitory concentration (FIC) and fractional bactericidal concentration (FBC)

Briefly, the different microorganism’s suspensions in PBS, 20 µl of each EO concentration based on the MIC values and 1,760 µl of yeast extract were prepared. Afterward, the incubation was done at 37°C for 24 hr. The combination effect of both essential oils based on FIC value was reported as: antagonism, indifferent, additive, and synergy according to the method of Hylgaard, Mygind, and Meyer (2012). For determination of the minimal bactericidal combination of essential oils, a method suggested by Mosquera, Sharp, Moore, Warn, and Denning (2002) was carried out to measure the FBC indicator. The following formula used for calculation of FIC and FBC:

\[
\text{FIC or FBC} = \frac{\text{MIC or MBC of Marzeh khuzestani EO in combination}}{\text{MIC or MBC of Marzeh bakhtiari EO alone}} + \frac{\text{MIC or MBC of Marzeh bakhtiari EO in combination}}{\text{MIC or MBC of Marzeh khuzestani EO alone}}
\]

The antimicrobial interaction was calculated as FIC or FBC ≤ 0.5: synergic effect (where the combined antibacterial activity is greater than the sum of activity of both EOs when used separately); 0.5 < FIC or FBC ≤ 1: additive effect (where the combined antimicrobial activity is equal to sum of the activity of EOs acting jointly); 1 < FIC or FBC ≤ 4: no interactive effect; FIC or FBC > 4: antagonistic effect (where the combined antibacterial activity is lower than the sum of activity of both EOs used separately).

2.6 | Combinatorial agar diffusion assays

Both essential oils were mixed at 25, 50, and 75% (v/v) concentration, and agar diffusion test was carried out for 100 µl of each bacterial suspension (10^6 CFU/ml). Subsequently, 10 µl of each essential oil mixture was added to sterile filter disk (10 mm diameter) and placed on the nutrient agar or MRS + nutrient agar medium. After that, plates were incubated at 37°C for 24 hr (Hashemi et al., 2018).

2.7 | Antibacterial activity of essential oils in table cream

The antibacterial activity of mixture of essential oils was measured on table cream. Table cream was purchased from Pegah Dairy Company and autoclaved for 15 min. After cooling, table cream was inoculated with 9.1–9.3 log CFU/g of each target strain separately and the mixture of essential oils (1 µl) was added to the samples. Samples were placed in polystyrene plastic tray covered by a lid and sealed with parafilm. Then, samples were kept at 4°C for 10 days and the count of each microorganism was detected during storage. For microbial determination, each sample was blended with peptone water in a stomacher (BagMixer 400 W, Interscience Co.) and following serial dilution; plating was carried out onto suitable medium. Afterward, plates were incubated at 37°C for 24–48 hr.

2.8 | Sensory evaluation

Sensory assessment of table cream supplemented with essential oils was done by using a twelve-member (six male and six female) semi-trained panel. The panelists scored the overall acceptability aspects by using a 9-point hedonic scale, where 1 = unacceptable and 9 = very acceptable, whereas the limit of acceptability was 6 (Hashemi, Amininezhad, Shirzadinezhad, Farahani, & Yousefabad, 2016).

2.9 | Statistical analysis

Statistical analyses were performed with ANOVA and significant differences at p < .05 were carried out by Duncan’s multiple range tests using SPSS package program (Version 22, SPSS Inc.).

3 | RESULTS AND DISCUSSION

3.1 | EOs composition

The composition of Marzeh khuzestani and Marzeh bakhtiari EOs is listed in Table 1. Carvacrol was the principle component of Marzeh khuzestani (86.5%), and the main components of Marzeh bakhtiari were thymol (33.5%), carvacrol (14.2%), borneol (13.4%), and linalool (11.5%). Thymol and carvacol are the most active oxygenated monoterpenes (Asensio, Grosso, & Juliani, 2015). Babadi et al. (2012) reported that the main constituent groups of S. bachtiarica EOs were monoterpenes and sesquiterpenes with the carvacrol (44.8%), gamma terpinene (18.7%), and thymol (14.95%) as the major components. Sefidkon and Jamzad (2000) also reported that thymol (44.5%), gamma terpinene (23.9%), p-cymene (7.3%), β-caryophyllene (5.3%), and borneol (4.2%) were the main components of Marzeh Bakhtiari EOs. Sefidkon, Jamzad, and Barazandeh (2005) reported that the EOs composition for S. bachtiarica was dependent on the region, so that for the Yazd population, the major components were carvacrol (66.5%), p-cymene (15.2%) and linalool (4.6%) while for Fars population was carvacrol (49.3%), p-cymene (12.7%), and trans-α-bergamotene (5.8%).

Hadian, Hossein Mirjalili, Reza Kanani, Salehnia, and Ganjipoor (2011) reported that carvacrol was the major component of the eight populations of S. khuzistanica. Farsam, Amanlou, Radpour, Salehnia, and Shafiee (2004) also reported that carvacrol was the
The antimicrobial activity of Marzeh khuzestani and Marzeh bakhtiari EOs was determined against L. plantarum as a common food probiotic strain and against S. flexneri and E. coli as food-borne bacteria. The MIC and MBC obtained are presented in Table 2. The results showed that both EOs were effective against tested bacteria. However, the difference in MIC and MBC of EOs observed. Marzeh Khuzestani EO with MIC ranging from 3.125 to 12.5 (µg/ml) exhibited a higher antibacterial property. However, the MIC for Marzeh bakhtiari EO was ranged from 6.25 to 25 (µg/ml). EOs containing aldehydes or phenols such as cinnamaldehyde, citral, carvacrol, eugenol, or thymol as the major components have been reported to have the highest antibacterial activity, followed by EOs containing terpene alcohols (Bassolé & Juliani, 2012). S. flexneri was the most sensitive pathogen strain to both EOs but L. plantarum and E. coli had a similar sensitivity to the EOs. These results are promising in term of production probiotic food and EOs have less inhibitory effect on the LABs that are the main probiotic culture in dairy products. Pirbalouti et al. (2010) observed a higher antimicrobial activity against gram positive bacteria and yeasts than gram negative bacteria for S. bachtiarica EOs and they reported that Bacillus cereus and Candida albicans were most sensitive strains against the EO. Babadi et al. (2012) reported that carvacrol and thymol are the major bactericidal components of S. bachtiarica EO and result in disturbance in bacteria membrane layer and leakage of intercellular ATP and potassium ions that cause cells death.

Although a higher concentration of EOs required to exhibit bactericidal activity (MBC) than MIC, a similar pattern observed for the MBC of the EOs and it ranged from 6.25–25 and 12.5–100 for the Marzeh khuzestani and Marzeh bakhtiari, respectively. A higher bactericidal activity observed for the Marzeh khuzestani EO and S. flexneri was the most sensitive strain.

### 3.3 Fractional inhibitory concentration (FIC) and fractional bactericidal concentration (FBC)

The combination effect of the Marzeh khuzestani and Marzeh bakhtiari EOs evaluated, and the fractional inhibitory effects (FIC) and fractional bactericidal concentration (FBC) against the selected bacteria are presented in Table 3. The values of FIC and FBC were uniformly coincident and the results showed synergic effect of the EOs combination against both S. flexneri and E. coli with the FIC and FBC values of 0.5 but additive effect against L. plantarum strain observed (FIC and FBC of 0.75). It can be concluded that the combination of both EOs had no synergistic effect on the probiotics while it significantly prohibited the pathogen bacteria and it is promising in term

### Table 1: Essential composition of Marzeh khuzestani and Marzeh bakhtiari

| Compound         | RI      | Marzeh khuzestani | Marzeh bakhtiari |
|------------------|---------|-------------------|------------------|
| α-Pinene         | 939     | 0.4 ± 0.1         | 0.5 ± 0          |
| Camphene         | 948     | —                 | 0.6 ± 0          |
| β-Pinene         | 978     | 0.4 ± 0.1         | 0.3 ± 0.1        |
| Myrcene          | 983     | 1.1 ± 0.1         | —                |
| p-Cymene         | 1,018   | 0.5 ± 0.0         | 3.1 ± 0.1        |
| Limonene         | 1,026   | 0.8 ± 0.2         | —                |
| γ-Terpine        | 1,063   | 1 ± 0.1           | —                |
| trans-Sabinene hydrate | 1,075 | 0.4 ± 0.0         | 0.8 ± 1          |
| Linalool         | 1,099   | 0.5 ± 0.1         | 11.5 ± 0.3       |
| Camphor          | 1,123   | —                 | 1.6 ± 0.2        |
| Borneol          | 1,163   | 0.6 ± 0.1         | 13.4 ± 0.3       |
| Terpine-4-ol     | 1,164   | 0.7 ± 0.2         | 2.1 ± 0.1        |
| Carvacrol methyl ether | 1,242 | 0.4 ± 0.0         | —                |
| Thymol           | 1,292   | 1.4 ± 0.2         | 33.5 ± 0.4       |
| Carvacrol        | 1,302   | 86.5 ± 0.5        | 14.1 ± 0.3       |
| Carvacryl acetate| 1,348   | —                 | 5.1 ± 0.2        |
| β-Caryophyllene  | 1,432   | 0.5 ± 0.0         | 0.9 ± 0.1        |
| β-Bisabolene     | 1,528   | 0.8 ± 0.1         | —                |
| Caryophyllene oxide | 1,586 | —                 | 12.3 ± 0.2       |

Note: Values are means ± standard error.

### Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Marzeh khuzestani and Marzeh bakhtiari against tested bacteria

| Bacterial strains | Marzeh khuzestani | Marzeh bakhtiari |
|-------------------|-------------------|------------------|
|                   | MIC (µg/ml) | MBC (µg/ml) | MIC (µg/ml) | MBC (µg/ml) |
| L. plantarum LU5  | 12.5        | 25           | 25           | 100          |
| S. flexneri       | 3.125       | 6.25         | 6.25         | 12.5         |
| E. coli           | 12.5        | 25           | 25           | 100          |

The main component in S. khuzistanica (93.9%) followed by eugenol (1.0%), and p-cymene (0.8%). Carvacrol is a monoterpenoid phenol, and it is one of the major component of EOs from Lamiaceae family (such as thyme, oregano, and savory oil) (Hadian et al., 2011). A wide range of activities such as antimicrobial, anti-oxidant, anti-candidal, and anti-inflammatory characterization are attributed to the carvacrol (Di Pasqua et al., 2007). Other researchers have reported carvacrol as the major compound in S. khuzistanica EOs (Hashemi et al., 2012; Mazarei & Rafati, 2019). The hydrophobicity of EO components such as carvacrol due to their lipophilic nature possesses a high affinity for cell membranes and causes physicochemical changes in microorganisms’ membrane that cause the decrease in membrane integrity and potential depolarization (Mazarei & Rafati, 2019).
of application the EOs mixture in probiotic food products. These observations affirm the potential use of combination of Marzeh khuzestani and Marzeh bakhtiari EOs on food pathogen bacteria.

Synergistic effects between carvacrol and other hydrocarbon monoterpense (e.g., α-pinene, camphene, myrcene, α-terpinene, and p-cymene) have been reported previously by other researchers that hydrocarbons by interacting with bacterial cell membrane facilitate the carvacrol penetration into the cells (De Azeredo et al., 2011; Ultee, Slump, Steging, & Smid, 2000). Minor components by modulating the activity of the major components help the synergism of EOs. Moon and Rhee (2016) reported a synergistic effect between carvacrol and thymol against E. coli, S. Typhimurium and L. monocytogenes.

### 3.4 Combinatorial agar diffusion assays

Three different combinations of Marzeh khuzestani:Marzeh bakhtiari (1:1, 1:2, and 2:1) were prepared, and their antimicrobial inhibition zone against selected bacteria is presented in Table 4. As can be seen, the combination of 2:1 Marzeh khuzestani:Marzeh bakhtiari exhibited the strongest antibacterial activity compared to the other treatments (p ≤ .05) and reduced the growth of bacteria strains with the inhibition zones from 24.7 mm for L. plantarum to 32.7 mm for S. flexneri. However, the combinations of 1:2 and 1:1 of Marzeh khuzestani:Marzeh Bakhtiari exhibited the similar antibacterial activities against the selected bacteria (p > .05). This observation affirms a higher antimicrobial activity of Marzeh khuzestani compared to Marzeh bakhtiari EOs on food pathogen bacteria.

| Bacteria        | FIC  | FBC  |
|-----------------|------|------|
| L. plantarum LU5| 0.75 | 0.75 |
| S. flexneri     | 0.5  | 0.5  |
| E. coli         | 0.5  | 0.5  |

FIC ≤ 0.5: synergic effect; 0.5 < FIC≤1: additive effect; 1 < FIC≤4: no interactive effect; FIC > 4: antagonistic effect.

### Table 4 Antibacterial activity of Marzeh khuzestani and Marzeh bakhtiari against tested bacteria

| Bacterial strains | Marzeh khuzestani + Marzeh bakhtiari (1:2 (v/v)) | Marzeh khuzestani + Marzeh bakhtiari (1:1 (v/v)) | Marzeh khuzestani + Marzeh bakhtiari (2:1 (v/v)) |
|-------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
|                   | Inhibition zone (mm)                              | Inhibition zone (mm)                              | Inhibition zone (mm)                              |
| L. plantarum LU5  | 21.5 ± 0.4bB                                     | 22.8 ± 0.5bB                                     | 24.7 ± 0.8aB                                      |
| S. flexneri       | 28.4 ± 0.5bB                                     | 29.6 ± 0.3ab                                     | 32.7 ± 0.3aA                                      |
| E. coli           | 22.1 ± 0.3bB                                     | 23.3 ± 0.5ab                                     | 28.4 ± 0.7aA                                      |

Note: Values are means ± standard error. Within each column means with the same lowercase letters are not significantly different (p > .05). The same uppercase letters are not significantly different between different concentrations for each method used (p > .05).

### Figure 1 The population change for L. plantarum in table creams treated with different concentrations of Marzeh khuzestani (k) and Marzeh bakhtiari (b) during 10 days of storage

![Graph showing population change for L. plantarum in table creams treated with different concentrations of Marzeh khuzestani (k) and Marzeh bakhtiari (b) during 10 days of storage](image-url)
3.5 | Antibacterial activity of essential oils in table cream

The population changes for *L. plantarum*, *S. flexneri*, and *E. coli* strains in table creams containing different combinations of EOs during storage period have been depicted in Figures 1–3, respectively. The initially recorded population for *L. plantarum* in control samples was 9.3 log CFU/g and a reduction of 1.3 log CFU/g observed after 10 of storage. However, the addition of EOs significantly reduced the initial number of viable *L. plantarum* cells in compared to control samples and the treatments containing higher concentration of EOs exhibited a lower viable cells of *L. plantarum*. Treatments containing 1%k + 1%b EOs and 1%k + 0.5%b showed the lowest population during the storage up to the day 8 of storage. The lowest population of probiotic strain observed at the day 10 of storage and for the 1%k + 1%b treatment that contained the highest amount of EOs. Moritz, Rall, SaeKi, and Fernandes Júnior (2012) reported that the population for *L. rhamnosus* in fermented milk did not affect by the addition of clove and mint EOs.

Similar pattern observed for *E. coli* and *S. flexneri* population during the storage of table cream samples. The population of both bacteria strain decreased during the storage time, and for the treatments with higher amount of EOs addition (1%k + 0.5%b and 1%k + 1%b), the number of viable bacteria was significantly lower than the other treatments up to the day 6 of storage. However, the difference between treatments containing 0.5%k + 0.5%b and 0.5%k + 1%b EOs was not significant (p > .05). Treatment loaded with 1%k + 1%b EOs exhibited the lowest number of pathogen strains during the storage period so that, it decreased the viable number of *S. flexneri* and *E. coli* cells up to 1.9 and 2.3 log CFU/g by the end of storage time. Govaris, Botsoglou, Sergelidis, and Chatzopoulou (2011) reported that oregano and thymol EOs showed strong antimicrobial activity against the *L. monocytogenes* and *E. coli* in feta cheese.

3.6 | Sensory analysis

Sensory evaluation scores of the creams treated with different combination ratios of Marzeh khuzestani and Marzeh bakhtiari during refrigerated storage at 4°C are shown in Figure 4. Results showed that by increase in the EOs addition in the cream, a slight decrease in overall acceptability was observed. So that, the lowest acceptability
the table creams and it can significantly improve the microbiological properties of the product.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

ETHICAL APPROVAL
This study does not involve any human or animal testing.

INFORMED CONSENT
Written informed consent was obtained from all study participants.

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