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Design of microemulsion formulations loaded *Scutellaria salviifolia* Benth, *Sideritis libanotica* Labill. subsp. linearis (Bentham) Bornm, and *Ziziphora clinopodioides* Lam. extracts from Turkey and in vitro evaluation of their biological activities

Umay Merve GÜVEN[1,*], Serpil DEMİRCİ KAYIRAN[2], Abdurrahman AYGÜL[3], Merve NENNİ[4], Saliha KIRICI[5]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Cukurova University, Adana, Turkey
[2]Department of Pharmaceutical Botany, Faculty of Pharmacy, Cukurova University, Adana, Turkey
[3]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Cukurova University, Adana, Turkey
[4]Department of Analytical Chemistry, Faculty of Pharmacy, Cukurova University, Adana, Turkey
[5]Department of Field Crops, Faculty of Agriculture, Çukurova University,Adana, Turkey

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Abstract: The species belonging to the Lamiaceae family have been useful tools for many years as a folk remedy. In the present study, a microemulsion system was developed with extract of Scutellaria salviifolia Benth (endemic), Sideritis libanotica Labill. subsp. linearis (Bentham) Bornm (endemic), and Ziziphora clinopodioides Lam. Both and related microemulsion formulations were also investigated for their potential biological activities. Pseudo-ternary phase diagrams of formulations were constructed consisting of oil/mixed surfactant/water emulsion system and a quaternary component. Antioxidant activity was determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. For antimicrobial activity determination, on the other hand, microdilution method was performed with Escherichia coli 25922, Staphylococcus aureus 29213, Candida albicans 14053, and Candida krusei 6258 standard strains. Tested extracts showed S. aureus-specific antibacterial activity with MIC values 64 µg/mL, 256 µg/mL, and 512 µg/mL for S. libanotica subsp. linearis, S. salviifolia, and Z. clinopodioides, respectively. For any other tested strain, considerable antimicrobial activity was not observed (MIC values > 1024 µg/mL). Related formulations were not superior to extracts in terms of antimicrobial activity. All extracts and related formulations appear to have antioxidant activity, and the antioxidant capacity of all formulations is higher than the extracts. When compared to ascorbic acid, formulation of S. libanotica subsp. linearis extract has stronger antioxidant activity. Sideritis libanotica subsp. linearis extract had a remarkable antibacterial activity against S. aureus. It also had a stronger antioxidant capacity than ascorbic acid. Besides, all formulations developed from extracts had antioxidant activity enhancing properties, although they did not contribute to antimicrobial activity. In conclusion, considering the potential antiinflammatory activity due to antioxidant activity, it is predicted that especially the formulation developed with Sideritis libanotica subsp. linearis extract is worth exploring with further studies for topical treatment of S. aureus-infected wounds and burns.

Key words: Lamiaceae family, microemulsion formulation, drug delivery, antimicrobial activity, antioxidant activity

1. Introduction

Lamiaceae species are a well-known group of medicinal herbs that have been used in folk medicine worldwide and have frequently been subject to ethnopharmacological studies. The genus *Sideritis* L., *Scutellaria* L., and *Ziziphora* L. are all included in *Lamiaceae* family.

Sideritis L. genus comprises about 150 species all over the world and is represented by 40 endemic taxa out of 53 in Turkey. A member of the genus, *Sideritis libanotica* subsp. *linearis*, is a perennial herb (González-Burgos et al., 2011; Guner et al., 2012). The infusion of *S. libanotica* subsp. *linearis* obtained mainly from dried leaves and flowers has been widely used in food industry as teas or flavoring agents and in traditional medicine for the treatment of mild diseases such as common cold (Dincer et al., 2017). The antioxidant, antimicrobial, cytotoxic, and enzyme inhibitory activities of this herb was reported previously with other members of the genus by Tunalier et al., (2004); Tepe et al., (2006); Demirtas et al., (2011); Dincer et al., (2017); Atas et al., (2019). According to these previous studies, linoleic, oleic, 6-octadecenoic, palmitic, and linoleic acids were identified as the major chemical components of aerial parts at flowering stage (Demirtas et al., 2011; Dincer et al., 2017).

Scutellaria L. is a widespread genus throughout the world represented by 350 species (Paton, 1990). There are

* Correspondence: umaymerveguven@gmail.com

18 *Scutellaria* species with 39 taxa, 16 of which are endemic in Turkey (Guner et al., 2012; Çiçek and Yaprak, 2013). One of them, *Scutellaria salviifolia* Benth., is commonly known as "kaside, sancı otu, şimşek otu, korku otu" in Turkish and was found to contain several secondary metabolites such as flavonoids, phenolic acids (Zengin et al., 2019), and glycosides (Saracoglu et al., 1995; Dogan et al., 2015; Zengin et al., 2019). Antimicrobial, antioxidant, anticholinesterase, anti-tyrosinase, cytotoxic, enzyme inhibitory properties of *S. salviifolia* have been reported by many studies and have been used as carminative, astringent, abdominal pain relaxant, and externally for wound healing (Baytop, 1999; Altundag and Ozturk, 2011; Cakilcioglu and Turkoglu, 2010; Mukemre et al., 2015; Saracoglu et al., 1995; Senol et al., 2010; Dogan et al., 2015; 2017; Zengin et al., 2019; Arituluk et al., 2019).

The genus *Ziziphora* L. is represented by 27 taxa, and 6 of them are found in Turkey. *Z. clinopodioides* Lam. with the common Turkish name "dağ reyhanı, kır nanesi" is used for its carminative, degasifier, appetizer, and antiseptic properties in various conditions such as gastrointestinal disorders, wounds, diabetes, and asthma (Baytop, 1999; Maral et al., 2015; Mohammadhosseini, 2017). Phytochemical content investigations of *Z. clinopodioides* Lam. mainly focused on its essential oil composition, and these studies revealed the presence of flavonoids, caffeoyl derivatives, fatty acids, and sterols, some of which were found to possess antibacterial, antifungal, antioxidant, cytotoxic, and analgesic activities (Salehi et al., 2005; Öztürk 2007; Mohammadhosseini, 2017; Bagheri et al., 2019; Reçber et al., 2019). Notably, it is also known to be rich in pulegone content (Salehi et al., 2005; Öztürk and Ercisli, 2007; Aghajani et al., 2008).

Nano drug delivery systems have several advantages for herbal drugs, such as providing stability, increasing solubility and bioavailability, enhancement of pharmacological activity, and sustained delivery. The use of nanotechnology for drug delivery has dramatically increased over the past few decades because this technology has the potential to improve treatment strategies against many important diseases (Bitencourt et al., 2016; Dai and Si, 2019). Microemulsions are commercially feasible, simple, and convenient novel vehicles for the delivery of medications that can enhance drug absorption with reduced systemic side effects (Grampuroit et al., 2011). In microemulsion formulation theory, the arrangement of surfactans occurs spontaneously. However, in some cases, energy is provided to the system to speed up the rearrangement of the surfactants or to overcome a small kinetic energy barrier. There are three principal methods, which may be used in microemulsion formation: low energy emulsification method, phase inversion temperature (PIT) method, high-pressure homogenization. In this study, microemulsion preparation was achieved by low energy emulsification methods involving dilution of an oil surfactant mixture with water (Solans et al., 2005).

Microemulsions as a drug delivery system are perfect vehicles for the transport of water-soluble or insoluble compounds and are remarkable thanks to their thermodynamic stability, low viscosity, small droplet size (5–200 nm), low surface tension, ease of preparation, and manufacturing (Kahlweit et al., 1990; Jeirani et al., 2013; Maqsood et al., 2019). Thus, these systems have a wide range of applications in any area that needs nanostructure preparation from food products to drug delivery. Particularly for encapsulation of various medicinally active compounds in pharmaceutical industry, emulsion systems have proven their worth (Bonifacio and Silvia, 2014, Özyılmaz et al., 2020). In this context, transdermal microemulsions are expected to provide a safe and effective means for the supported bioavailability of plant extracts. Also, formulations prevent the degradation of active agents in extracts, extend their bioavailability for long period, and advance the biological activity of active materials. (Çomuoğlu and Gönül, 1997, Lawrence and Rees, 2000).

Free radicals are formed in living cells during the functioning of normal metabolic pathways or under the influence of various external factors such as environmental agents (eg pesticides, aromatic hydrocarbons, toxins), stress, radiation (Yeşilada et al., 1995). Free radicals and reactive oxygen types are also constantly manufactured in the human system. Oxidative stress due to free radicals can cause various disorders and aging by triggering tissue damage and cell death (Akkuş, 1995). Epidemiological studies have indicated that several plants can be beneficial for prophylactic purposes against oxidative stress when consumed as a food source. Those plants that contain ascorbic acid (vitamin C), α-tocopherol (vitamin E), carotenoids, glutathione, and phenolic compounds such as flavonoids, phenolic acids are known to be responsible for these antioxidant characteristics (Halvorsen et al., 2002). When free radicals and reactive oxygen types cause oxidation in biological systems, antioxidants can prevent or postpone this process through various mechanisms (Ulusoy et al., 2010). Revealing the antioxidant capacities of plants increases their use for this purpose in the pharmaceutical and cosmetic industry day by day. DPPH, one of the fast and simple antioxidant determination methods, was found appropriate to use for our study in antioxidant activity experiments.

In our study, designed within the framework of the mentioned theoretical background, we prepared and characterized microemulsion formulations containing *Scutellaria salviifolia*, *Sideritis libanotica* subsp. *linearis* and *Z. clinopodioides* extracts collected in Southern

Turkey. Antioxidant and antimicrobial activity studies are also conducted with both extracts and formulations.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

Plant materials were collected in Adana, Kozan and Çamlıyayla in Southern Turkey in July 2019. The voucher specimens have been deposited at the herbarium of the Pharmacy, Çukurova University (voucher numbers: CUEF 1668-1670). They were identified by Dr. Serpil Demirci Kayiran.

2.1.2. Solvents and other chemicals

Oleic acid, methanol, ethanol, 2,2-diphenyl-1-picrylhydroazyl (DPPH), Tween 20, and glycerin were provided from Sigma–Aldrich (Aldrich, St. Louis MO, USA). All other chemicals were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Extraction and fractionation

Aerial parts (stems, leaves, and flowers) were dried for one week under shade and powdered mechanically using a commercial electric blender. Air-dried plant material of *S. salviifolia* (20 g) was stirred with methanol solvent (420 mL); *S. libanotica* subsp. *linearis* (36 gr) with methanol solvent (720 mL); *Z. clinopodioides* (27 gr) with ethanol solvent (550 mL) for 24h. Then, the extracts were concentrated at 40 °C to dry using a rotary evaporator. The extracts were then stored in dark glass at 4 °C until further analyses.

2.2.2. Construction of pseudo-ternary phase diagrams

Phase diagrams were performed using the water titration method of the oil phase and surfactant/cosurfactant (S/Cos) mixtures at ambient temperature. A pseudo-ternary phase diagram was constructed to find the microemulsion region. The phase diagram was constructed using 2:1, 1:1, 1:2, and 1:3 weight ratio of S/Co (S_{mix}). Oil and S/Co ratios were mixed carefully at various weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. (w/w) in glass tubes. Then, the mixture was dropped with water under mechanical stirring (600 rpm), and the physical state of the system was observed. The transparent phase formed was an indication of stable microemulsions (Lim et al., 2012; Maqsood et al., 2019). During the titration, the amount of water added was recorded when the transparent microemulsion appeared. Then, the pseudo-ternary phase diagrams were plotted according to the proportion of each component. The phase diagrams have been created by means of a computer software, and all tests were repeated three periods (Üstündağ Okur et al., 2015). The area covered by these points was assumed as a microemulsion area. The composition of the microemulsion was chosen from phase diagrams that gave the largest microemulsion region (Öztürk and Güven, 2019). After the microemulsion regions were identified from the phase diagrams, the formulations were prepared at ambient temperature with mechanical stirring. Then, 3, 6, 9 wt % of extract was added to the microemulsion formulation. So, concentrations 3, 6, 9 wt% were used for all three extracts. And then, a total of 9 formulations were developed.

2.2.3. Characterization of microemulsion formulations

The developed formulations were characterized for visual clarity, centrifugation, pH measurement, droplet sizes, and polydispersity indexes. The stability of microemulsion was checked by centrifuging at 3500 rpm for 30 min at room temperature, and the occurrence of phase separation was examined visually. The formulation was incubated at 4 and 40 °C (48 h) for 3 cycles, respectively and evaluated for phase separation (ElShafei et al., 2010; Khan et al., 2015; Ali et al., 2017). The droplet size and polydispersity index of the microemulsion were examined at 25 °C by dynamic light scattering (Nano ZS, Malvern, UK). The pH value was evaluated using a pH meter. Each sample was measured in triplicate (Güven and Yenilmez, 2019).

2.2.4. Antimicrobial activity experiments

Antimicrobial activity experiments were performed with bacterial (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213) and fungal (*Candida albicans* ATCC 14053, *Candida krusei* ATCC 6258) standard strains. Mueller-Hinton broth (MHB), Mueller-Hinton agar (MHA), Sabouraud dextrose broth (SDB), and Sabouraud dextrose agar (SDA) (BD Biosciences, USA) were used as a medium whenever needed. The microdilution method in 96-well microplates (Orkim, Turkey) was preferred for the determination of MIC values of extracts and related formulations (CLSI, 2012). Firstly, serial dilutions of extracts and formulations were performed with MHB or SDB. After that, inoculums with $1x10^6$ CFU/mL density for bacteria and $1–5×10^3$ CFU/mL density for fungi were prepared from overnight fresh cultures and added on serial dilutions with an equal volume. Thus, the final inoculums of $5×10^5$ CFU/mL for bacteria and $0.5–2.5 ×10^3$ for yeast were obtained. Finally, microplates were incubated at 36 ± 1 °C for 18,24, and 48 h according to the tested strains. At the end of the incubations, all microplates were evaluated visually, and the lowest concentrations without any visible growth were determined as MIC values.

2.2.5. Antioxidant activity experiments

Antioxidant activity of *S. salviifolia, Z. clinopodioides, S. libanotica* extracts and of their formulations was measured. Antioxidant activity was performed by 2,2-diphenyl-1-picrylhydroazyl (DPPH) radicals scavenging method which is a colorimetric method. The changes in color (from violet to yellow) were measured with a UV/visible

light spectrophotometer. DPPH assay was established by Blois (1958), Brand-Williams et al. (1995) and slightly modified by Wróblewska et al. (2019) and adapted for 96-well plates. Ascorbic acid was used as a positive control (Ghasemi et al., 2009). While methanol was used in the negative control of plant extracts, blank formulations without plant extract were used in the negative control of the formulations.

A total of 50 µL of 0.1 mM DPPH radical solution, which was freshly made in methanol, was added to 150 µL of extracts or standards in 96-well plates. The plates were shaken for 1 min with the microplate reader (Thermo Scientific, Multiskan Sky Microplate Spectrophotometer, Waltham, MA, USA) and were incubated for 45 min in the dark at room temperature. The absorbance was measured at 517 nm by a microplate reader. The experiment was repeated three times. The % DPPH radical scavenging activity of samples and standards were calculated from the decrease in absorbance in comparison with the negative control. The percentage of DPPH scavenging activity was calculated according to % DPPH Scav. Act. = $[(A_{Control} - A_{Sample})/A_{Control}] \times 100$ formula. A sample is the absorbance measured for the sample and AControl is the absorbance measured for the control. Half-maximal inhibitory concentration (IC_{50}) value is calculated as, the concentration of sample required to scavenge 50% of DPPH radicals. While the IC_{50} value is decreased, the antioxidant ability of extracts is increased. A lower IC_{50} value reveals a higher antioxidant activity. Amount of antioxidant spent to decrease the initial DPPH concentration by 50% is given with the value of EC_{50} (effective concentration). EC_{50} was calculated according $EC_{50}=IC_{50}/[DPPH]$ in mg/mL formula. Radical scavenging capacity demonstrated as antiradical power (ARP) were calculated $ARP=(1/EC_{50})\times100$ formula. The results were expressed as ascorbic acid equivalent antioxidant capacity ($AEAC=(IC_{50}(AA)/IC_{50}(sample))\times10^5$) (Kroyer, 2004).

2.2.6. Statistical analysis

All data were expressed as mean and standard deviation (mean ± SD) of IC50. Student`s t-test has been down to compare the data, and all tests were considered statistically significant at $p < 0.005$.

3. Results and discussion

3.1. Construction of pseudo-ternary phase diagrams

Microemulsions have been as favorite colloidal carriers for poorly soluble active agents due to their simple and economical preparation, long-term stability, biocompatibility, and high solubility degree (Üstündağ Okur et al., 2017).

Oleic acid, Tween 20, glycerin, ethanol, and distilled water were used as ingredients for the preparation of microemulsions in our study. The selection of the components for microemulsion formulations is often a balance between the nontoxic compounds and ingredients (Jeirani et al., 2013). Oleic acid is a well-established penetration enhancer. Oleic acid was selected as the oil phase because it is reported to disturb the lipid barrier in the stratum corneum by forming a separate area that induces highly permeable pathways (Aggarwal et al., 2013). Between nonionic surfactants with lower toxicity and lower skin irritation, Tween 20 was selected as the main surfactant due to its safe and biocompatible excipient property (Azeem et al., 2009).

Surfactants are unable to decrease the interfacial tension and form stable microemulsion. Therefore, cosurfactants should be used with low surfactant concentration to form microemulsion system. Glycerin was selected as a cosurfactant because of its great biocompatibility and high polarity. It has three hydroxyl functional groups, which increases the water solubility of compounds (Azeem et al., 2009; Golmohammadzadeh et al.,2017). Ethanol, which is commonly used in topical microemulsions, was selected as another cosurfactant.

The components of microemulsions are usually optimized by using pseudo-ternary phase diagrams, which present the suitable amount of oil, water, and surfactant mixtures. Three pseudo-ternary phase diagrams using different mixtures are given in Figure. The marked area represents the microemulsion region, while the rest either represents turbid phases or liquid crystalline phases observed. From this region, four different compositions of oil, surfactant, and cosurfactant were selected to make four microemulsion formulations. The largest region was observed at the S_{mix} ratio of 1:2, followed by 1:1, 1:3, and 2:1, respectively. These results indicated that the S_{mix} ratio of 1:2 sufficiently reduced interfacial tension of the microemulsion systems (Aggarwal et al., 2013; Öztürk and Güven, 2019). The microemulsion for further studies was selected from the phase diagram. The central point of phase diagrams provided the percentage of components in order to prepare the microemulsion formulations. Studying the different combinations of ingredients, it was observed that the best ratio of oil, surfactants, and distilled water for making optimum microemulsion was 24.5: 59.8: 15.7. This ratio was used to develop the blank formulation.

3.2. Characterization of microemulsions

The choice formulation was characterized with respect to the appearance, droplet size, polydispersity index, pH value. Microemulsion formulation was examined visually for their color, consistency, and homogeneity. The microemulsion was stable, with no phase separation or creaming at ideal concentrations of oil, water surfactant, and cosurfactant. The formulation was found to be stable at stress conditions like heat and cool cycles. A centrifugation study was carried out by centrifuging the microemulsions

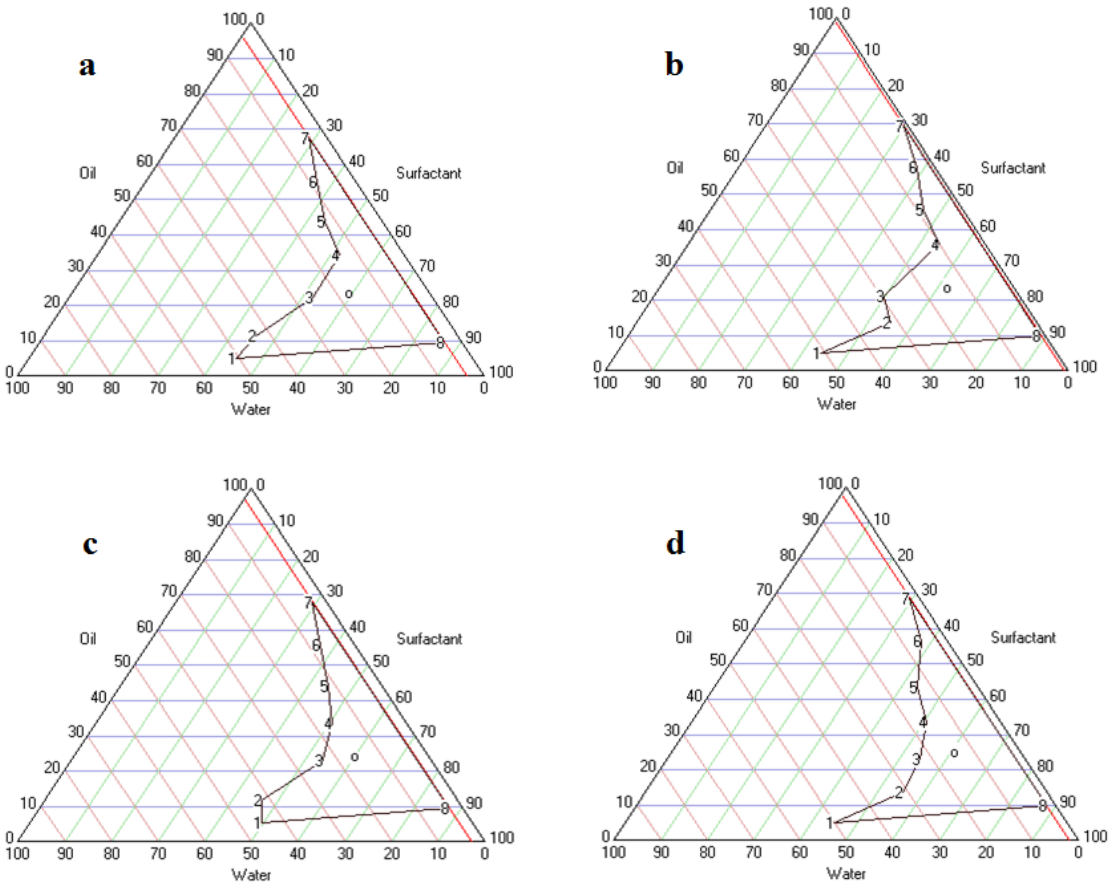

Figure. Pseudoternary phase diagrams for microemulsion systems. (S_{mix} ratio a. 2:1, b. 1:1, c.1:2, d. 1:3)

at 3500 rpm for 30 min. The microemulsions stayed clear and transparent after centrifugation, which confirmed the mechanical stability of the microemulsion (Zhao et al., 2020). Thermodynamic stability data of the microemulsion system is demonstrated in Table 1. These data suggested the compatibility and stability of the microemulsions. The formulation ruled its stability in terms of thermodynamic properties. No significant difference was achieved in terms of phase behavior ($p > 0.05$).

Characterization studies of microemulsion systems are demonstrated in Table 2. The optimum blank formulation had droplet size of 146.6 ± 6.6 nm and polydispersity index of 0.257 ± 0.036. Most droplet sizes of extract loaded microemulsions were in the range of 246–314 nm. A total of 9% of extract-loaded microemulsions data could not be retrieved or suitable. The small droplet size was explained due to the cosurfactant molecules that penetrate the surfactant film. It is important that droplet size influences drug penetration of microemulsion formulation. An increase in total surface area is observed with a smaller droplet size of microemulsion system for contacting the skin and there is more opportunity for drug transfer into the skin (Kumar et al., 2013; Mishra et al., 2016). Polydispersity index is a measure of globule homogeneity, and it varies from 0.0 to 1.0. Small PDI of the developed microemulsions showed uniformity in the size distribution of droplets. Zeta potential values of microemulsions were measured neutral because of microemulsion components like nonionic surfactants (Mishra et al., 2016).

The pH value of a topical preparation is an important factor for patient compliance. Blank formulation of pH was 6.2 ± 0.10. pH values of the extract loaded microemulsions were found to be in the range of 5.88 ± 0.06 to 6.18 ± 0.06. This pH range is suitable for topical applications (Üstündağ Okur et al., 2017). The results of the characterization study indicate the development of successful microemulsion formulations with optimum characteristics.

3.3. Antimicrobial activity

According to the microdilution results, all extracts showed moderate antibacterial activity against *S. aureus* 29213, notably the MIC value of *S. libanotica* was determined as 64 µg/mL (Table 3). As the MIC values for *E. coli* 25922, *C. albicans* 14053, and *C. krusei* 6258 is 1024 µg/mL or more, extracts are ineffective against them compared to typical antimicrobials. On the other hand, it was observed that all microemulsion formulations had the same MIC value as

Table 1. Thermodynamic stability data of microemulsions.

Thermodynamic stability		**Time (days)**		
		0.	**15.**	**30.**
5±1°C	Visual appearance	Clear - transparent	Clear - transparent	Clear - transparent
	Centrifugation	No seperation	No seperation	No seperation
	Heating-cooling cycles	No seperation	No seperation	No seperation
25±2°C	Visual appearance	Clear - transparent	Clear - transparent	Clear - transparent
	Centrifugation	No seperation	No seperation	No seperation
	Heating-cooling cycles	No seperation	No seperation	No seperation

T**able 2.** Characterization studies of microemulsions.

Characterization studies			
Code / (mean±S.D)	**Droplet size (nm)**	**Polydispersity index (PDI)**	**pH value**
F0 Blank Formulation	146.6 ± 6.6	0.257 ± 0.036	6.20 ± 0.01
F3 *Scutellaria salviifolia*	266.8 ± 6.2	0.188 ± 0.012	6.02 ± 0.04
F6 *Scutellaria salviifolia*	290.2 ± 3.2	0.236 ± 0.024	6.00 ± 0.08
F9 *Scutellaria salviifolia*	*no data*	*no data*	5.96 ± 0.06
F3 *Sideritis libanotica*	314.0 ± 4.2	0.172 ± 0.008	6.18 ± 0.06
F6 *Sideritis libanotica*	298.4 ± 8.0	0.214 ± 0.010	6.12 ± 0.02
F9 *Sideritis libanotica*	618.6 ± 20.2	0.866 ± 0.096	6.14 ± 0.06
F3 *Ziziphora clinopodioides*	257.8 ± 3.0	0.242 ± 0.028	5.92 ± 0.04
F6 *Ziziphora clinopodioides*	246.0 ± 5.2	0.226 ± 0.014	5.88 ± 0.06
F9 *Ziziphora clinopodioides*	*no data*	*no data*	5.98 ± 0.08

*F3: 3 % extract loaded microemulsion, F6: 6 % extract loaded microemulsion, F9: 9 % extract loaded microemulsion.

extracts for all tested strains. Therefore, formulations were not superior to extracts in terms of antimicrobial activity against any tested microorganisms, and MIC values of the formulations were not included in Table 3 for simplicity.

In a Turkey addressed previous research, which was very similar to our study, the MIC values of *S. salviifolia* methanol extract for *E. coli, S. aureus, C. albicans,* and *C. krusei* were reported as 1024 µg/mL, 1024 µg/mL, 512 µg/mL, and 256 µg/mL, respectively (Arituluk et al., 2019). Extracts prepared from *Scutellaria* species and related seconder metabolites were also previously tested for their antibacterial activity against other pathogens such as *Acinetobacter baumanni, Salmonella typhimurium, Streptococcus mutans*. In studies with *A. baumanni*, the MIC value for *Scutellaria barbata* (Tsai et al., 2018) and *Scutellaria baicalensis* (Miyasaki et al., 2013) extracts were reported as 4 mg/mL and ~1 mg/mL, respectively. One of the major components of *Scutellaria* species, baicalein, on the other hand, was found to be effective against *S. typhimurium* at 64 µg/mL concentration (Wu et al., 2018). In another study (Duan et al., 2017) conducted with *S. baicalensis*, it has been reported that the MIC values of methanolic and ethanolic extracts were 0.5 mg/mL against dental streptococcus species such as *Streptococcus mutans*.

Our result is fully compatible with a previous study (Basile et al., 2006), investigating *Sideritis italica* essential oils against various bacterial pathogens, in terms of *S. aureus* (MIC value of essential oil from leaves: 62.5 µg/mL). On the other hand, when similar studies in the literature observing *Sideritis* extracts with polar solvents (ethanolic, methanolic, aqueous) were examined, they generally found higher MIC values for *S. aureus* than in our study. To exemplify, for aqueous and methanolic extracts of *Sideritis raeseri* (Stagos et al., 2012) and ethanolic extract

Table 3. MIC values of extracts for tested bacteria and fungi. Formulations prepared from extracts have the same MIC value with extracts themselves for all tested strains.

Bacteria	MIC values (µg/mL)				
	F6 *Scutellaria salviifoli*a	**F6 *Sideritis libanotic*a**	**F6 *Ziziphora clinopodioide*s**	**Cefaclor**	**Fluconazole**
***E. coli* 2592**2	1024	1024<	1024<	1	-
***S. aureus* 29213**	256	64	512	2	-
***C. albicans* 14053**	1024<	1024	1024<	-	0.5
***C. krusei* 6258**	1024<	1024	1024<	-	1

of *Sideritis scardica* (Tadić et al., 2012), MIC values in *S. aureus* were determined as 3 mg/mL, 4< mg/mL, and 1.28 mg/mL, respectively, in such studies. Considering the applied methods are the same or similar, such serious differences regarding MIC values can be attributed to the probability that secondary metabolites in the extracts and their amounts are highly variable.

Ziziphora species, like other plants examined in our study, are herbs whose antibacterial activity was the subject of many pieces of researches, particularly for their essential oil. *Ziziphora clinopodioides* essential oil had MIC values as low as 0.01 µg/mL against clinical *S. aureus* isolates (Pakdel et al., 2017). Similarly, *Ziziphora tenuior* essential oil was found effective with 0.25 µg/mL MIC value against *Enterococcus faecalis* in another study (Nazemisalman et al., 2012). Considering that essential oils are rich in terpenic compounds that have prominent antimicrobial activity, such results can be expected. Herbal extracts other than the essential oils of *Ziziphora* species, on the other hand, are generally not a subject of antimicrobial activity researches.

3.4. Antioxidant activity

3. Today, because of the widespread use of synthetic drugs, significant side effects can occur. Medical and economic problems caused by these side effects have made herbal treatment agenda again (Özbek, 2005). Concern about whether synthetic antioxidants and antimicrobial agents are safe or not has also increased. Therefore, research in the field of medicinal and aromatic plants is becoming popular, as they are often rich in antioxidant and antimicrobial bioactive compounds (Rojas et al., 2003; Ivanova et al., 2005; Chanwitheesuk et al., 2005). Antioxidant activity has been widely studied in literatures. Antioxidant therapy is believed to have significant benefits for improving oxidative stress-related cutaneous wound healing (Cao et al., 2017).

The studies carried out suggest that the decreased oxidative damage in the wound tissue could be due to increased quenching or scavenging of oxygen free radicals by the elevated levels of antioxidants.

DPPH radical is one of the most common radicals in studies investigating the antioxidant capacities of plant extracts. DPPH testing is routinely used in laboratories to determine the free radical scavenging potential of plant extracts as it is fast, easy, and inexpensive (Mishra et al., 2012). By drawing a logarithm graph of scavenging capacity against sample concentration, IC_{50} value was calculated (Dhar et al., 2013). Afterwards, consecutively, EC_{50} value was calculated from IC_{50}, ARP value was calculated from EC_{50}, and AEAC value was calculated from ARP value. These parameters are used to express the antioxidant potency. If low IC_{50} – EC_{50} and high ARP – AEAC values are the case, an antioxidant is reported to be stronger (Kedare and Singh, 2011). In this study, the results are given as IC_{50}, EC_{50}, ARP, and AEAC values and are listed in Table 4. Table 4 shows the DPPH radical scavenging activity of plants and their formulations compared to ascorbic acid. It also shows the difference between antioxidant activities of formulations relative to their plant extract.

When antioxidant parameters of *S. salviifolia* extract (IC_{50}: 0.6814±0.1197 mg/mL, EC_{50}: 0.0173 mg/mg DPPH, ARP: 57.87 and AEAC: 1421.75) and formulation of *S. salviifolia* extract (IC_{50}: 0.2500±0.0308 mg/mL, EC_{50}: 0.0063 mg/mg DPPH, ARP: 157.75 and AEAC: 3875.75) are compared, it is seen that the formulation had higher antioxidant power. When antioxidant parameters of *Z. clinopodioides* extract (IC_{50}: 0.0515±0.0021 mg/mL, EC_{50}: 0.0013 mg/mg DPPH, ARP: 766.13 and AEAC: 18822.84) and formulation of *Z. clinopodioides* extract (IC_{50}: 0.0283±0.0049 mg/mL, EC_{50}: 0.0007 mg/mg DPPH, ARP: 1391.71 and AEAC: 34192.83) are compared, it is seen that the formulation had higher antioxidant power. When antioxidant parameters of *S. libanotica* extract (IC_{50}: 0.0133±0.0014 mg/mL, EC_{50}: 0.0003 mg/mg DPPH, ARP: 2961.70 and AEAC: 72765.82) and formulation of *S. libanotica* extract (IC_{50}: 0.0050±0.0005 mg/mL, EC_{50}: 0.0001 mg/mg DPPH, ARP: 7860.54 and AEAC: 193125.23) are compared, it is seen that the

Table 4. Antioxidant activities of extracts and of their formulations.

	IC_{50} (mg/mL)	EC_{50} (mg/mg DPPH)	ARP Values	AEAC Values
Scutellaria salviifolia	0.6814 ± 0.1197	0.0173	57.87	1421.75
Ziziphora clinopodioides	0.0515 ± 0.0021	0.0013	766.13	18822.84
Sideritis libanotica	0.0133 ± 0.0014	0.0003	2961.70	72765.82
F6 *Scutellaria salviifolia*	0.2500 ± 0.0308*	0.0063	157.75	3875.75
F6 *Ziziphora clinopodioides*	0.0283 ± 0.0049*	0.0007	1391.71	34192.83
F6 *Sideritis libanotica*	0.0050 ± 0.0005*,#	0.0001	7860.54	193125.23
Ascorbic acid	0.0097 ± 0.0010	0.0002	4070.18	-

*IC50 values expressed are means±SD of three measurements. The values with small characters were significantly different ($p \leq 0.05$). * compared with its own extract # compared with ascorbic acid.

formulation had higher antioxidant power. As a result, plant extracts appear to have antioxidant activity and the antioxidant power of the formulations is higher than the extracts. When the antioxidant capacity of plant extracts and formulations relative to ascorbic acid are compared, it is seen that the formulation of *S. libanotica* extract has stronger antioxidant activity than ascorbic acid.

4. Conclusion

The most remarkable results of our study were clearly obtained with *Sideritis libanotica* subsp. *linearis*. Its extract had a remarkable antibacterial activity against *S. aureus* and a stronger antioxidant capacity than ascorbic acid. Following antistaphylococcal and antioxidant activity observations in preliminary studies, *S. salviifolia, S. libanotica* subsp. *linearis* and *Z. clinopodioides* loaded microemulsion formulations developed for the first time in our study. These formulations had antioxidant activity-enhancing properties, although they did not contribute to antimicrobial activity. Due to its hydrophobic characteristics, it is inherently difficult to test the activity of extracts and especially microemulsion formulations with in vitro experimental conditions. However, this does not necessarily lead to the conclusion that observed biological activities will be at the same level for in vivo conditions. Invasive wound infection is influenced by the type and quantity of microorganisms that colonize the burn wound. All formulations are likely to further potentiate the antibacterial activity if tested in topical use. In conclusion, considering the potential anti-inflammatory activity due to its antioxidant activity, it is predicted that especially the formulation developed with *Sideritis libanotica* subsp. *linearis* extract is worth exploring with further studies for topical treatment of *S. aureus*-infected wounds and burns**.**

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