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Original Article

Thermosensitive *in situ* gel formulation and characterization studies of *Sambucus ebulus* L. extract

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ABSTRACT

Background and Aims: *Sambucus ebulus* L. has been used to treat inflammation-related gastrointestinal disorders, influenza, kidney ailments, lung diseases, rheumatoid arthritis, and snake and insect bites. Our study provides important ethnobotanical information about *S. ebulus* with the aim of developing a formulation with increased extract bioavailability, diminished side effects, and easy drug loading and dose adjustment as an effective local therapy for dermatologic diseases.
Methods: Twelve *in-situ* gels with *S. ebulus* were prepared as antifungal treatments in accordance with the cold method and formulated using poloxamer and hydroxypropyl methylcellulose (HPMC). The formulations were characterized in terms of pH, gelling capacity, swelling degree, spreadability, and rheological properties.
Results: Among the prepared *in situ* gel formulations, the Poloxamer 407 and 407-HPMC mixtures of P14H1 and P15 demonstrated acceptable gelation temperatures for dermal use.
Conclusion: Thermosensitive *in-situ* gels containing *S. ebulus* may be a viable alternative for treating fungal diseases.

Keywords: *Sambucus ebulus* L., *in-situ* gel, gelling system, topical antifungal

INTRODUCTION

The genus *Sambucus* L. belongs to the *Adoxaceae* family, which is comprised of 30 species worldwide, including two (i.e., *Sambucus nigra* L. and *Sambucus ebulus* L.) that have been recorded in Turkey (Scopel et al., 2007; Senica, Stampar, & Mikulic-Petkovsek, 2019). *S. ebulus*, some of whose common names are elderberry, dwarf elder danewort, dane weed, danesblood, dwarf elder/European dwarf elder, walewort, dwarf elderberry, elderwort and blood hilder, is a type of shrub that is widely distributed in southern and central Europe and southwest Asia, especially in Iran and Turkey (Shokrzadeh & Saravi, 2010). The rootsstem barks, aerial parts, leaves, flowers, and fruits of *S. ebulus* have long been used to treat different ailments (Vallès, Bonet, & Agelet, 2004; Cvetanović, 2020; Charlebois, Byers, Finn, & Thomas, 2010). Due to its extensive usage by Anatolian people for medicinal purposes, they have given *S. ebulus* the name *hekimana* (mother of the physician) (Jabbari et al., 2017; Yeşilada, Gürbüz, & Toker, 2014). The traditional uses of the *S. ebulus* fruit for treating nail fungus infections were reported for the first time by Demirci & Özhatay in 2012. The design of the current study was inspired by this information after reviewing the remarkable knowledge obtained from the countryside in Kahramanmaraş, Turkey. Topical drug delivery is recognized as an effective local therapy for many dermatologic diseases. Active antifungal agents are commercially available in classic dosage forms such as creams, ointments, gels, and pastes (Hudson, Langer, Fink, & Kohane, 2010).

The roots of *S. ebulus* have been used for rheumatic diseases and arthritis, while its leaves are used in preparations for improving liver and kidney function and kidney functions. Its fruit is effective as a laxative and immune modulator. (Cvetanović, 2020). Re-

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ports exist of *S. ebulus* being used as a sedative, antispasmodic, diuretic, emetic, and laxative for treating asthma, bronchitis, cancer, edema, epilepsy, fever, gut issues, head pain, neuralgia, psoriasis, rheumatism, wounds, throat aches, and tooth pains (Kültür, 2007; Kaileh et al., 2007; Passalacqua, Guarrera, & De Fine, 2007; Charlebois et al., 2010; Melikoğlu, Kurtoğlu, & Kültür, 2015). In folk medicine, *S. ebulus L* is the most commonly used species for rheumatism, which is often observed in northern Anatolia due to the high humidity (Yeşilada et al., 1999). The traditional medicinal uses of *S. ebulus* L. in Anatolia are listed in Table 1.

S. ebulus berries are rich in several important secondary metabolites such as anthocyanins (cyanidin-3,5-diglucoside, cyanidin-3-sambubioside-5-glucoside, cyanidin-3-O-sambubioside, and cyanidin-3-O-glucoside), flavonoids (isorhamnetin-3-O-β-D-glucopyranoside, isorhamnetin-3-O-rutinoside, hyperoside, and isoquercitrin), iridoid glycosides (sambulin A, sambulin B), lectins (ebulin), phytosterols, phenols, triterpenes, tannins, cardiac glycosides, and phenolic acids (caffeic acid derivatives, chlorogenic acid, ursolic acid; (Atay, Kirmizibekmez, Gören, & Yeşilada, 2015; Cvetanović, 2020; Kaya et al., 2019; Shokrzadeh & Saravi, 2010).

Numerous studies have investigated the biological activities of these plants with the intention of discovering new pharmaceutical applications (Table 2). As such, anti-inflammatory (Ahmadiani et al., 1998; Ebrahimzadeh, Mahmoudi, & Salimi, 2006; Yeşilada, 1997), anti-nociceptive (Ahmadiani et al., 1998; Ebrahimzadeh et al., 2006), antimicrobial (Rodino et al., 2015; Salehzadeh, Asadpour, Naeemi, & Houshmand, 2014), anti-herpes simplex (Zahmanov et al., 2015), antiulcerogenic (Yeşilada et al., 2014), antioxidant (Cvetanović, 2020; Hashemi, Ebrahimzadeh, & Khalili, 2019), antihypoxic (Kaveh, Mohamadyan, & Ebrahimzadeh, 2019), hypolipidemic (Ivanova, Tasinov, & Kiselova-Kaneva, 2014), and wound healing (Süntar et al., 2010) activities have been demonstrated.

Table 1. Traditional medicinal uses of *Sambucus ebulus* L. in Turkiye.

Province	Local name	Parts used	Use	Literature
Giresun	Yabani mürver, Yer mürveri, Yivdim, Yivdin	flower, leaves radix	digestion analgesic, itching rheumatism, hemorrhoids, diuretic	Karaköse et al., 2017
Rize	İnçiyi, Levor	leaves, fruit	rheumatism, hemorrhoids	Saraç, 2013
Ordu	Yivdin, Yer mürveri, Yabani mürver	leaves, flower, fruit, aerial parts	diuretic, expectorant, flu treatment, antiparasitic, purgative, rheumatism, skin diseases	Gül et al., 2016 Aydın et al., 2018
Trabzon	Livor, Levor	leaves, flower	wounds, hemorrhoids, analgesic	Sağıroğlu et al., 2012 Bozkurt et al., 2017
Artvin	Anzili	leaves, fruit, radix	rheumatism	Eminağaoğlu et al., 2017
Samsun	Yivdin, Sultan otu	leaves, flower	burn treatment, eczema, antifungal, wounds, expectorant, antipyretic, analgesic, rheumatism, anti-inflammatory, hemorrhoids, animal bites	Karcı et al., 2017
Sakarya	Livor, Şahmelek, Şahmelik, Yiğdin, Yiğdün	leaves, flower aerial parts	diuretic, rheumatism, burn treatment	Koyuncu et al., 2009 Göç et al., 2019
Edirne	Sultan otu, Bizga, Mülver	leaves, fruit, herb	rheumatism, hemorrhoids	Güneş, 2018
Kırklareli	Sultanotu, Pıyran, Piran Haptoyina,	leaves, stem	rheumatism, hemorrhoids, wounds, snake bites	Kültür, 2007
İzmit	Lor, Lüver, Piran, Sultan, Şahmelek	leaves, stem	antiparasitic	Kızılarslan, 2012
Balıkesir	Bodur mürver	flower	antipyretic	Güner et al., 2016
Çanakkale	Sultan otu	leaves	rheumatism	Tuzlaci, 2015
Adana	Ayı otu	leaves, flower	hemorrhoids	Güneş et al., 2017
Kahramanmaraş	Ayıboğan, Yir otu	seed	rheumatism, hemorrhoids	Demirci et al., 2012

Table 2. Traditional medicinal uses of *Sambucus ebulus L.*

Country	Parts	Use	Literature
France	flower	Diuretic	Wichtl & Bisset, 1994
	leaves	Digestion	Shokrzadeh et al., 2010
	fruit	Purgative	Rigat et al., 2018
	seed	Analgesic	Shokrzadeh et al., 2010
Belgium	flower	Diuretic	Wichtl & Bisset, 1994
Spain	herb	veterinary medicine	Rigat et al., 2018
	leaves	Expectorant	
	radix	snake bites	
Iran	leaves	anti-inflammatory, burn treatment	Jabbari et al., 2017
	herb	analgesic, uterus diseases, burn treatment, dental infections, diuretic, purgative, animal bites	
Bulgaria	fruit	diuretic, purgative, antiseptic	Kaya et al., 2019
	radix	Digestion	
Romania		rheumatism, diuretic	Chirigiu et al., 2011
Croatia	fruit	rheumatism, antipyretic	Pieroni et al., 2003
	seed / radix	liver diseases	Charlebois et al., 2010
Serbia	herb	anti-inflammatory, antioxidant	Popović et al., 2020
Germany	flower fruit	digestion, neuropathic pain, dental diseases	Bradley et al., 1992 Mahboubi, 2020
USA	flower	flu treatment, analgesic	Mahboubi, 2020

Topical treatments are frequently used for preventing or treating diseases locally. In this regard, gel systems have advantages such as high patient compliance and ease of use and preparation. For these reasons, drugs that can be topically applied are often preferred (Nirmal, Bakliwal, & Pawar, 2010). *In-situ* gel systems provide various advantages such as prolonged skin contact and ease of drug loading and dose adjustment. Biocompatibility of polymeric delivery systems is also clinically important (Kang & Singh, 2005). Gel systems can control and sustain drug release, which increases bioavailability, decreases side effects, reduces systemic absorption, and improves patient resistance by consolidating dosing frequency (Agrawal, Das, & Jain, 2012; Bhattacharjee, Beck-Broichsitter, & Banga, 2020).

In-situ gel formation involves several mechanisms, including pH change, ionic cross-linkage, and temperature modulation. Innovative polymeric systems represent a promising method of applying drugs, as these polymers transform from a solution into a gel state once administered (Nirmal et al., 2010; Khode & Dongare, 2019; Khule & Vyavahare, 2021). Thermosensitive polymers are probably the most studied class of environment-sensitive polymer systems in drug delivery research. A polymer is a solution at room temperature that gels at body temperature. Poloxamers usually have an efficient thermo-reversible property with a characteristic sol-gel transition temperature that is used widely in *in-situ* gelling systems (Güven, Berkman, Şenel & Yazan, 2019; Xie et al., 2019; Niyompanich, Chuysinuan, Pavasant, & Supaphol, 2021).

Topical delivery of *S. ebulus* is very suitable for antifungal treatment because the first-pass effect is preventable, and a topical application can directly target the skin. As a main physiological skin barrier, the stratum corneum limits the absorption of foreign materials into the body. (Güngör, Erdal, & Aksu, 2013; Rezaei-Moshaei et al., 2021). Therefore, the efficiency of topical treatment depends on penetration capability of the active agent through the *stratum corneum*. The formulation may play a key role in drug penetration into the skin, *in-situ* gel systems for topical antifungal treatment may improve skin penetration of active compounds (Hudson et al., 2010; Güngör et al., 2013) and, directly target lesions for maximum local therapeutic effect. Applying drugs in this way is beneficial for skin disorders where the treatment should ideally accumulate on the skin surface and not pass deeper so as to avoid systemic side effects (Erol et al., 2020).

This study proposes a new approach for the topical delivery of *S. ebulus* by employing *in-situ* gel formulations. The main aim is to develop and characterize a in situ gel formulation of *S. ebulus* that may have a potential to increase its topical efficacy and, to diminish systemic side effects.

MATERIALS AND METHODS

Chemicals and plant material

Poloxamer 407® and hydroxypropyl methylcellulose (HPMC; Sigma-Aldrich, Germany) are used as the *in-situ* gel formulation polymers. Glycerin (Sigma-Aldrich, Germany) is the preferred plasticizer. Benzalkonium chloride (Fluka, Germany) is used as the preservative, and distilled water has been pre-

ferred as the solvent. Plant material and fruit from *S. ebulus* have been collected from Andırın in Kahramanmaraş Province in June 2018. The voucher specimen was deposited at the Faculty of Pharmacy of Cukurova University Herbarium (CUEF 1671).

Extraction

The fresh fruit were divided into two groups. The first group was dried at room temperature in the shade, and then the dried material was extracted with a methanol-water (50:50; v/v) mixture using a shaker at 25°C for 24 hours. The procedure was repeated four times until the samples were exhausted. After filtration, the solvent was removed by rotary, and the water was removed by lyophilization. The extract (DFM) was stored at -20°C until analysis. The second group was squeezed.

Preparing the *in-situ* gel formulation

The *in-situ* gel formulations were prepared using the cold method (Güven et al., 2019). First, Poloxamer 407® was dissolved in distilled water at 13–20% (w/w) concentrations. Then, 1% (w/w) of the HPMC was added drop by drop into the polymer solution under magnetic stirring (500 rpm) at 4°C. The gels were left at 4°C until the poloxamer completely dissolved. Lastly, 0.005% benzalkonium chloride and 5% glycerin were added to the solution. The gel formulation compositions are provided in Table 3. All final formulations have been evaluated for their gelation temperature.

Physicochemical characterization of *in situ* gels

Formulation appearance

The clarity, visual appearance, and particle content of the gels were determined using optical tests under a dark background and rated as follows: turbid = +; clear = ++; and very clear = +++ (Okur, Yoltaş, & Yozgatlı, 2016). Accelerated stability studies were performed by exposing ideal formulations at various temperatures between 8 and 40°C. After a week of storage for assessing stability, the *in-situ* gel was then evaluated for its and physicochemical characteristics.

Gelation temperature measurement (Tsol-Gel)

To determine the gelation temperature, a glass test tube containing the gel formulation was put in a water bath (25°C) and warmed in 2°C increments. The temperature at which the flow of the formulation stopped upon turning over the container was measured as the gelation temperature. The temperature at which the solution was converted to a gel was determined using a thermometer placed in the test tube. The formulations were evaluated using the test tube tilting method between the temperatures of 25-40°C. The results are an average of three determinations (Xie et al., 2019). The study proceeded with the gelling systems that had undergone gelation at the appropriate temperature.

Measuring pH

The pHs of the prepared formulations were checked using a calibrated pH meter (WTW ProfiLab pH 597 Meter, Germany) at room temperature. All analyses were conducted in triplicate.

Gelling capacity

The formulations were dropped into 2 ml of distilled water, and the gelling time was visually recorded to determine gelling capacities. The code system on the table was used to determine the gel formation capacity (Yara, 2019).

Swelling Studies

Distilled water was used at 37±1°C for the formulation swelling studies. 1 ml formulation sample was put on a dialysis membrane and fixed to prevent leakage, after which the gel's weight was measured, noted, and reweighed after being kept in distilled water for a certain period. The following formulation was used to calculate the swelling rates (Güven et al., 2019):

Swelling ratio (t) = {[gel weight (t) - gel weight (t0)] / gel weight (t0)} x 100 (1)

Determining Rheological Properties

The rheology of the developed formulations was performed using a digital cone-plate rheometer (Brookfield, USA). The rheological tests were done at two different temperatures) (25±1°C and 37±1°C. Shear stress values were recorded by determining the difference in viscosity change and shear rate between the two temperature measurements as 0 and 2000 1/s (second) (Güven et al., 2019).

Table 3. Composition of the tested topical *in-situ* gel formulations.

Code / ingredients (%)	*Sambucus ebulus* L. extract	Poloxamer 407	HPMC	Glycerin	Benzalkonium chloride
P13	5	13	-	5	0.005
P13H1	5	13	1	5	0.005
P14	5	14	-	5	0.005
P14H1	5	14	1	5	0.005
P15	5	15	-	5	0.005
P15H1	5	15	1	5	0.005
P16	5	16	-	5	0.005
P16H1	5	16	1	5	0.005
P17	5	17	-	5	0.005
P17H1	5	17	1	5	0.005

Spreadability

Spreadability is the area over which the gel formulation diffuses per unit time (cm^2/min). The spreadability of the prepared formulations was determined using filter paper. A 1-ml calibrated pipette was fixed to the stand so that the last point of the pipette was 2 cm above the filter paper. The liquid formulation (0.1 ml) was dripped onto the middle of the filter paper. After a fixed period of time, the diameter of the surface area covered by the spreading of the formulation was determined and measured in triplicate (Chaudhary & Verma, 2014).

Accelerated stability

The optimized formulations were maintained at 40±2°C and 8±2°C for one month. Samples were withdrawn weekly and tested for pH, visual appearance, color, and gelling capacity (Mandal et al., 2012).

Statistical analysis

Data were shown as the mean ± *SD* (n = 3). Statistical data were analyzed using the Student's t-test with a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Preparing the *in situ* gel formulation

The thermosensitive *in-situ* gel formulations that were prepared using the cold method included Poloxamer 407 and HPMC. The cold method is a formulation technique favorable for use because it provides transparent formulations for gel systems and does not form the polymer globs that occur when the hot method is applied (Choi et al., 1998).

Poloxamer 407 has been demonstrated to have low side effects, perfect solubility, good drug release characteristics, and conformity with other formulation ingredients. This study uses Poloxamer 407 for its temperature-dependent gelation properties as a base for the semisolid drug delivery system with suitable sol-gel temperatures (36–38°C; Liu et al., 2019).

Poloxamer 407 and HPMC were selected as the polymers, as these polymers provide prolonged residence time at the application site (Nirmal et al., 2010). The neutral polymer, HPMC, was selected for its mucoadhesive properties, good swelling characteristic, low toxicity, and low irritancy (Liu et al., 2019).

Physicochemical characterization of *in situ* gels

Formulation appearance

The thermosensitive *in-situ* gel systems were visually evaluated in terms of color, transparency, and dissolution state in both solution and gel form. All *in-situ* gels showed good homogeneity without globs, and all formulations had sufficient clarity (Table 4).

Determining solution gelation temperature (Tsol-gel)

The transformation from solution to gel after applying the formulation is important for thermosensitive *in-situ* gel formulations. The impact of polymers on gelation temperature is based on chemical characterizations and the concentration in the formulations. The formulations were therefore examined to determine whether they had reached a sol-gel temperature suitable for topical application (Yuan et al., 2012).

Solutions containing less than 13% Poloxamer 407 did not form a gel over the evaluated temperature ranges. The gelation temperatures of poloxamer solutions containing 13-17% Poloxamer 407 and P407-HPMC mixtures ranged from 25-40°C, with several formulations gelling at body temperature (Table 4).

Gelation temperatures above 38°C saw the formulation remain a liquid at body temperature. Gelation temperatures of the *in-situ* gel lower than 25°C saw gelation occur at room temperature, leading to difficulty administering to the diseased area. Therefore, *in-situ* gel formulations with an average gelation temperature between 32°C-35°C are preferred, as they are likely to spread easily at room temperature but gel rapidly when in contact with skin (Liu et al., 2019).

Measuring pH

A pH meter was used to measure pH values to investigate the compatibility of the developed *in-situ* gel formulations with dermal surfaces. The pH of these formulations ranged between 6.6-6.8 (Table 5), which is close to the natural pH of the skin surface. The results of the pH analysis showed that the tested *in-situ* gel formulations to be compatible with the skin and to be unlikely

Table 4. Evaluation of the *in-situ* gel formulation data.

Code	Clarity	T (sol-gel,°C)
P13	+++	sol state
P13H1	++	38.4 ± 0.2
P14	+++	35.4 ± 0.1
P14H1	++	34.2 ± 0.1
P15	+++	32.8 ± 0.2
P15H1	++	30.8 ± 0.1
P16	+++	28.2 ± 0.2
P16H1	++	26.5 ± 0.2
P17	+++	25.1 ± 0.2
P17H1	++	gel state
P18	+++	gel state
P18H1	++	gel state

Table 5. Physicochemical properties of the selected formulations.

Code	pH	Gelling capacity	Spreadability (cm^2/min)
P14H1	6.6 ± 0.2	+++	6.76 ± 0.60
P15	6.8 ± 0.2	+++	4.84 ± 0.80

to exert any local irritation or inflammation on pH-sensitive skin (Yara, 2019; Salatin, Lotfipour, & Jelvehgari, 2020).

Gelling capacity

Gelling capacities of the formulations have been visually scored according to the following grades: (-) no gelation, (+) gelation after a few minutes followed by fast dissolution, (++) immediate gelation remaining for a few minutes, and (+++) immediate gelation remaining for nearly an hour. A high gelling capacity was obtained with the evaluated formulations. The gelling capacity data of the prepared formulations reveal the formulations to have immediate gelation that could be maintained for nearly an hour (Table 5). The inclusion of HPMC improved the gelling capacity of the formulations compared to Poloxamer 407 alone.

The gelling capacity is based on gelation time and dissolution time of the formed gel due to environmental properties. Thus, by increasing the concentration of the polymer, the transition time was decreased, and the dissolution time of the formed gel was extended (Gugleva et al., 2020).

Swelling studies

The *in-situ* gels were observed to be stable throughout the period of swelling (6 hours). The rate of swelling from P14H1 was slower than from P15 (Fig. 1). The results show the *in-situ* gels to be less swollen than the poloxamer gel alone due to the formation of very hard gels when HPMC is combined with the poloxamer (Güven et al., 2019).

Determining the rheological properties

The rheological behaviors of semisolid formulations are important for dosing, flowability, drug release, and patient compliance with topical applications. The selected formulations have been subjected to rheological studies. The rheograms of the formulations are shown in Figure 2. The *in-situ* gels have been demonstrated to display pseudo-plastic flow and shear-thinning rheological behaviors. The *in-situ* gel systems should not have a high viscosity when administered to the skin. Once administered, the *in-situ* gel formulation is desired to have a high viscosity in order to maintain contact with the area for a more extended period of time so as to provide a therapeutic effect (Öz et al., 2020).

Developed formulations were Newtonian below the gelation temperature (25°C), but achieved pseudo-plastic flow upon reaching body temperature, as was expected due to their thermosensitive properties (Gugleva et al., 2020). The formulations

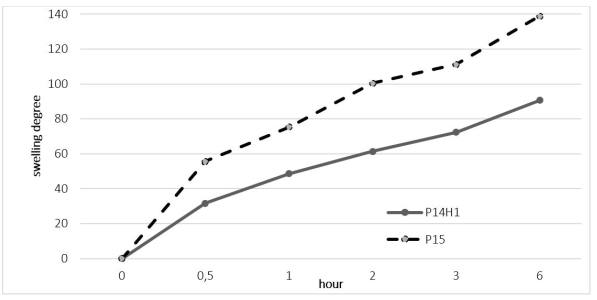

Figure 1. Swelling profiles of the selected formulations.

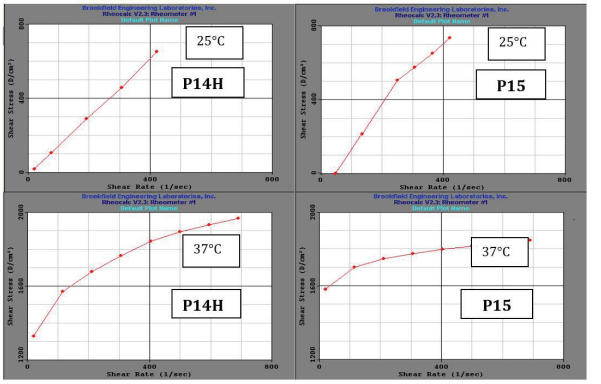

Figure 2. Rheograms of selected formulations (25°C and 37°C).

showed characteristic rheological responses of hard gels at physiological temperatures. This thermosensitive formulation showed Newtonian flow at 25°C and a rapid rise in viscosity at 37°C.

Spreadability

Spreadability is an important parameter for patient conformity and helps provide uniform gel application to the skin. High gel spreadability limits the time it can be spread on dermal surfaces. Spreadability refers to the distance traveled by the formulations before they transition to a gel. An increase in the poloxamer concentration reduces the spreadability of the formulations due to the reduced gelation temperature (Rençber et al., 2017). Table 5 shows the spreadability of formulations P14H1 and P15. Formulation P14H1 shows good spreadability compared to P15. Lastly, the two formulations both show good spreadability for topical application.

Accelerated Stability

Accelerated stability studies were performed by exposing the ideal formulations to various temperatures between 8 and 40°C. After a week of storagef, the *in-situ* gel was evaluated for its appearance and physicochemical characteristics. No major changes were observed regarding its physicochemical characteristics or appearance.

CONCLUSION

The topical treatment of cutaneous infections is preferred compared to oral treatments. The benefits of topical drugs include avoiding systemic adverse effects, directly targeting the site of infection, and high patient compliance. *In-situ* gel systems have superiorities such as providing easy topical application, increased residence time and prolonging drug release. In this study *in-situ* gels with *S. ebulus* was prepared for topical antifungal treatment and, focused on the characterization of thermosensitive *in-situ* gels containing the *Sambucus* extract as designed for topical administration. Among the 12 different *in-situ* gel formulations prepared using mixtures of Poloxamer 407 and 407-HPMC, the P14H1 and P15 formulations are seen to demonstrate an acceptable gelation temperature for dermal use.

The formulations have been characterized in terms of pH, gelling capacity, swelling degree, spreadability, and rheologi-

cal properties. Based on the conducted study, the following conclusions have been drawn. The gels that were prepared by using the cold method with the *Sambucus* extract show good physicochemical proportions. As a result, the Poloxamer 407-HPMC-based *in-situ* gel formulation containing the *Sambucus* extract was determined to be an efficient alternative for treating dermal fungal diseases. The developed formulation can extend the antifungal activity of the *Sambucus* extract for a longer period of time. Future controlled *in-vivo* trials are needed to evaluate the efficacy of the plant extract in formulations.

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Conflict of Interest: The authors have no conflict of interest to declare

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