

## PHARMACEUTICAL TECHNOLOGY

### QUALITY ANALYSIS OF SEMISOLID FORMULATIONS WITH THE LIQUID EXTRACT OF ELDERFLOWER (*SAMBUCUS NIGRA* L.)

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**Abstract:** The active ingredients in the liquid extract of elderflower are responsible for the antibacterial, antioxidant and anti-inflammatory effects of the plant. The main objective of the investigation was to design semisolid formulations with the elderflower extract and evaluate their quality using the biopharmaceutical research method *in vitro*. The quality of the elderflower (*Sambucus nigra* L.) extracts was evaluated spectrophotometrically by determining the total amount of polyphenols and flavonoids. The antiradical activity was determined spectrophotometrically by employing the DPPH free radical scavenging method. The agar diffusion method was used to determine antibacterial activity *in vitro*. The modified diffusion cells of Franz type were used to perform the biopharmaceutical experiment *in vitro* of semisolid pharmaceutical formulations. The results revealed that the highest amount of active compounds were found in the extracts when 70% (v/v) ethanol was used for the extraction of raw material. The obtained extracts had antiradical activity and antimicrobial effects against *S. aureus* and *B. cereus*. An inverse correlation was found between the total amount of flavonoids released after 6 hours *in vitro* experiment and the dynamic viscosity of the formulations. The base and the amount of excipients affected the release of active compounds from the formulations during the experiment. The results of the study showed that, as the lipophilicity and viscosity of the base increased, the performance in formulations slowed down. The released amount of active compounds decreased when oleogel concentration in the formulation increased. The hydrophilic base was confirmed as the best carrier of the liquid extract of elderflower.

**Keywords:** elderflower, extract, bigel, biopharmaceutical *in vitro*

The skin is the largest organ of the body that performs protective, sensory and thermoregulatory functions. Damage to the skin caused by temperature, various trauma, chronic ulcers or vein stasis, affects the skin, and it can become a colonization site for various bacteria (1). Various pathogenic microorganisms present on the skin can cause inflammatory tissue reactions, the body's immune response and the development of skin diseases (1, 2). In the case of skin infections, topical or systemic antibiotic treatment is commonly prescribed. With the use of these agents, skin hypersensitivity reactions, various allergies can develop, and normal skin microflora may be disrupted (1, 3). One of the major disadvantages of antibiotics use is the emergence of resistant microorganisms, especially when administered topically. Finding new effective antimicrobials

is crucial to address this issue. C. Hearst et al. (2010) carried out a study to investigate the antimicrobial activity of elderflower blossoms, leaves and berries. Researchers found that ethanol blossom extract had the strongest antimicrobial activity when compared to leaf and berry extracts. The blossom extract of *Sambucus nigra* L. stopped the growth of methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Salmonella poona* and *Pseudomonas aeruginosa* (4). The flavonoids in the plant have antibacterial properties due to their ability to combine complexly with extracellular, soluble proteins and thereby destroy the bacterial wall by interacting with enzymes responsible for the structure of the cell. Antibacterial effects may also occur due to the ability of the compounds to damage membrane by causing perforation or altering the membrane flow. For

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these reasons, the plant blossom extracts could be used as an active ingredient in semisolid forms for the prevention of skin infections (1). Phenolic compounds have antioxidant properties that inhibit the harmful effects of free radicals on the skin.

For these reasons, the extract of elderflower could be used as an active ingredient in the pharmaceutical forms of protective semisolids (5). The extract of elderflower contains flavonoids, which have an anti-inflammatory, blood-stimulating effect. Due to this reason, the extract, as an active ingredient, can be used to prevent cellulite, to reduce the severity of the lower extremities and to reduce fatigue when used externally (4). Because of these properties, it is important to simulate semisolid pharmaceutical forms with the active ingredient of the elderflower extract. The effectiveness of the semisolid preparation used on the skin depends not only on the condition of the patient's skin but also on the properties of the product, which are determined by the materials used in the production (6). It is important to select the proper base for the semisolid preparation, which not only ensures the stability of the drug substance in it but also performs the role of the drug substance carrier. Properly selected drug carriers should ensure the proper release of the drug substance from the substrate and therapeutic efficacy, as well as acceptable organoleptic properties. A modern patient usually chooses preparation which is easily removed from the containers, which is easily lubricated and absorbed quickly into the skin, leaving no visible, sticky residue on the skin and being easily rinsed. It is also important to model

a semisolid preparation with the elderflower extract of positive sensory properties, but also to study the influence of the carrier on the release of active compounds. We consider that gels and bigels are suitable carriers for the incorporation of the elderflower extract to ensure the positive organoleptic properties of the product and the release of the active compounds. Hydrophilic gels are easily distributed on the skin, have a cooling effect and moisturize the horny skin layer. Two-phase gels are capable of penetrating the lipophilic skin barrier, they can contain both hydrophilic and the lipophilic active substances (7). The purpose of our study was to model semisolid formulation with liquid extracts of the elderflower (*Sambucus nigra* L.) and to carry out their biopharmaceutical evaluation.

## EXPERIMENTAL

### Materials

Elderflower: LSMU Pharmacy, Lithuania; Ethanol 96.6%: Stumbras Company, Lithuania; Aluminum trichloride hexahydrate ( $\text{AlCl}_3 \times 6\text{H}_2\text{O}$ ): Sigma-Aldrich® Chemie GmbH, Germany; Carbormer 980: Fagron, USA; Triethanolamine: Appli-Chem GmbH, Germany; Liquid paraffin: Sigma-Aldrich® Chemie GmbH, Germany; Sorbitan monostearate (Span 60): Sigma-Aldrich® Chemie GmbH, Germany; Folin-Ciocalteu phenol reagent: Sigma-Aldrich®, Switzerland; Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ): Sigma-Aldrich®, France; Actonic acid = 99.8%: Sigma-Aldrich® Chemie GmbH, Germany; DPPH (2,2-diphenyl-1-picrylhydrazine): Sigma-Aldrich®,

Table 1. The composition of semisolid formulations (100 g).

Formulation	Elderflower liquid extract (mL)	Purified water (mL)	Span 60 (g)	Liquid paraffin (g)	Carbormer 980 (g)	Triethanolamine
N1	30	67	-	-	1	qs ad pH = 7
N2	20	77				
N3	15	82				
N4	30	18.5	7.5	42.5		
N5	20	28.5				
N6	15	33.5				
N7	30	38.5	4.5	25.5		
N8	20	48.5				
N9	15	53.5				
N10	30	58.5	1.5	8.5		
N11	20	68.5				
N12	15	73.5				

Germany; Rutin trihydrate: Sigma Aldrich®, Germany; Muller-Hinton agar: Oxoid, England; Bacterial cultures: Reference *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Pseudomonas aeruginosa* ATCC 27853, and Clinical *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*.

#### The production of liquid extracts of elderflower

Liquid extracts of elderflower are made by a maceration method. The extraction time is 5 days. The ratio of raw material and the extraction solvent is 1 : 1. Ethanol of 40, 70 and 80% (v/v) was used as the solvent in the production of the extracts.

#### The production of gels and bigels

First of all, carbomer hydrogels (N1-N3) were produced, in which the extract content was 15%, 20% and 30%. Triethanolamine was used as a carbomer-neutralizing agent (8). The production of bigels begins with the preparation of oleogel. The porcelain plate is filled with a suitable amount of liquid paraffin and heated on a water bath. While heating in a liquid paraffin an appropriate amount of Span 60 is dissolved. The mixture is heated to a homogeneous mass. The cooled oleogel is added to the carbomer gel containing the active ingredient. It is mixed up to a homogeneous mass.

In the process of the production of the bigels, the ratio between hydrogel and oleogel was 1 : 1 (N4 – N6), 7 : 3 (N7 – N9), and 9 : 1 (N10 – N12). The amount of the extracts in semisolid preparations was 15%, 20%, or 30%. The compositions of the designed hydrogels and bigels are presented in Table 1.

#### The determination of the total phenol content of elderflower extracts

The total amount of phenolic compounds is determined using the Folin-Ciocalteu reagent. All samples were analyzed with a spectrophotometer (Agilent 8453, Australia) at 765 nm wavelength. During the reaction, the phenolic compounds are capable of reacting with the Folin-Ciocalteu reagent to form blue complex compounds (9-11).

#### The determination of the total flavonoid content of elderflower extracts

The total amount of flavonoids is determined by reacting with  $AlCl_3$  in an acidic environment and measuring absorbance at 415 nm wavelength with the Agilent 8453 UV-Vis spectrophotometer (12). The total amount of flavonoids was determined according to rutin equivalents.

#### The determination of antiradical activity of elderflower extracts

The antiradical activity of extracts was determined by employing the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical binding method (13). To determine the antioxidant activity, a DPPH solution of 0.1 mmol/L was made in 96% ethanol 24 h before the study. The solution was stored in a dark, cool place all the time. Applying this method, 0.1 mL of the extract is mixed with 2.9 mL of DPPH solution and after 30 min the absorbance of the solution is measured with the spectrophotometer at the wavelength of 518 nm (14). In order to evaluate the antiradical activity, Equation 1 is used (15):

$$\text{Inactivated DPPH content (\%)} = \frac{A_0 - A_t}{A_0} \times 100 \quad (1)$$

Where  $A_0$  is the absorption of DPPH comparative solution,  $A_t$  is the absorption of the test solution.

#### Evaluation of antibacterial activity

The antibacterial properties of the elderflower extracts and semisolid formulations were evaluated *in vitro* using the agar diffusion method. Müller-Hinton agar was used (Mueller-Hinton agar Oxoid LTD (CM 0337), Basingstoke, Hampshire, England). *In vitro* studies were performed with the referent bacteria strains *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Pseudomonas aeruginosa* (ATCC 27853) and the clinical bacteria strains: *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*. For the positive control, there was 1% chlorhexidine (CHL) gel used and for negative on there was 70% ethanol used.

In accordance with the standard approved by the Clinical and Laboratory Standards Institute (CLSI), there was the fluid Mueller-Hinton agar prepared and it was poured in 10 cm diameter Petri dishes 35 mL each and left in the horizontal position to thicken. There were bacterial strains spread out on the surface of the thickened medium and there were 6 wells (of 7 mm diameter) made in every Petri dish, which were filled with 0.1 mL of elderflower extract, ethanol, semisolid formulation and 1% chlorhexidine gel. The plates were incubated for 24 h at 36°C. The antibacterial activity of extracts *in vitro* was evaluated after 24 h of cultivation, calculating the diameter of the transparent areas in millimeters, occurring around the wells. If the transparent area around any well did not appear, it has been concluded that the substance investigated has not got the bactericidal effect on the strain bacteria.

### The determination of the dynamic viscosity

The viscosity of the produced hydrogels and bigels was measured using a viscosimeter Vibro Viscometer SV-10 (A & D Company Ltd., Japan). The measurements were carried out at room temperature (16).

### The determination of the pH value

The pH values of the manufactured semisolids were determined using a pH meter, which is specifically used for determining the pH of semisolid pharmaceutical form (pH meter 766 with Knick SE 104N electrode) (17).

### Realese study of active compounds *in vitro*

The modified Franz-type diffusion cells were used by performing release studies *in vitro* of flavonoids from the experimental semisolid formulations with elderflower extract (12). The semisolid sample ( $1.00 \pm 0.02$  g) was placed into the cell with a dialysis membrane Cuprophan® (Medicell International Ltd., UK). The diffusion area was  $1.77 \text{ cm}^2$ . Thermostated ( $32 \pm 0.2^\circ\text{C}$ ) ethanol 70% (V/V) acted as the acceptor medium. The medium was stirred using a magnetic stirrer (IKAMAG® C-MAG HS7) (18). Samples from the acceptor solution were taken at 1, 2, 4, and 6 h and were immediately replaced with the same volume of fresh

acceptor solution. The quantity of flavonoids was determined using Agilent 8453 UV-Vis spectrophotometer at 415 nm wavelength (Agilent Technologies, Inc., Santa Clara, USA) according to rutin equivalents.

### Statistical analysis

All the trials were repeated three times. Statistical analysis was carried out by employing Microsoft Office Excel 2013 and IBM SPSS 20.0 programmes. The mean and standard deviations of the results were calculated, significance of differences in the results of the research was estimated by employing a single-factor dispersion analysis model (One-way ANOVA), the significance level is  $p < 0.05$ . The correlation of the results was estimated by the Spirmen's ranking correlation coefficient.

## RESULTS AND DISCUSSION

### The quality analysis of liquid extract of elderflower

In order to select a suitable extraction solvent for the extraction of active compounds from the elderflower, the solvents of 40%, 70%, and 80% (v/v) ethanol were used in the production of the extracts. Table 2 presents the research results of the elderflower liquid extract quality.

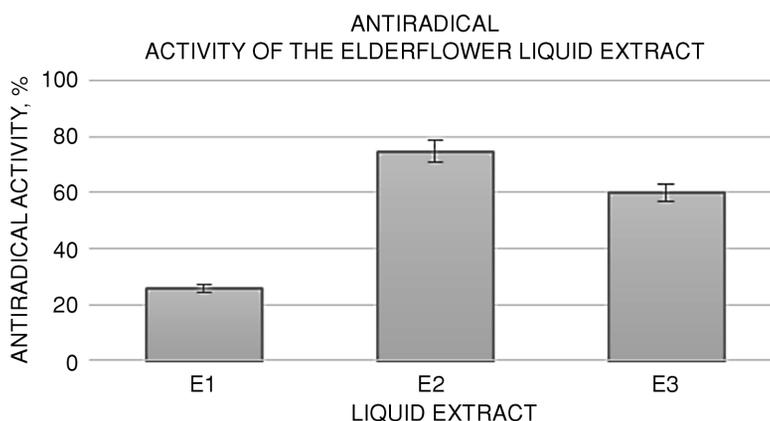


Figure 1. Antiradical activity (%) of elderflower liquid extracts by DPPH assay, (mean  $\pm$  SD,  $n = 3$ ).

Table 2. Quality research of the elderflower liquid extract.

Liquid extract	Extraction solvent (% v/v ethanol)	The ratio of raw materials and extraction solvent	The appearance of the produced extract	Total phenolic compounds (mg/mL)	Total flavonoid content (mg/mL)
E1	40		Brownish	$3.78 \pm 0.289$	$1.49 \pm 0.245$
E2	70	1 : 1	green, specific	$7.48 \pm 0.105$	$5.11 \pm 0.211$
E3	80		odour liquid	$5.21 \pm 0.227$	$4.10 \pm 0.149$

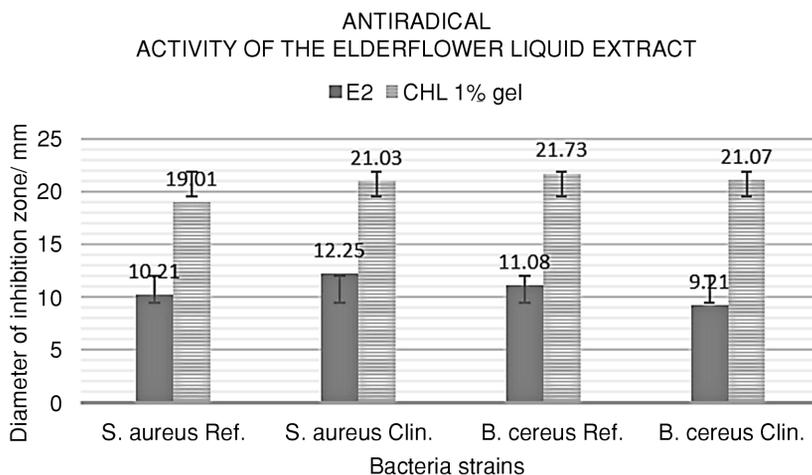


Figure 2. Evaluation of antibacterial activity (mean of the diameter of the transparent areas, mm  $\pm$  SD, n = 3) of elderflower extracts.

The results of the research revealed that the ethanol concentration had no effect on the appearance of liquid extracts; all the extracts had a brownish-green colour and a specific odour. The highest content of phenolic compounds and flavonoids was found in the extracts when 70% (v/v) ethanol was used for the extraction of the raw material. A statistically significant difference ( $p < 0.05$ ) was found among the total compounds in extracts E1, E2, and E3. While comparing the experimentally obtained results with the ones obtained by S. A. Socaci, the total amount of the flavonoids in extracts of the elderflower was 4.31 mg/mL and phenolic compounds – 5.43 mg/mL. The results obtained could vary due to the location of raw material growth, geographic or climate factors as well as an extraction technology (19).

#### Antiradical activity of elderflower liquid extract

In the process of planning the production of semisolid forms, which have a protective effect, one of the objectives of the study was to evaluate the antiradical activity of elderflower extracts. The results of the studies are presented in Figure 1.

The results are expressed as the percentage of inactivated DPPH. Extract E2 had the highest amount of flavonoids and phenolic compounds and exhibited the highest antiradical activity (Fig. 1). Compared to the extracts (E1, E3), which contained a lower total amount of active compounds, the percentage of inactive DPPH was lower, respectively. The Spearman ranking correlation coefficient between the amount of active compounds and the antiradical activity  $r = 1,000$  was determined. On the basis of the statistical analysis it can be stated that

the antiradical properties of the extracts are directly related to the amount of phenolic compounds and flavonoids contained in the extract. Based on the experimental data comparison with I. Stoilova et al. (2007), it was found that the extract of elderflower was inactivated by 97.7%. DPPH solution (9). A. L. Dawidowicz et al. (2006) also conducted a study to evaluate the antiradical activity of blossom, leaves and berries of elderflower using the DPPH radical binder method. The study showed that the highest antiradical activity was found in plant flowers, inactivated DPPH content in percentage  $91.95 \pm 0.12\%$ . The lowest inactivated DPPH content was found on the leaves –  $16.76 \pm 0.32$  percent (20). The obtained results of the study confirm the literature data that the extracts of elderflower have antiradical activity (9, 20).

In order to simulate the qualitative semisolid forms, the liquid extract E2 was selected as an active ingredient. This extract was characterized not only by the largest total amount of flavonoids, but also by the strongest antiradical properties. Based on the results obtained, the liquid extracts of elderflower as an active ingredient are suitable for the production of semisolid formulations that could be used to protect the skin against harmful effects of free radicals.

#### Antibacterial activity of the elderflower liquid extract

In order to evaluate the quality of the elderflower liquid extract (E2) antibacterial activity determination *in vitro* was performed using diffusion to agar method. The results are presented in Figure 2.

The data presented (Fig. 2) reveals that E2 extract had antibacterial effect. In the wells where the E2 extract was added, zones of different diame-

ter were visible, depending on the type of bacteria. Extract E2 was probably characterized by suppressing effect on the bacterial inhibitors *S. aureus* and *B. cereus* growth. It was also found that the extract had no effect on the growth of *P. aeruginosa* bacteria. Reference and clinical *S. aureus* strains of extract E2 showed similar activity. There was no statistically significant difference between the clinical and referential strain growth inhibition ( $p > 0.05$ ). Used as a control 70% ethanol did not inhibit the growth of bacteria that were tested. When comparing the obtained results with the ones obtained by C. Hearst et al. (2010), the study found that the elderflower extract inhibited the growth of the *S. aureus*, *B. cereus* bacteria (4). The results coincid-

ed with the data obtained in the study and confirmed that the extract from the elderflower inhibited the growth of bacteria *S. aureus* and *B. cereus*. Since skin infections are often associated with *Staphylococcus* infections, and when the immunity and the natural skin protection barrier are weakened, the skin can also be damaged by *B. cereus* microorganisms, and, thus, the extract of elderflower, as an active ingredient, is suitable for the production of preparations with antibacterial properties (1, 2).

#### Assessment of the physical properties

In order to produce high quality and acceptable semisolid forms for the consumer, an assessment of their physical properties is performed.

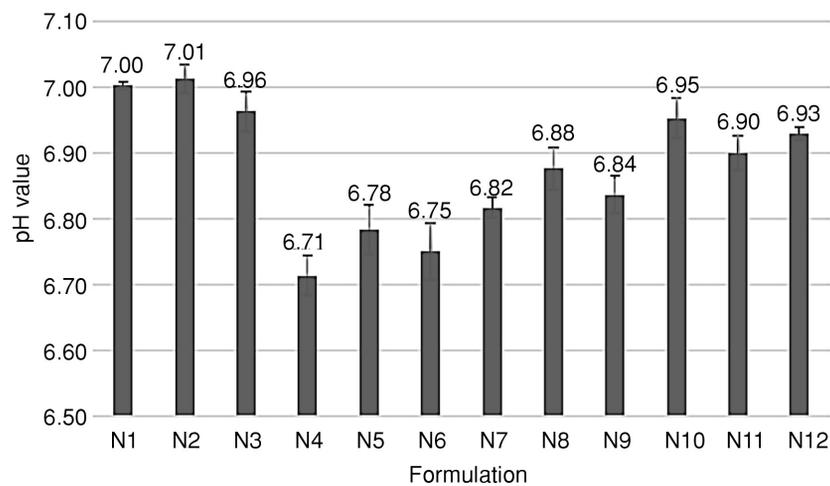


Figure 3. pH values of the formulated gels and bigels (mean  $\pm$  SD,  $n = 3$ ).

Table 3. The results of the physical properties of semisolid formulations.

Formulation	Colour	Odour	Phase separation after production and after a month
N1	Brown	Pleasant	Not present
N2			
N3			
N4	White	Specific	Not present after production, present after a month
N5			
N6			
N7	Yellowish	Pleasant	Not present
N8			
N9			
N10	Yellow		
N11			
N12			

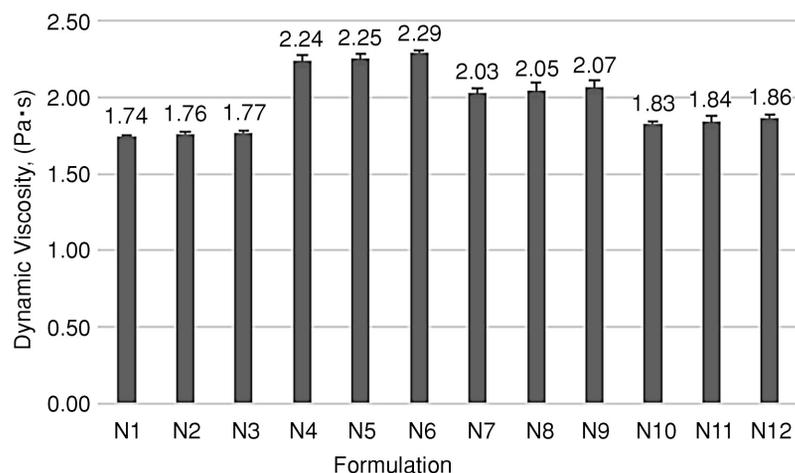


Figure 4. The results of dynamic viscosity (Pa·s) of semisolid formulations with liquid elderflower extract, (mean  $\pm$  SD, n = 3).

Table 4. Correlation coefficients between released total flavonoids and dynamic viscosity of semisolid preparations.

Formulations	Spearman's rank correlation coefficient
N1, N4, N7, N10	r = - 0.809
N2, N5, N8, N11	r = - 0.909
N3, N6, N9, N12	r = - 0.944

It is evident from the data obtained (Table 3) that the colour of biphasic gels depends on the amount of oleogel, and as it increases, the colour of the preparation becomes brighter. Hydrogels (N1 – N3) and bigels (N7 – N12) have a pleasant odour. Meanwhile, biphasic gels containing a 1 : 1 ratio of hydrogel and oleogel have a specific odour.

Semisolid forms should be characterized by proper functioning: the preservation of the homeostasis of skin barrier, the integrity of the horny layer, antimicrobial protection and skin anti-irritation (21). The standardized formulations should have a pH value similar to that of the skin: from 4.5 to 7 (22). When the pH value of the preparation is 9 – 10, the skin is irritated, the protective barrier properties are lost, and appropriate conditions for bacterial reproduction are created (23). The production of semisolid formulations with the elderflower extract has a purpose that a pH value of produced preparations would ensure their safe use on the skin. The results obtained are presented in Figure 3.

The results have shown (Fig. 3) that the hydrogels (N1 – N3) had the highest pH values. A similar

pH was found in semisolid forms, with a ratio of a hydrogel to an oleogel of 9 : 1 (N10 – N12). A lower pH value was observed in biphasic gels (N7 – N9) containing a 7 : 3 ratio of hydrogel to oleogel. The lowest pH value was found in biphasic gels (N4 – N6) with a 1 : 1 ratio of phases. The results shown in Figure 3 reveal that the increase in the oleogel content in the semisolid form significantly decreases the pH value of the preparation ( $p < 0.05$ ). When evaluating the effect of the extract on the pH value of the formulations produced, no statistically significant difference was found ( $p > 0.05$ ). For this reason, it can be stated that the amount of the inserted extract of elderflower preparation has no influence on the pH value. Scientific literature states that preparations with neutral or acidic pH (pH = 7) are safer than those that are alkaline. Skin semisolid preparations with an alkaline pH can cause swelling of the skin, affect the lipid barrier of the skin, increase the likelihood of skin conditions such as candidiasis, atopic dermatitis, and acne (21). The pH value of all the semisolid preparations produced with the liquid extract of elderflower ranged from 6.71 to 7.01, therefore they are suitable for the use on the skin, avoiding irritant effects.

The viscosity of semisolid forms affects opacity of the product, contact with the skin, release of the active compounds from the formulation and penetration through the skin (24). For these reasons, it is advisable to determine the viscosity of the simulated preparations. The results of the dynamic viscosity of the produced hydrogels and bigels are presented in Figure 4.

The results presented in Figure 4 show that the highest dynamic viscosity was observed in bigels

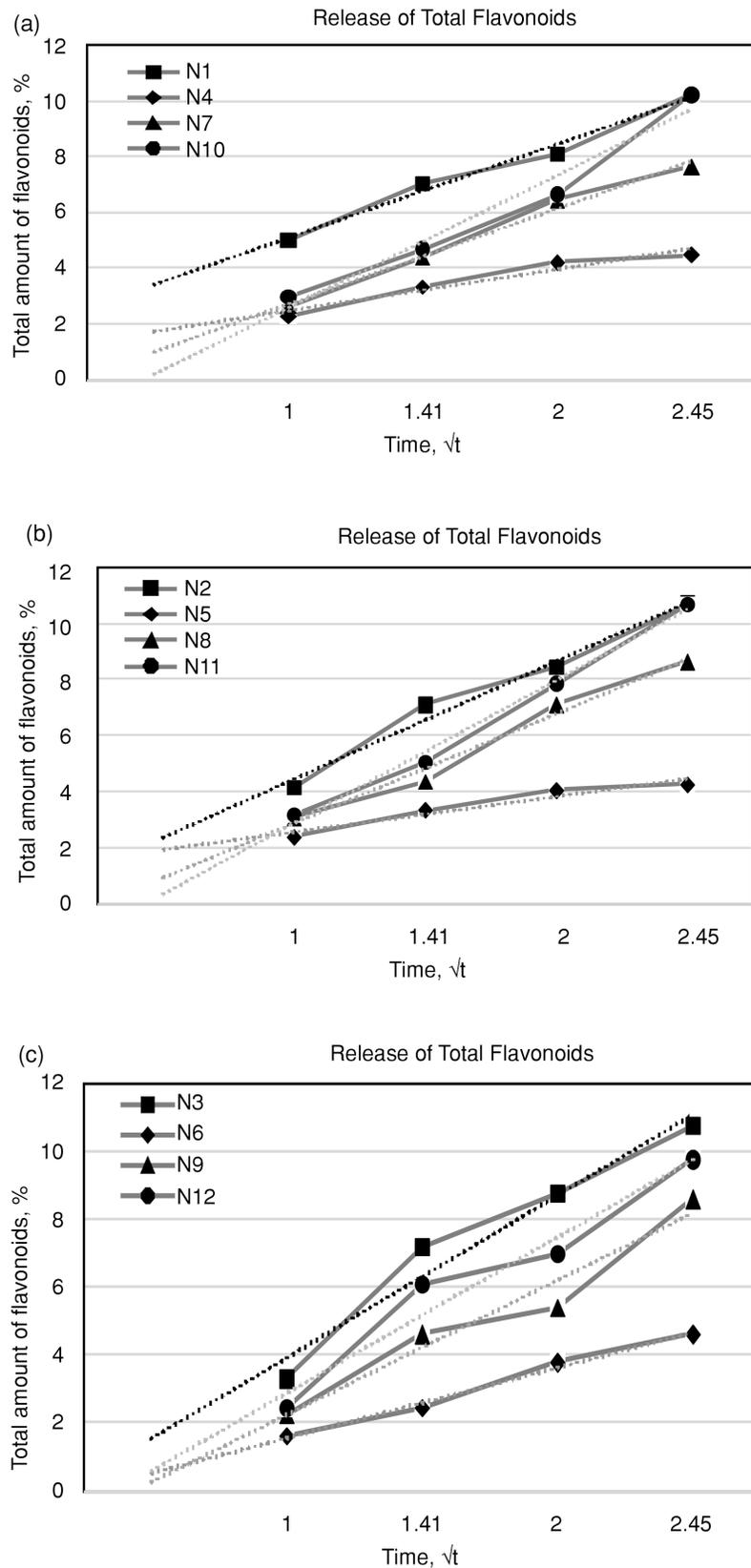


Figure 5. The release of total flavonoids (%) according to the Higuchi mathematical model; (a) formulations N1, N4, N7, N10; (b) formulations N2, N5, N8, N11; (c) formulations N3, N6, N9, N12.

N4 – N6 containing 1 : 1 oleogel-hydrogel proportion. Low viscosity was observed in gels N7 – N9, in which the amount of hydrogel and oleogel was 7 : 3. The minimum dynamic viscosity of bigels was found in gels N10 – N12. The lowest viscosity of all the formulations containing the liquid extract of elderflower was determined in hydrogels N1 – N3 from. Statistically viscosity significantly ( $p < 0.05$ ) increases with the increasing oleogel amount in the bigel.

In order to determine whether the amount of the extract introduced influences the viscosity of the products, the statistical analysis of the results have shown that there is no statistically significant difference ( $p > 0.05$ ) between the viscosity of the formulations, where the content of the base is the same, but the amount of the extract is different. Based on the presented results it can be stated that the amount of extract in the formulation has no significant effect on the dynamic viscosity. The results of the research confirmed the data published in the scientific literature that the viscosity of bigels depends on the amount of oleogel: the increase in the oleogel content in the bigel increases the dynamic viscosity (25).

#### *In vitro* study

After the biopharmaceutical release study it was found that the highest amount of flavonoids is released from hydrophilic gels, while the amount of released flavonoids decreases with the increasing oleogel content in a bigel. According to the linearity of the obtained Higuchi curves (Fig. 5), it can be stated that the release of flavonoids from the semisolid formulations was influenced by the diffusion process of the compounds at the base (26, 27).

An inverse correlation between the total amount of released flavonoids and the dynamic viscosity of semisolid forms was calculated by statistical analysis. Table 4 presents the calculated ratios of Spearman's rank correlation coefficients for semisolid forms depending on the amount of the extract they contain.

A strong reciprocal correlation was found between the total amount of flavonoids released during the *in vitro* study and the dynamic viscosity of

the preparations. From the results presented in Table 4, it can be argued that the viscosity of the carrier may influence the results of *in vitro* studies. As the viscosity increases, the particle diffusion process decelerates and the active compounds are released more slowly. Based on the *in vitro* release results, it can also be said that the amount of the released compounds depends on the oleogel-hydrogel ratio in the bigels. As the amount of oleogel increases in bigels, the reduced total amount of flavonoids decreases. The results of the study revealed that the therapeutic efficacy of semisolid formulations depends not only on the active ingredient but also on the chosen carrier.

Mathematical analysis of the kinetic profile of the flavonoids for the release study immediately after production of formulation showed that the Higuchi model regression coefficients for formulations N1, N4, N7, N10 (Fig. 5 (a)) were 0.9842, 0.9662, 0.9898 and 0.9440, for formulations N2, N5, N8, N11 (Fig. 5 (b)) were respectively, 0.9803, 0.9914, 0.9799, 0.9320 and for formulations N3, N6, N9, N12 (Fig. 5 (c)) were respectively 0.9563, 0.9533, 0.9527 and 0.9892.

The performed experimental studies confirm that the importance of biopharmaceutical science in the implementation of pharmaceutical modeling plays an important role in the development process of the preparation (28). This study shows how properties of the active substance, viscosity of the formulations, excipients and their amounts affect the *in vitro* release of the active compounds. The research helps to not only select the most suitable auxiliary substances, but also to monitor how the amount of active compounds release over time intervals (29). The goal of antibacterial study *in vitro* was to evaluate whether the active substances released from formulations can cause biological effects.

The data in the Table 5 shows that the formulations N1 and N10 have inhibited the growth of on the tested microorganisms *in vitro*. The research results may be caused by the quantity of active compounds released from semisolid formulations in order to cause the antibacterial effect for bacteria. Thus, our test results revealed that the antibacterial activity of semisolid preparations depends not only on the

Table 5. Evaluation of antibacterial activity (mm) of formulations with elderflower liquid extracts, (mean  $\pm$  SD, n = 3).

Formulations	The zone of inhibition (mm $\pm$ SD)					
	N1	N2	N3	N10	N11	N12
Staphylococcus aureus ATCC 25323	7.10 $\pm$ 0.55	0	0	5.6 $\pm$ 0.48	0	0
Bacillus cereus ATCC 8035	7.43 $\pm$ 0.12	0	0	5.49 $\pm$ 0.23	0	0

carrier properties, but also on the active compounds amounts in the formulation. It is noteworthy that formulation with 15% and 20% liquid extract have not inhibited the growth of bacteria. The study results confirmed data presented in the literature that elderflower preparations have antibacterial effects (1, 2). The test results showed that hydrophilic base and bigel with the smallest amount of oleogel are suitable for introduction of elderflower extract, because the tested formulations (N1, N10) have antibacterial activity.

## CONCLUSION

The highest total content of phenolic compounds and flavonoids was found in extracts when 70% (v/v) ethanol was used in production and the lowest content was found when 40% (v/v) ethanol was used. This shows that the extraction solvent is an important technological step in the production of extracts in order to efficiently extract the active compounds from the plant raw material. The *in vitro* results of a biopharmaceutical semisolid formulation study showed that the base and the amount of excipients affected the release kinetics of the active compounds from the formulations. The results of the study showed that, as the lipophilicity and viscosity of the base increased, the performance in formulations slowed down. The released amount of active compounds decreased when oleogel concentration in the formulation increased. The best carrier of elderflower liquid extract was confirmed the hydrophilic base.

## Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

This study was totally funded by a grant from the University.

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*Received: 25.09.2019*