APPLICATION OF DRY HAWTHORN (CRATAEGUS OXYACANTHA L.) EXTRACT IN NATURAL TOPICAL FORMULATIONS

ADA STELMAKIENE1*, KRISTINA RAMANAUSKIENE1, VILMA PETRIKAITE2, VALDAS JAKSTAS3 and VITALIS BRIEDIS1

1Department of Clinical Pharmacy, 2Department of Drug Chemistry, 3Department of Pharmacognosy, Lithuanian University of Health Sciences, Mickevicius 9, Kaunas, Lithuania

Abstract: There is a great potential for a semi-solid preparation for topical application to the skin that would use materials of natural origin not only as an active substance but also as its base. The aim of this research was to model semisolid preparations containing hawthorn extract and to determine the effect of their bases (carriers) on the release of active components from experimental dosage forms, based on the results of the in vitro studies of the bioactivity of hawthorn active components and ex vivo skin penetration studies. The active compounds of hawthorn were indentified and quantified by validated HPLC method. The antimicrobial and anti-radical activity of dry hawthorn extract were evaluated by methods in vitro. The penetration of active substances into the full undamaged human skin was evaluated by method ex vivo. Natural topical composition was chosen according to the results of release of active compounds. Release experiments were performed with modified Franz type diffusion cells. B. cereus was the most sensitive bacteria for the hawthorn extract. Extract showed antiradical activity, however the penetration was limited. Only traces of hyperoside and isoquercitrin were founded in epidermis. Protective topical preparation with shea butter released 41.4-42.4% of active substances. Four major compounds of dry hawthorn extract were identified. The research showed that extract had antimicrobial and anti-radical activity, however compounds of hawthorn stay on the surface of the undamaged human skin. Topical preparation containing beeswax did not release active compounds. Beeswax was identified as suspending agent. Topical preparations released active compounds when shea butter was used instead of beeswax.

Keywords: hawthorn, natural, release, skin penetration, topical

The base of semisolid preparations is an active factor that may affect the release of the drug substances from a pharmaceutical form and their absorption through biological membranes, which in turn may have an effect on the pharmacokinetic parameters of a pharmaceutical preparation (1, 2). The base composition of a semi-solid preparation affects its stability and drug release kinetics. As products from natural materials are becoming increasingly popular, scientists and manufacturers face a challenging task of creating stable products that meet modern requirements by using materials of natural origin (2). There is a great potential for a semi-solid preparation for topical application to the skin that would use materials of natural origin not only as an active substance but also as its base. When modeling an ointment basis, the following components were selected: beeswax, shea butter, cholesterol, cocoa butter, honey, avocado and olive oils (3).

Dry hawthorn (Crataegus oxyacantha L.) extract was used as an active substance for formulations modeling. Crataegus spp. is a member of the rose family which has been used for its medical properties since ancient times. It is well known that bioactivity of hawthorn is associated with the treatment of cardiovascular diseases (4-7), however recently, a broader spectrum of the effect of hawthorn bioactivity has been found. Hawthorn extracts were incorporated into semisolid drug forms (8). Crataegus spp. is indeed one of the species that is highly recommended in folk medicine, being regarded as particularly important in the management and prevention of age-related diseases (for instance, cardiovascular disease, atherosclerosis, arthritis, and hypertension), nervous system disorders (such as migraines, confusion, irritability and memory loss) and treatment of upper respiratory infections, cellulite, obesity and menopause disturbances (9, 10). The juice of its fruits is a topical preparation for skin application that relieves pain and stiffness (10). C. monogyna extracts have been verified to be highly effective against Candida albini-
cans and Herpes simplex virus (11). However, no effect of hawthorn extracts on C. albicans was observed by other authors (4). The O-glycosidic flavonoids and the oligomeric proanthocyanidins from Crataegus sinaica exhibited significant inhibitory activity against Herpes simplex virus type 1 (HSV-1), which was shown to be due to an extracellular mechanism for procyanidin C-1 (12). Extracts of Crataegus spp. have anti-inflammatory, gastro-protective and antimicrobial properties (4, 13-15). Antioxidant, radical-scavenging activity of different parts of hawthorn have been evaluated by a number of authors (16-19).

Research has shown that C. monogyna extract can be used as a photoprotective agent, which provides stronger protection against UVA than UVB radiation (20). Crataegus monogyna has been shown to be cytoprotective by scavenging free radicals. Jalali et al. study has shown that Crataegus monogyna fruits aqueous extract with antioxidant properties could serve as a protective agent against reproductive toxicity during cyclophosphamide treatment in a rat model (21). Furthermore, Crataegus pinnatifida extract significantly inhibited the generation of reactive oxygen species and the phenomena of inflammation induced by TPA. Also, extract inhibited benzoprene/TPA-induced skin tumor formation and decreased the incidence of tumor (22). The extracts obtained from C. monogyna parts revealed antiproliferative activity associated with cancer progression and development, such as cell proliferation, apoptosis, cell differentiation and neovascularization, and did not show toxicity for non-tumor cells (23). Other authors also found antitumor activity of Crataegus spp. demonstrated on various cell lines (24).

There are little data published in the scientific literature about the use of hawthorn extract in the production of topical preparations. The literature search did not yield any data pertaining to the skin penetration of the active components of hawthorn extract or the effect of carriers on the release of active components from topical preparations. The physical and chemical properties of the drug substance in the topically applied pharmaceutical forms determines the permeation of a given drug substances through the skin from applied carrier matrix (25). Topical bioavailability of a drug depends on the amount of drug diffusing from the dosage form and reaching the surface of the skin where it can be absorbed. Thus, it is relevant not only to produce stable semisolid preparations but also to study the skin penetration of hawthorn active components and to select suitable carriers that would ensure a proper release of active compounds.

The aim of this research was to model semisolid preparations containing hawthorn extract and to determine the effect of their bases (carriers) on the release of active components from experimental dosage forms, based on the results of the in vitro

Figure 1. Chemical structure of active compounds of hawthorn flowers and leaves extract. A - chlorogenic acid; B - vitexin-2'-O-rhamnoside; C - hyperoside; D - isoquercitrin
studies of the bioactivity of hawthorn active components and ex vivo skin penetration studies.

MATERIALS AND METHODS

Materials

Vitexin-2'-O-rhamnoside, HPLC grade acetonitrile and acetic acid were purchased from Sigma Aldrich Chemie GmbH (Steinheim, Germany). Hyperoside, isoquercitrin and chlorogenic acid were purchased from Carl Roth. Standardized dry hawthorn extract was purchased from Naturex (France).

Methods

Preparation of hawthorn extract for analysis

The hawthorn leaf and flower dry extract was dissolved in purified water at the ratio 1 : 20 (w/v) by mixing it with a magnetic stirrer for one hour at room temperature.

HPLC

Amounts of hawthorn active components were evaluated by HPLC method with Agilent 1260 Infinity Capillary LC System (Agilent Technologies, Santa Clara, USA) using the Agilent diode array detector (DAD) and applying validated HPLC method for quantification. The separation was performed in C18 ACE (5 µm) 150 × 0.5 mm i.d. column. The mobile phase consisted of the solvent (A) 0.5% aqueous acetic acid (v/v) and (B) acetonitrile, using gradient elution of 10% (B) at 0-3 min, 14% (B) at 3.1-25 min, and 70% (B) at 26-29 min and then returned to the initial conditions with 10 min re-equilibration, with total 40 min run time. The analysis was carried out at a flow rate of 10 µL/min with the detection wavelength set at 340 nm.

Total phenolic content

Total phenolic content was determined according to the method of Ramanauskiene et al. (26). The quantity (n = 3) of phenolic compounds was determined using Agilent 8453 UV-Vis spectrophotometer (Agilent Technologies, Inc., Santa Clara, USA) according to p-coumaric acid equivalents after reaction with Folin-Ciocalteu phenol reagent.

Antiradical scavenging activity

A 0.1 mmol/L DPPH solution was prepared in 96% ethanol. The solution was kept in a cool, dark place for 24 h. The reaction (n = 3) was initiated in a cuvette to which 2.9 mL of DPPH solution and 0.1 mL of the analyzed solution were added. The absorbance of the reaction mixture was measured at the wavelength of 518 nm 30 min after the start of the reaction. The results were expressed in percentage of bound DPPH according to Molyneux (27).

Antimicrobial activity

The microbiological examination (n = 5) was performed in aseptic conditions. During the microbiological study, the antimicrobial activity of the studied preparation was determined using solid growth media and the well technique. Resistance to preparations from natural material was examined in Mueller-Hinton agar (Mueller-Hinton Agar II, BBL, Cockeysville, USA) with standard cultures of Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 12459, Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 12228, Escherichia coli ATCC 25922, Bacillus cereus ATCC 8035 and Candida albicans ATCC 60193. Agar was poured into sterile Petri dishes 85 mm in diameter (20 mL in each dish). Agar wells were 7 mm in diameter and 8 mm deep. The density of microorganism suspensions applied in the test was 0.5 Mac Farland standard scale (5.107 n 1.108 CFU/mL). The density of the bacterial suspension in the Mueller-Hinton agar was 106 CFU/mL. After the agar solidified, wells were formed in the medium and were filled with the studied preparation. The cultures were incubated for 24 h in a thermostat at
37°C, and then the microorganism growth in the whole agar volume was evaluated.

**Rheological flow behavior test**

The test (n = 5) was performed using the Carri-med CSL 2 500 rheometer (TA Instruments, USA), by applying the cone-and-plate geometry system (cone diameter = 60 mm, angle = 2°, sample thickness = 150 µm). The shear rate was increased for 2 min from 0 to 500 s⁻¹. Rheological characteristics were calculated according to the Ostwald de Waele’s mathematical model.

**In vitro release study**

**In vitro** release experiments (n = 3) were performed using the modified Franz type diffusion cells. The semisolid sample (1.00 ± 0.02 g) was placed into these cells with a dialysis membrane. The Cuprophan® dialysis membrane (Medicell International Ltd., London, UK) was made of natural cellulose. Area of the diffusion was 1.77 cm². Purified water was used as an acceptor medium. The temperature of acceptor medium was kept on 37 ± 0.2°C. The medium was stirred using magnetic stirrer. The samples from the acceptor solution were taken at 1, 2, 4, 6 h and immediately replaced with the same volume of fresh acceptor solution.

**Penetration experiment through the full-thickness undamaged human skin**

Abdominal skin of Caucasian women (age range: 25-40 years) was obtained from the Department of Plastic and Reconstructive Surgery (the Hospital of the Lithuanian University of Health Sciences, Lithuania) after cosmetic surgery. It was stored at -20°C for not longer than 6 months before use. Kaunas Regional Biomedical Research Ethics Committee has approved the use of human skin for transdermal penetration studies. A Bronaugh-type flow-through diffusion cell with full-thickness human skin was used for ex vivo skin penetration experiments (n = 3). The experiment was performed according to the methods proposed by Kezutyte et al. and Zilius et al. (25, 28, 29).

Statistical data evaluation was performed using the SPSS software with one-way ANOVA. Tukey’s post hoc test was performed for multiple comparisons. Level of significance was determined as p < 0.05.

**RESULTS AND DISCUSSION**

The experimental research found phenolic compounds of different classes in the standardized extract of dried hawthorn leaves and flowers: phe-

<table>
<thead>
<tr>
<th>Analyzed sample</th>
<th>Total phenolic content based on p-coumaric acid equivalent, mg/g</th>
<th>Chlorogenic acid, mg/g</th>
<th>Vitexin-2’-O-rhamnoside, mg/g</th>
<th>Hypersoside, mg/g</th>
<th>Isoquercitrin, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry hawthorn extract</td>
<td>92.00 ± 0.53</td>
<td>7.91 ± 0.52</td>
<td>23.00 ± 1.38</td>
<td>3.65 ± 0.43</td>
<td>1.95 ± 0.89</td>
</tr>
</tbody>
</table>

Figure 3. Inhibition zones (mm) of bacterial growth including well diameter (7 mm)
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nolic acid, flavone-C-glycoside, flavonol-O-glycoside. The prevailing compounds, the chemical structure of which is provided in Figure 1, were quantitatively assessed in the hawthorn extract.

A typical chromatogram of active components of hawthorn dried leaves and flowers extract was obtained (seen in Fig. 2), based on the adapted and validated HPLC method. The quality of the dry hawthorn extract was assessed by measuring the amounts of prevailing active components as well as the total phenolic content (Table 1). It has been determined that the hawthorn leaf and flower extract contained the highest levels of vitexin-2"-O-rhamnoside.

While there are research data indicating that hawthorn extracts show antimicrobial activity, the activity against specific microorganisms varies between different authors (4, 14, 15). The aforementioned differences are probably determined by different hawthorn species analyzed, different hawthorn plant materials as well as different solvents. The antimicrobial research carried out by the authors of this study analyzed an aqueous solution of *Crataegus oxyacantha* dried flowers and leaves extract. The results of the antimicrobial activity *in vitro* studies showed that the active components of the hawthorn extract possess antimicrobial activity. Seven microorganisms were analyzed during the antimicrobial study. The extract of hawthorn flowers and leaves inhibited the growth of *S. epidermidis*, *B. cereus*, *E. coli* and *S. aureus*, whereas *P. mirabilis*, *P. aeruginosa* and *C. albicans* remained resistant. *B. cereus* showed the highest degree of sensitivity (the widest zone of inhibition) to the hawthorn extract (Fig. 3).

Different methods can be found in scientific literature to determine antiradical and antioxidant activity of hawthorn extract (14, 16-19). Differences in values of DPPH antiradical activity occur as a result of methodological differences. The study results of antiradical activity showed that the hawthorn extract possesses antiradical activity (Fig. 4). The binding of the free radical DPPH depends on the concentration of compounds: the greater amount of phenolic compounds leads to more powerful radical binding effect.

The results of the bioactivity tests have confirmed the data reported in scientific literature (4, 16) that hawthorn extract is a suitable component for topical preparations due its antimicrobial and antioxidant properties.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chlorogenic acid</th>
<th>Vitexin-2&quot;-O-rhamnoside</th>
<th>Hyperoside</th>
<th>Isoquercitrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard mix and taped skin</td>
<td>-</td>
<td>-</td>
<td>tr</td>
<td>-</td>
</tr>
<tr>
<td>Standard mix</td>
<td>-</td>
<td>-</td>
<td>tr</td>
<td>tr</td>
</tr>
</tbody>
</table>

tr = traces

Figure 4. Antiradical activity (n = 3) of the total phenolic content of hawthorn based on the binding of DPPH radical.
Ex vivo penetration experiment was conducted using full-thickness undamaged human skin to determine the capacity of hawthorn active compounds for skin penetration. The study results (Table 2) showed that the epidermis acts as a permeability barrier; no analyzed compounds were found in the dermis. After 24 h of testing, it was determined that the more lipophilic hyperoside and isoquercitin accumulate in the epidermis but their amounts are below the limit of quantification (Table 2). This can be associated with the fact that the solubility of hyperoside and isoquercitin is higher in a lipophilic environment than in a hydrophilic one (logP is 0.4), as compared to the predicted hydrophilic properties of chlorogenic acid and vitexin-2′-O-rhamnoside because their logP is -0.4 and -1.3, respectively. The permeation test results showed that the hawthorn active compounds do not permeate across the undamaged human skin and their activity is limited to the surface of the skin.

Table 3. Compositions of modelled natural topical formulations (g).

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample no.</th>
<th>Beeswax</th>
<th>Honey</th>
<th>Butter cacao</th>
<th>Cholesterol</th>
<th>Avocado/Olive oil</th>
<th>Shea butter</th>
<th>Dry hawthorn extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>N1</td>
<td>5.0</td>
<td>10.0</td>
<td>10.0</td>
<td>3.0</td>
<td>72.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>7.0</td>
<td>10.0</td>
<td>10.0</td>
<td>3.0</td>
<td>70.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>9.0</td>
<td>10.0</td>
<td>10.0</td>
<td>3.0</td>
<td>68.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>N4</td>
<td>11.0</td>
<td>10.0</td>
<td>10.0</td>
<td>3.0</td>
<td>66.0</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>N5</td>
<td>13.0</td>
<td>10.0</td>
<td>10.0</td>
<td>3.0</td>
<td>64.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>N6</td>
<td>15.0</td>
<td>10.0</td>
<td>10.0</td>
<td>3.0</td>
<td>62.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>N7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>98.0</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>N8</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>97.5</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>N9</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>97.0</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>N10</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>96.5</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>N11</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>96.0</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>N12</td>
<td>3.0</td>
<td>-</td>
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<td>-</td>
<td>95.0</td>
<td>-</td>
<td>2.0</td>
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<tr>
<td></td>
<td>N13</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>93.0</td>
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<tr>
<td></td>
<td>N14</td>
<td>-</td>
<td>10.0</td>
<td>10.0</td>
<td>3.0</td>
<td>60.0</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td>III</td>
<td>N15</td>
<td>-</td>
<td>10.0</td>
<td>10.0</td>
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<td>60.0</td>
<td>15.0</td>
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<td></td>
<td>N16</td>
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<td>60.0</td>
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<td>2.0</td>
</tr>
<tr>
<td></td>
<td>N17</td>
<td>1.0</td>
<td>10.0</td>
<td>10.0</td>
<td>3.0</td>
<td>60.0</td>
<td>14.0</td>
<td>2.0</td>
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</table>
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This is determined by their lipophilicity and skin layer properties. In order to determine whether the stratum corneum limits the penetration of hawthorn active compounds into the human skin, this skin layer was removed. *Ex vivo* penetration results showed that the permeability of active compounds across the human skin with no stratum corneum did not increase over 24 h of the test. No active compounds were found in the dermis layer of the skin. Meanwhile, hyperoside and isoquercitrin (Table 2) were identified in the epidermis with no stratum corneum. The study results showed that the stratum corneum does not limit the permeability of the studied compounds. Based on the results obtained, it can be assumed that the permeability of active compounds is affected by the viable epidermis which is more lipophilic, the closer it is to the stratum corneum (30). The study results have supported the data reported in scientific literature that the physical and chemical properties of the drug substance in the topical dosage forms determines the permeation of the drug substance across the skin (31, 32). Based on the results of these hawthorn skin penetration tests, it is appropriate to select carriers that act in the epidermis and on its surface and allow modeling a stable protective semisolid preparation for topical application (33). The ointment base for incorporating active substance was selected by combining components of natural origin (Table 3).

Beeswax and olive oil were the main components of semisolid bases of groups I and II. Also, a N7 suspension and a N14 formulation without beeswax were prepared to determine the effect of this material on the release of active substances. Shea butter and olive oil were used as the main components for the base of group III semisolids. An emulsifier – cholesterol for enhanced stability and cocoa butter for better spreadability were incorporated into the base of I and III group semisolids (34). Furthermore, honey was incorporated for its antibacterial, anti-inflammatory and wound-healing properties (35, 36). According to the data reported in scientific literature, honey, olive oil and beeswax mixture is useful on patients with atopic dermatitis, psoriasis vulgaris and eczema (37, 38). Shea butter has moisturizing, anti-inflammatory, UV protection, anti-age-

![Figure 6. The kinetics of hawthorn active compounds release (n = 3) from group II formulations](image)
Two percent of active substance, extract of dried hawthorn leaves with flowers, was incorporated into the bases (Table 3).

A comparative analysis of rheological characteristics (Fig. 5) between samples of group I revealed a high variety of consistency index from 1.33 to 129.40 (Pa s). It was noticed 2-fold increase of the consistency index when increasing the amount of beeswax by 2 grams (the amount of beeswax range was between 5 to 15 grams) compared with a sample containing less than 2 grams of beeswax. An exception was the range of beeswax between 11 to 13 grams that had a slight effect on rheological characteristics. The highest change (decrease) of flow behavior index was seen when increasing the amount of beeswax from 5 to 7 grams, and later, a consistent reduction of the flow behavior index to 0.1 was observed. All tested samples had pseudoplastic characteristics. Statistical evaluation of rheological characteristics revealed that samples can be divided into four homogeneous subsets (N1-N2, N2-N3, N4-N5 and N6) according to the consistency index. On the other hand, there was five homogeneous subsets (N1, N2, N3, N4-N5 and N6) according to the flow behavior index.

The 2% of dry hawthorn extract were added to the semisolid formulations, however the results of the in vitro release testing of the active compounds of group I formulations with 2% of dry hawthorn extract showed that these bases are not suitable carriers because the analyzed hawthorn compounds were not released after 6 h of testing. The hypothesis that beeswax may suspend active compounds has been suggested. Therefore, samples of group II (Table 3) were modeled. The experimental tests determined the effect of beeswax on the release of hawthorn active compounds by analyzing the samples of group II (Fig. 6). The results demonstrated that an increase in beeswax content had a statistically significant effect on the release of active substances. The samples (N10-N13) with 1.5-5% of beeswax did not release active substances. The data presented in Figure 5 show that formulations N7 and N8 released significantly higher amounts of active

![Figure 7. The kinetics of hawthorn active compounds release from group III formulations](image-url)
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substances compared to the formulations N9 and N14. The results demonstrated that 0.5% of beeswax did not affect the release of active compounds, whereas 1% of this material caused a statistically significant reduction in the amounts of active compounds released. Other components contained in the semisolid formulations (Table 3) decelerate the release of active compounds but their cumulative effect is not significant as compared to the effect of beeswax. The mathematical analysis were performed for cumulative amounts of active substances released per square cm from modeled formulations. It revealed that $R^2$ values of Higuchi model were 0.829, 0.952, 0.974, 0.830 of formulations N7, N8, N9 and N14, respectively. The study results showed that the release of a drug substance is determined by the components of the selected carrier matrix (41). After analyzing the results obtained from the tests, shea butter was chosen as a thickening agent for the semisolid formulations of group III (Table 3) as a full or partial substitution for beeswax. An *in vitro* release test of hawthorn active compounds from formulated semisolid formulations was conducted (Fig. 7).

The results of the release test (Fig. 7) showed that the formulation N15 without beeswax released the highest amounts (41.4-42.4%) of active substances, and the formulation N17 with 1% of beeswax released the lowest amounts (5.37-6.63%). Formulation N16 released moderate amounts (32.0-35.4%) of active compounds. Moreover, the amount of hyperoside (N17) and amounts of isoquercitrin released from all the formulations were lower than the limit of quantification. According to the release kinetic profile given in Figure 7, the most rapid processes occurred during the first and the second hour. The deceleration of release was observed after 4 h of the test and stabilization was found after 6 h. The results of statistical analysis showed that amounts of released substances differed statistically significantly between all the tested formulations. The mathematical analysis of kinetic profile of active substances released per square cm from modeled formulations revealed that $R^2$ values of Higuchi model were 0.864, 0.981, 0.892 of formulations N15, N16 and N17, respectively. The analysis of rheological characteristics (Fig. 8) of group III formulations confirmed literature data that higher amounts of active compounds were released from formulations with lower consistency indices and higher flow behavior indices (42). The rheological characteristics of group III formulations showed that even small amounts of beeswax (up to 1%) statistically significant influenced rheological characteristics of formulations (Fig. 8).

The study results confirmed the hypothesis that the amount of beeswax had an effect of modeled formulation on active substance release. A statistically significant difference was found between group III samples and the sample N14. The amounts of active compounds released from the formulation N14 were lower than those released from the formulations N15 and N16, and higher than those released from the formulation N17. The test results demonstrated that shea butter does not limit the release of hawthorn active components from semisolid forms.

The *ex vivo* skin penetration tests of semisolid samples containing shea butter (Table 3, group III)
revealed that hawthorn active substances do not penetrate into the skin. The test results demonstrated that the formulated semisolid preparation can only affect the surface of the skin. The test results supported the data found in the scientific literature that the physical and chemical properties of a drug substance in topical dosage forms determines the permeability of that substance across the skin from the applied carrier matrix (31, 32).

CONCLUSIONS

The study analyzed the extract of dried hawthorn (Crataegus oxyacantha L.) leaves with flowers in which the following were identified as the main phenolic compounds: chlorogenic acid, vitexin-2′-O-rhamnoside, hyperoside and isoquercitrin; the extract possesses antibacterial activity against B. cereus, S. aureus, S. epidermidis, and E. coli, and antiradical activity based on the DPPH radical binding capacity, and can be used as an active ingredient in the topical formulations. The *ex vivo* penetration test using undamaged human skin demonstrated that the permeability of hawthorn active substances is limited: only the traces of hyperoside and isoquercitrin were found in the epidermis. The *in vitro* release tests revealed that beeswax in semisolid formulations has a limiting effect on the release of hawthorn active components.

Declaration of interest

The authors report no declarations of interest.

REFERENCES


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