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APPLICATION IN MODELING OF PROPOLIS OIL EXTRACT IN SEMISOLID PHARMACEUTICAL FORMS

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Summary

Bee products are widely used in the production of pharmaceutical and cosmetic products. The purpose of this study is to extract propolis by oil and apply the oil extract to compound the semisolid preparations from natural components. The selected solvent and extraction conditions ensure the isolation of phenolic compounds from the raw material, the total amount of phenolic compounds in the oil extract was determined to be 35.82 ± 1.12 mg/ml. Propolis oil extract is a suitable component in the production of ointments due to its lipophilic properties. The modeled formulations had a homogeneous, semisolid consistency. The viscosity of the produced ointments depends on the amount of wax. As the amount of wax increases, its viscosity increases. All modeled in research formulations were temperature-sensitive. The pH value of the formulations was in the range of 4.17 - 5.77. As the amount of wax increases, less active compounds are released from the formulations.

Keywords: propolis, honey, beeswax, phenolic compounds, ointments

Introduction

Bee products, propolis, and honey are traditionally widely used components in the production of pharmaceutical and natural cosmetic products. Propolis is a complex of biologically active substances with mutually synergistic effects. Propolis can be used in various forms: thick extract, liquid ethanolic extract, and aqueous extract (De Clermont-Gallerande, 2022). When creating propolis products for external use, the optimal form of propolis must be selected for introduction into dermatological semisolid pharmaceutical and cosmetic preparations. Therefore, it is relevant to produce an oil extract from Lithuanian propolis raw material and to evaluate its quality. It is important to choose the right extraction technology so that the produced oil extract is dominated by the same biologically active substances as in the raw material. In the production of propolis oil extract, olive oil was chosen as a solvent, considering its positive properties - it mixes well with solid substances, animal fats, waxes, and paraffins (Gottschlack and McEwen, 2010). Propolis is compatible with olive oil and has even been shown to protect olive oil from autoxidation and lipolysis (Jankowski et al., 2017). Olive oil is used in the production of pharmaceutical and cosmetic products as an auxiliary component and as an active ingredient, as the therapeutic effect of olive oil, when used internally and externally, is proven. Components of olive oil: fatty acids, vitamins (E and carotene), and water-soluble substances. The main fatty acids are oleic (55-58 percent of all olive oil), linoleic acids (9 percent), and linolenic (linoleic) acid (up to 1.5 percent). Olive oil also contains phenolic compounds, etc. of reactive substances (Gottschlack and McEwen, 2010; Jankowski et al., 2017). Olive oil used externally has skin moisturizing properties; due to its antioxidant action, it reduces the skin-damaging effect of free radicals; brightens and evens skin color and the appearance of wrinkles; pleasant to use and non-irritating, suitable for sensitive skin; works against skin aging. Olive oil can be used to treat dermatitis, atopic dermatitis, xerosis, eczema, rosacea, seborrhea, psoriasis, burns, and various skin inflammations (Jankowski et al., 2017; Nilforoushzadech et al., 2018).

Honey is listed in the International Nomenclature of Cosmetic Ingredients (INCI) as a moisturizing, humectant, and emollient product (Öğütcü and Yılmaz, 2014). Honey is useful in skin care products, and its regular use contributes to the youthfulness of the skin and the reduction of wrinkles. Honey is used in different proportions depending on the type of dermatological preparations. Honey is commonly used at 1 - 10 % in products such as lip balms, moisturizers, and gels (Servili, 2013). Cosmetic products are currently using synthetic spermaceti to contribute to animal safety. Spermaceti is a hard wax rich in high molecular weight esters. It is most often found in the composition of ointments, as one of the components of the base, characterized by a moisturizing effect (Silici and Baysa, 2020; Šuran, 2021). Cocoa butter is used in the production of cosmetic products due to its protective and moisturizing properties.

All selected components of the semisolid base must be compatible with each other and ensure the stability and release of the biologically active substances from the modeled semisolid matrix. Therefore, biopharmaceutical release tests *in vitro* are currently performed to assess the quality of semisolid pharmaceutical forms, during which it is determined

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how the modeled semisolid base is appropriate to release active substances, and how the active substances penetrate through biological membranes. This work aims to produce an oil extract of propolis and apply it in the modeling of ointments, using natural components for the formation of a semisolid matrix and to evaluate their influence on formulation quality.

Methods of research

Propolis oil extract technology. Propolis (UAB Medicata Filia (Vilnius, Lithuania) 30.0 g was extracted by maceration for 7 days in a dark place with 70.0 g of olive oil.
Determination of the amount of phenolic compounds in propolis oil. 0.3 g of propolis oil was mixed with 50 ml 96% ethanol. Samples were held at 9 p.m. at -12 ºC. Pour 1 ml of the test solution into a 50 ml volumetric flask containing 15 ml of water and 4 ml of Folin-Ciocalteu reagent. Then 6 ml of 20% sodium carbonate solution was added. Water was added up to the 50 ml mark. The prepared solution was left for 2 hours. Absorbance was measured at a wavelength of 765 nm.
Modeling of ointment formulations with propolis oil extract. Beeswax, spermaceti, and cocoa butter were melted in a water bath, with adding the appropriate amount of olive oil propolis extract and mixing until homogeneous mass. The compositions of the formulations with propolis oil extract are presented in Table 1.

Table1. Composition of ointment formulations with propolis oil extract

Formulation substances	Mass, g		
	F1	F2	F3
yellow beeswax	10.0	5.0	2.svi
honey	2.0	2	2.0
propolis oil extract	50.0	50.0	50.0
spermaceti	0.25	0.25	0.25
cocoa butter	10.0	10.0	10.0
olive oil	till 100.0	till 100.0	till 100.0

Studying rheological properties. The consistency coefficient of the simulated semisolid formulations was determined using a Carri-Med CSL100 rheometer (TA Instruments, Germany).
Quality analysis. The pH value was determined by the potentiometric method (pH-meter 766 Knick SE 104 N, Knick Elektronische Meßgeräte GmbH & Co, Germany). The release of phenolic compounds from the formulations was evaluated *in vitro*. Acceptor phase – 30% ethanol solution. Samples were taken after 1, 2, 3, and 6 hours. The total amount of phenolic compounds was assessed according to p-coumaric acid equivalent by spectrophotometric method, wavelength 765 nm. The organoleptic indicators of the modeled semisolid formulations with propolis oil extract were evaluated visually: color, smell, phase stability, and spreadability/washability.
Student's t-test and $p<0.05$ was used as the research level of significance. All tests were repeated three times.

Result and discussion

Propolis oil extract was produced, and organoleptic properties were determined. Propolis oil extract is characterized by a yellowish-brown color and a specific smell of propolis, it is a viscous oily liquid.
Propolis oil extract is intended for inclusion in semisolid formulations, as well as for external use as a final product. Therefore, it is appropriate to determine the amount of biologically active substances - polyphenolic compounds in propolis oil. The total amount of phenolic compounds in propolis oil extract was determined. The research results showed that the total amount of phenolic compounds according to p-coumaric acid in propolis olive oil extract is 35.82 ± 1.12 mg/ml.
One of the application possibilities of propolis oil is the formulation of a dermatological semisolid preparation. Materials of natural origin were chosen for the ointment modeling: yellow beeswax and olive oil, propolis, honey, spermaceti, and cocoa butter.
The quality evaluation results of the produced propolis oil ointment formulations are presented in Table 2. The research results show that the formulations of the modeled compositions remained stable, and phase separation did not occur. The color and smell corresponded to the characteristics of the ingredients.

Table 2. pH values and organoleptic evaluation of ointment formulations with propolis oil extract

Formulation	pH value	Phase separation	Spreadability / Washability	Colour	Odour
F1	4.17 ± 0.04	unnoticed	spreadable / hard to wash off	yellowish	pleasant, characteristics of propolis
F2	4.69 ± 0.02				
F3	5.77 ± 0.08				

The pH values of the ointment formulations were set within the limits of 4.17-5.77. The research results showed that the lowest pH value was typical for formulation F1, and the highest for F3. The pH value of all modeled formulations with propolis oil extract was weakly acidic, close to the physiological pH value of the skin.
The research results showed that formulation composition and temperature influence the structural properties of modeled ointments (Table 3).

Table 3. Consistency coefficient of semisolid formulations with propolis oil extract in different temperatures

Formulations	*Consistency coefficient* (K) Pa·s^n		
	20 °C	32 °C	37 °C
F1	0.2204 ± 0.041	0.1893 ± 0.024	0.0291 ± 0.032
F2	0.1258 ± 0.033	0.0974 ± 0.028	0.0501 ± 0.028
F3	0.0999 ± 0.027	0.0330 ± 0.031	0.1002 ± 0.024

During the experimental study, it was found that the rheological properties of semisolid formulations - the consistency coefficient depend on the amount of wax in the base of the ointment. A statistically significant difference was found between all the tested formulations. It was also found that the viscosity of semisolid formulations decreases with increasing temperature.
During the *in vitro* release study, the influence of the semisolid formulation substances on the release of phenolic compounds from propolis oil extract was determined (Figure 1).

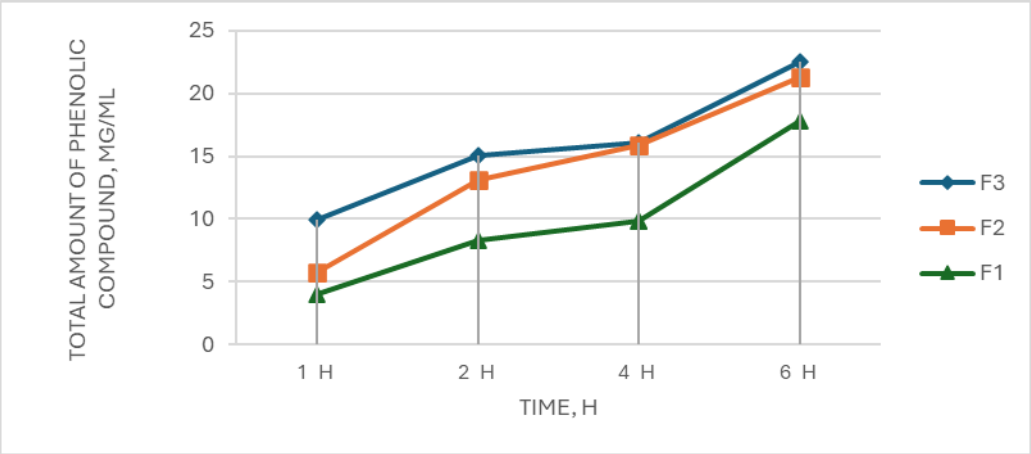

Figure 1. Release of total phenolic compounds from semisolid formulations with propolis oil extract

During the research, it was found that the auxiliary substances forming the basis of the formulations influence the release of bioactive substances from such systems. The compositions of the studied semisolid systems differ in terms of wax content. Therefore, the influence of wax content on the release of phenolic compounds *in vitro* was observed. The systems (F2 and F3) with the least amount of wax release the most phenolic compounds. Statistically significantly less ($p<0.05$) was released by the F1 formulation. This supports the view that due to their lipophilic nature, phenolic acids are difficult to release from the lipophilic semisolid base (Šuran, 2021).

Conclusions

1. The selected propolis extract olive oil and propolis oil technology are suitable for the extraction of biologically active propolis substances from propolis raw material.
2. Propolis oil extract is compatible with the selected base materials and is suitable for inclusion in lipophilic semisolid preparations, for use on the skin due to established organoleptic properties, close to the physiological skin pH value and consistency coefficient at body and skin temperature.
3. Ointment formulations with propolis oil extract ensure the release of propolis bioactive substances from the system.

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