Streszczenie rozprawy doktorskiej w języku angielskim

Development of dermocosmetic products based on selected plant extracts, essential oils and substances with a high soothing and regenerative potential in relation to injuries and degenerative diseases of the joints

In recent years an increase in the incidence of osteoarthritis (OA) has been observed, which has been diagnosed not only in the elderly, but also in the younger generation, especially in athletes, which usually means the end of their career. As yet, no effective treatment for osteoarthritis has been developed, so only therapies that slow down the progression of the disease or protect against the deepening of existing damage can be applied. Typically, conservative therapies are based on analgesic therapy and aim at maintaining mobility and soothing effects in the joints as long as possible. Therefore, the search for alternative forms of bringing relief to people struggling with OA is continued.

The studies presented in this doctoral dissertation were aimed at development of dermocosmetic products based on selected plant extracts, essential oils and substances with high soothing and regenerative potential for injuries or degenerative joint diseases, and to conduct all necessary research to enable the products introduction on the market. The presented dissertation contains a presentation of the topic based on literature along with a methodological part as well as the results of the conducted research and their discussion.

The introductory part presents the structure of joints with an indication of problems related to osteoarthritis. The problems of this disease are described, including presentation of etiopathogenesis, clinical picture, epidemiological data and currently available treatment methods. This chapter indicates substances with high regenerative potential for OA, their mechanism of action and possible side effects of their use (with particular explication of the characteristics of glucosamine sulfate and icariin derived from the *Epimedium Koreanum* extract). The structure of the skin is also presented focusing on the possibility of using agents with local epidermal application and the possibility of transepidermal transport of active ingredients. The next chapter of the dissertation presents the possible *in vitro* and *in vivo* methods of testing the skin permeation of substances, the problems of designing new formulations, and analysis of the legal aspects regarding the implementation of new products in terms of the safety of their use. Moreover, this chapter also describes all the tests required for the implementation procedure emphasizing the importance for the safe use of the cosmetic products.

The methodological part presents the methodology for quantitative determination of the active substance - icariin, in the *Epimedium Koreanum* extract, needed to determine the final compositions of the developed products. A list of all raw materials used in the new formulations is attached, and the technology for producing the formulations is described on the micro scale for three application forms (ointment, gel, emulsion), as well as for increasing the scale of gel and emulsion production from the micro to semi-technical scale. Then, the methods applied to determine the physicochemical parameters used to develop specifications for cosmetic products are presented, including those for measuring pH, density, viscosity, sensory evaluation, centrifuge stability, stability by multiple light scattering, microscopic analysis and water activity measurement. The conditions of the release tests carried out in the paddle apparatus

and the *in vitro* permeation tests through the skin-imitating membranes in the Franz chamber are described. This chapter also presents the implementation tests methodologies. The procedures for microbiological purity tests and the cosmetic mass preservation test, stability, dermatological and application tests are described in detail along with the procedure of toxicological assessment. Key toxicological parameters have been determined indicating the sources or calculation methods.

The results section presents an analysis of available raw materials used to support the treatment of osteoarthritis, based on which the main ingredients of the proposed formulation were selected: glucosamine sulfate as an endogenous component of the polysaccharide chains of the cartilage matrix and glucosaminoglycans of synovial fluid, along with icariin derived from the extract of *Epimedium Koreanum* with a potentially high ability to activate chondrogenesis and regeneration of the intercellular matrix by chondrocytes. Then, the problems encountered during the preparation of the proposed formulations are described and their possible causes are identified. The new formulations have been characterized in terms of physicochemical properties, on whose basis product specifications necessary for production quality control have been developed. The same chapter also presents the results characterizing the release and *in vitro* permeation through the skin-imitating membranes. The use of both carriers in the form of a gel or emulsion has made it possible to achieve the penetration of icariin above the assumed level of 20%. On the basis of the release tests of active substances from the ointment, due to the lowest release percentage values, this carrier has been excluded from further tests. The next section presents the results of the process of optimizing the production of cosmetic mass on a semi-technical scale, indicating the influence of the mixing and homogenization process parameters (time and number of revolutions per minute) on the final physicochemical properties or stability of the produced mass. This section also presents the results of implementation tests of selected formulations. Microbiological purity has been checked on the basis of determination of the total number of mesophilic aerobic microorganisms, yeasts and molds, as well as pathogenic strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*, showing no deviations from the permissible number of microorganisms, and no presence of any of the tested pathogenic strains. The findings of the cosmetic products' preservation system examination confirmed its effectiveness against the multiplication of artificially introduced microorganisms. Results of the examination and calculation of the irritation potential, indicate that both products (cooling gel (joint injuries) and warming emulsion (joint degeneration)) are well tolerated by the skin and do not show irritating or sensitizing properties, therefore the products can be classified as non-irritating. The final part of this chapter presents the results of an application survey, assessment of the effectiveness of the products and the overall impression of potential consumers. The panel of volunteers participating in the study is characterized and a statistical analysis of the responses obtained in the surveys has been performed giving high ratings of the sensory properties of both products with no objections regarding their color, smell or application. The 12-week application tests confirmed all the declarations promised by the manufacturer, and thus the effectiveness of the products in the entire range, hence suggesting their high implementation potential. The last subsection of this chapter presents in great detail the toxicological study of each of the components of the warming emulsion formulation, in the form of a literature research on toxicological data based on available *in vitro* and *in vivo* tests.

Then, all ingredients have been analyzed as a mixture in terms of the possible risks when applied. The product has been assessed as a safe cosmetic one under normal and reasonably foreseeable conditions of use. Based on the above analysis, the information mandatory on the product label has been prepared, including the warnings, instructions for use, notes, labeling and INCI composition.