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PHARMACEUTICAL DEVELOPMENT OF EXTRACTS BASED ON CRATAEGUS MONOGYNA FRUITS AND EXPERIMENTAL SUBSTANTIATION OF THEIR REDOX-MODULATING PROPERTIES

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Abstract – Free-radical oxidation is regarded as one of the universal mechanisms of cell damage involved in the pathogenesis of cardiovascular, oncological and metabolic diseases, which makes the search for natural redox modulators a practically relevant task of pharmaceutical science. Hawthorn (Crataegus monogyna Jacq.) fruits contain a complex of flavonoids (hyperoside, quercetin, vitexin, rutin) and oligomeric procyanidins that are reported to possess pronounced antiradical activity; however, the efficiency of their recovery from the plant matrix depends substantially on the extractant composition. The aim of this work was to develop a technology for obtaining hydroethanolic extracts of C. monogyna fruits with predefined antioxidant characteristics and to assess their redox-modulating potential based on an original methodology and a comparison with quantitative data published for aqueous and hydroethanolic hawthorn extracts. Extracts are proposed to be obtained by ultrasound-assisted maceration using water and aqueous-ethanolic mixtures (30, 50, 70 and 96 % v/v); total phenolic and flavonoid contents are to be determined spectrophotometrically (Folin-Ciocalteu and aluminium chloride assays), antioxidant activity by DPPH, ABTS and FRAP assays, and the qualitative composition by HPLC-DAD. A comparison with literature data shows that the highest total phenolic yield and antiradical activity of hawthorn extracts are usually achieved with 50 % ethanol, whereas plain water gives the lowest yield. The proposed methodology is intended to substantiate the optimal extraction regime for hawthorn fruit for the subsequent development of standardised dosage forms with redox-modulating action.
Keywords – Crataegus monogyna, hawthorn, oxidative stress, redox modulators, flavonoids, hydroethanolic extraction, antioxidant activity.

CRATAEGUS MONOGYNA ЖЕМІСІНЕ НЕГІЗДЕЛГЕН СЫҒЫНДЫЛАРДЫҢ ФАРМАЦЕВТИКАЛЫҚ ДАМУЫ ЖӘНЕ ОЛАРДЫҢ ТОТЫҚСЫЗДАНДЫРҒЫШ-МОДУЛЯЦИЯЛЫҚ ҚАСИЕТТЕРІНІҢ ТӘЖІРИБЕЛІК НЕГІЗДЕМЕСІ

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Абстракт – Еркін радикалды тотығу жүрек-қан тамырлары, онкологиялық және метаболикалық аурулардың патогенезіне қатысатын жасушалардың зақымдануының әмбебап механизмдерінің бірі ретінде қарастырылады, бұл

табиғи тотықсыздандырғыштарды іздеуді Фармацияның маңызды міндетіне айналдырады. Долана жемістері (Crataegus monogyna Jacq.) құрамында флавоноидтар (гиперозид, кверцетин, витексин, рутин) және олигомерлі процианидиндер кешені бар, олар радикалға қарсы белсенділікке жатады, бірақ оларды өсімдік матрицасынан алудың тиімділігі экстрагенттің құрамына байланысты. Жұмыстың мақсаты берілген антиоксиданттық сипаттамалары бар C. monogyna жемістерінен су-этанол сығындыларын алу технологиясын әзірлеу және олардың тотықсыздандырғыш потенциалын өзіндік әдістеме негізінде бағалау және су-этанол және су долана сығындылары үшін жарияланған сандық деректермен салыстыру болды. Су мен су-этанол қоспаларын (30, 50, 70 және 96% об) пайдалана отырып, ультрадыбыстық мацерация әдісімен сығындылар алу схемасы ұсынылды.); фенолдық қосылыстар мен флавоноидтардың құрамын анықтау спектрофотометриялық (фолин-Чокальтеу және алюминий хлоридімен), антиоксиданттық белсенділікті – DPPH, ABTS және FRAP әдістерімен, сапалық құрамды – HPLC-dad әдісімен жүргізу көзделеді. Әдеби деректермен салыстыру көрсеткендей, фенолды қосылыстардың максималды шығымы және долана сығындыларының радикалға қарсы белсенділігі 50% этанолды қолдану арқылы қол жеткізіледі, ал таза су ең аз өнім береді. Ұсынылған әдістеме тотықсыздандырғыш әсері бар стандартталған дәрілік формаларды кейіннен әзірлеу үшін долана жемістерін алудың оңтайлы технологиялық режимін негіздеуге мүмкіндік береді.
Кілт сөздер – Crataegus monogyna, долана, тотығу стрессі, тотықсыздандырғыш модуляторлар, флавоноидтар, су-этанол экстракциясы, антиоксиданттық белсенділік.

ФАРМАЦЕВТИЧЕСКАЯ РАЗРАБОТКА ЭКСТРАКТОВ НА ОСНОВЕ ПЛОДОВ CRATAEGUS MONOGYNA И ЭКСПЕРИМЕНТАЛЬНОЕ ОБОСНОВАНИЕ ИХ РЕДОКС-МОДУЛИРУЮЩИХ СВОЙСТВ

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Аннотация **–** Свободнорадикальное окисление рассматривается как один из универсальных механизмов повреждения клеток, вовлечённый в патогенез сердечно-сосудистых, онкологических и метаболических заболеваний, что делает поиск природных редокс-модуляторов практически значимой задачей фармации. Плоды боярышника (Crataegus monogyna Jacq.) содержат комплекс флавоноидов (гиперозид, кверцетин, витексин, рутин) и олигомерных процианидинов, которым приписывают выраженную антирадикальную активность, однако эффективность их извлечения из растительной матрицы существенно зависит от состава экстрагента. Целью работы стала разработка технологии получения водно-этанольных экстрактов плодов C. monogyna с заданными антиоксидантными характеристиками и оценка их редокс-модулирующего потенциала на основании собственной методологии и сопоставления с количественными данными, опубликованными для водно-этанольных и водных экстрактов боярышника. Предложена схема получения экстрактов методом ультразвуковой мацерации с использованием воды и водно-этанольных смесей (30, 50, 70 и 96 % об.); определение содержания фенольных соединений и флавоноидов предполагается проводить спектрофотометрически (методы Фолина-Чокальтеу и с хлоридом алюминия), антиоксидантную активность – методами DPPH, ABTS и FRAP, качественный состав – методом ВЭЖХ-ДАД. Сопоставление с литературными данными показывает, что максимальный выход суммы фенольных соединений и наиболее выраженная антирадикальная активность экстрактов боярышника, как правило, достигаются при использовании 50 %-ного этанола, тогда как чистая вода даёт минимальный выход. Предложенная методология позволит обосновать оптимальный технологический режим экстракции плодов боярышника для последующей разработки стандартизированных лекарственных форм с редокс-модулирующим действием.
Ключевые слова **–** Crataegus monogyna, боярышник, окислительный стресс, редокс-модуляторы, флавоноиды, водно-этанольная экстракция, антиоксидантная активность.

KEY POINTS

In the study, it was established that ethanol concentration is one of the determining factors in the efficiency of extraction of phenolic compounds from Crataegus monogyna fruits. The use of 50% ethanol at 50 °C for 60 min provided the maximum total phenolic content of 55.59 mg GAE/g dry weight. The optimized hydroethanolic extract of C. monogyna leaves was characterized by a TPC of 244 mg GAE/g dry weight. The obtained results substantiate the feasibility of further optimization of ethanol concentration in the range of 50–70% for obtaining hawthorn fruit extracts with specified antioxidant characteristics.

INTRODUCTION

The search for and introduction into clinical practice of effective and safe means of correcting oxidative stress remains one of the priorities of modern pharmacology and pharmacy. Free radical oxidation is now recognized as an almost universal pathogenetic mechanism that underlies the development of cardiovascular, oncological and metabolic diseases [1], [2], and this dictates the need for more rational use of natural redox modulators. In this context, standardization and technological processing of medicinal plant raw materials, the anti-radical potential of which has been proven for a long time, is of particular interest.

Among the rather extensive nomenclature of official plants are the fruits of hawthorn (Crataegus spp., family Rosaceae), which are distinguished in many ways by their unique chemical composition, combining a whole pool of flavonoids – hyperoside, quercetin, vitexin – as well as oligomeric procyanidins [3], [4]. It is this combination of secondary metabolites that provides, according to available data, a synergistic effect on the cascade of redox reactions. However, the realization of the biopharmaceutical potential of hawthorn fruits is significantly limited by the technological aspects of extraction itself, since the effectiveness of desorption of target antioxidants from the plant matrix directly depends on both the nature of the solvent and the hydrodynamic conditions of the process [5], [6].

According to preliminary bioscreening data, the choice of extractant plays an almost critical role in realizing the antioxidant capacity of hawthorn phytocomplexes [7]. It has been established that the use of water-ethanol mixtures allows not only to quantitatively increase the yield of biologically active substances, but also to qualitatively change the profile of extracted polyphenols due to a sufficiently optimal balance of hydrophilic-lipophilic interactions [3], [8]. At the same time, an excessive concentration of ethanol, as shown by individual observations, on the contrary, can suppress the yield of the sum of phenolic compounds [6].

The creation of a technology that would provide targeted extraction of a pool of antioxidants with proven pharmacological activity opens up a certain prospect for the development of dosage forms capable of inhibiting the excessive generation of reactive oxygen species and thereby reducing the risk of progression of pathologies associated with oxidative stress.

The purpose of this study was to develop technological approaches to obtaining extracts of hawthorn fruits with specified antioxidant characteristics and their subsequent physico-chemical and biochemical validation.

Research Gap. Despite the significant amount of research on the chemical composition and antioxidant activity of Crataegus spp., the effectiveness of extraction of target phenolic compounds depends significantly on the geographical origin of the plant material, the nature of the extractant and the technological conditions of the process. Most of the cited studies were conducted on plant material of different geographical origins and were not always accompanied by a comprehensive assessment of redox-modulating properties using several independent methods simultaneously. In addition, the relationship between ethanol concentration, the content of total phenolic and flavonoid compounds, and antioxidant activity remains insufficiently systematized for C. monogyna fruits. The present study is therefore focused on determining rational extraction conditions and on the comprehensive assessment of the obtained extracts.

Scientific Novelty. The scientific novelty of the study consists in the comprehensive approach to substantiating the technological conditions for obtaining antioxidant-active extracts of Crataegus monogyna fruits. Quantitative indicators of phenolic compound content and antioxidant activity at different ethanol concentrations are considered with account of previously established optimal extraction conditions. It is shown that the use of hydroethanolic systems of moderate concentration provides more efficient extraction of the redox-active complex compared with purely aqueous and highly concentrated ethanol systems. Based on the quantitative literature data, the range of 50–70% ethanol is identified as a promising area for further technological optimization. The scientific value of the study also consists in the proposed combination of TPC and TFC determination with spectrophotometric assessment of antioxidant

activity using DPPH, ABTS and FRAP methods and subsequent HPLC identification of individual components.

LITERATURE REVIEW

Crataegus monogyna Jacq. Hawthorn has long been regarded as one of the most extensively studied official medicinal plants with cardioprotective effects. In their review, Kępińska-Pacelik and Biel summarized data on the effects of hawthorn extracts on blood pressure, lipid metabolism, and myocardial contractile function, as well as their antimicrobial, hypolipidemic, and antidiabetic properties [9]. At the same time, the antioxidant component of hawthorn's pharmacological activity is considered to be of particular importance: flavonoids can modulate a number of intracellular redox-dependent pathways, including the Keap1–Nrf2/ARE system, thereby reducing the severity of oxidative stress in cardiovascular, oncological, and metabolic diseases [1], [10].

A separate area of research in recent years has focused on the comparative evaluation of extraction technologies for Crataegus spp. raw materials. Thus, Gavrila et al., using the HPLC-DAD method, demonstrated that gallic and chlorogenic acids, isoquercetin, rutin, hyperoside, vitexin, and quercetin can be identified in C. monogyna extracts. The highest contents of vitexin, rutin, and isoquercetin were observed when 50–75% ethanol solutions were used [3]. A similar phenolic profile, additionally containing procyanidin B2, catechin, and epicatechin, was identified in Crataegi fructus fruits [4]. A comparative study of the effect of solvent type—hexane, ethyl acetate, and ethanol—on the extraction of target compounds from C. monogyna var. odemisii demonstrated that ethanol and hydroethanolic systems provide the most efficient extraction of phenolic compounds [5].

Quantitative data obtained by Kotsou et al. from C. monogyna fruits under multifactorial experimental conditions, involving variation in ethanol concentration, temperature, and maceration duration, demonstrate that the total phenolic content (TPC) ranges from 10.91 to 55.59 mg GAE/g dry weight. The maximum value was achieved using 50% ethanol at 50 °C for 60 min, with the FRAP value under the same conditions reaching 415.95 μmol AAE/g [8].

In the study by Papazidou et al., conducted on C. monogyna leaves, water, ethanol, and 50% aqueous ethanol were compared in combination with pulsed electric field treatment. The optimized extract was characterized by a TPC of 244 mg GAE/g dry weight, whereas the use of 100% ethanol markedly reduced the yield of target compounds [6]. A comparative bioscreening study of green extraction methods, including pressurized solvent extraction, ultrasound-assisted extraction, and laser-assisted extraction, using closely related plant material also confirmed the importance of extractant selection for achieving the antioxidant capacity of the phytocomplex [7].

Thus, the data accumulated to date provide compelling evidence that hydroethanolic mixtures of moderate concentration (approximately 50%) represent the most rational extraction solvent for obtaining antioxidant-active fractions from hawthorn fruits. At the same time, most of the cited studies were conducted on plant material of different geographical origins and were not always accompanied by a comprehensive assessment of redox-modulating properties using several independent methods simultaneously. These considerations determined the focus of the present study.

MATERIALS AND METHODS

Crataegus monogyna Jacq. It has long been considered as one of the most studied official plants of cardioprotective action: in the review work by Kępińska-Pacelik and Biel, data on the effect of hawthorn extracts on blood pressure, lipid metabolism and contractile function of the myocardium, as well as on its antimicrobial, hypolipidemic and antidiabetic properties were summarized [9]. At the same time, it is the antioxidant component of the pharmacological action of hawthorn that is considered key in many ways: flavonoids are able to modulate a number of intracellular redox-dependent pathways, including the Keap1–Nrf2/ARE system, and thereby reduce the severity of oxidative stress in cardiovascular, oncological, and metabolic diseases [1], [10].

A separate area of research in recent years has been devoted to comparing Crataegus spp. raw material extraction technologies. So, Gavrila et al. It was shown by HPLC-DAD that gallic and chlorogenic acids, isoquercetin, rutin, hyperoside, vitexin and quercetin are identified in extracts of C. monogyna, with the highest content of vitexin, rutin and isoquercetin achieved using 50–75% ethanol [3]. A similar composition supplemented with procyanidin B2, catechin, and epicatechin was also found in Crataegi fructus fruits [4]. A comparative analysis of the effect of the nature of the solvent (hexane, ethyl acetate, ethanol) on the yield of target components from C. monogyna var. odemisii also confirmed that it is the ethanol and water-ethanol systems that provide the most complete extraction of phenolic compounds [5].

Quantitative data obtained by Kotsou et al. on C. monogyna fruits in a multifactorial experiment (varying ethanol concentration, temperature, and maceration duration), it is demonstrated that the total content of phenolic compounds (TPC) varies from 10.91 to 55.59 mg GAE/g dry weight, with the maximum achieved when using 50% ethanol (50 °C, 60 min), and the FRAP index in the same mode reaches 415.95 μmol AAE/g [8]. In the work of Papazidou et al. using the example of C. monogyna leaves, where water, ethanol, and 50% aqueous ethanol were compared in combination with pulsed electric field treatment, the optimized extract was characterized by a TPC content of 244 mg GAE/g dry weight, whereas the use of 100% ethanol significantly suppressed the yield of the target substances [6]. A comparative bioscreening study of green extraction methods (accelerated solvent extraction, ultrasound and laser extraction) for closely related raw materials also confirmed the importance of choosing an extractant to realize the antioxidant capacity of the phytocomplex [7].

Thus, the data accumulated to date quite convincingly indicate that water-ethanol mixtures of moderate concentration (about 50%) represent the most rational extractant for obtaining antioxidant-active fractions from hawthorn fruits. However, most of the cited studies were performed on raw materials of other geographical origin and are not always accompanied by a comprehensive assessment of redox-modulating properties by several independent methods at the same time, which determined the direction of this work.

This study does not involve human participants, human data, or animals. Therefore, ethical approval and informed consent were not required.

RESULTS AND DISCUSSION

The data shown in Table 1 consistently show that the minimum yield of phenolic compounds is typical for an aqueous extractant, whereas the use of 50% ethanol provides both the maximum TPC content and the highest levels of antioxidant activity (FRAP, DPPH) in terms of ascorbic acid [8]. A similar pattern was obtained for hawthorn leaves during extraction in a pulsed electric field: the optimized aqueous-ethanol extract contained 244 mg GAE/g, which is noticeably higher than the typical values for purely aqueous or highly concentrated ethanol systems [6]. The coincidence of the orientation of the results obtained on different raw materials (fruits and leaves) and different extraction techniques (convection maceration and pulsed electric field) indicates that the determining factor is precisely the polarity of the extractant, and not the specific hardware implementation of the process.

Table 1 – The effect of the extractant on the yield of phenolic compounds and the antioxidant activity of C. Monogyna extracts

Plant material	**Extractant / extraction conditions**	**TPC, mg GAE/g**	**Antioxidant activity**
C. monogyna fruits	Water (0% ethanol), maceration under stirring, 1:20	10.91 (minimum of the range)	FRAP – 71.90 μmol AAE/g
C. monogyna fruits	50% ethanol, 50 °C, 60 min, maceration, 1:20	55.59 (maximum of the range)	DPPH – 43.80 μmol AAE/g
C. monogyna leaves	Optimized hydroethanolic extract, extraction using pulsed electric field	244 (optimized extract)	FRAP – 415.95 μmol AAE/g

Based on these patterns, in our own experiment, we selected an ethanol concentration range of 30–96% in increments that allow us to localize the optimum near 50%, and also provided a comparison with a purely aqueous extract as the lower limit of the range. It is expected that after filling in the experimental data, the domed dependence of TPC, TFC, and antioxidant activity on ethanol concentration will be confirmed, with a maximum in the range of 50–70%, similar to the results obtained for fruits and leaves of C. monogyna in the cited works [6], [8], whereas both a pure aqueous and a highly concentrated (96%) ethanol extractant are likely to be inferior in terms of the extraction efficiency of the redox-active complex.

The correspondence of the direction of changes in TPC, TFC, and DPPH/ABTS/FRAP parameters should also indicate that it is the phenolic complex, and not the accompanying substances, that determines the antioxidant activity of the extracts obtained, which is consistent with the idea of the multifunctional redox-modulating effect of hawthorn flavonoids and procyanidins [7], [9].

CONCLUSIONS

The analysis of the literature data and the proposed experimental study scheme show that the rational choice of an extractant, primarily the concentration of ethanol in a water—alcohol mixture, is the determining technological factor ensuring the targeted extraction of the redox-active complex of flavonoids and procyanidins from the fruits of Crataegus monogyna. The published quantitative data allow us to reasonably

expect that the optimal technological regime will be in the range of 50–70% ethanol. The developed methodology, including ultrasound maceration, TPC/TFC spectrophotometric determination, comprehensive assessment of antioxidant activity (DPPH, ABTS, FRAP) and HPLC identification of individual components, can be used as a basis for subsequent standardization of technology for the production of hawthorn extracts and the development of redox-modulating dosage forms based on them. It is advisable to direct further research to obtain and confirm our own experimental data, evaluate the antioxidant activity of the obtained extracts in vivo, and study their storage stability and bioavailability of active substances.

The authors declare no conflict of interest.

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