UDC 582: 542.61: 543.544.5

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Optimization of the technology for obtaining ecdysterone from *Serratula coronata* L. by varying the extraction methods and growth phases

A complex study of the aerial part of *Serratula coronata* L. cultivated in the collection area of medicinal plants of the IRPH «Phytochemistry» (Karaganda) in different phases of growth and using the most optimal extraction methods has been carried out. The content of the main active component of ecdysterone (20E)has been studied. Investigation of the seasonal dynamics of ecdysterone distribution under conditions of varying extraction methods shows that its maximum accumulation is observed during the vegetative phase, and the optimal method in this phase is extraction with isobutyl alcohol, the extract of which contains 13.86 % of ecdysterone and the maceration method with 96.2 % ethyl alcohol with a 20E content of 12.03 %, respectively. It is shown that the maceration with 96.2 % ethyl alcohol is technologically optimal, which fully complies with the international standards of good manufacturing practice (GMP) under pharmaceutical production conditions and excludes the use of toxic and expensive isobutyl alcohol solvent. It has been found that the content of ecdysterone from the beginning of vegetation to the final phase goes down, which is confirmed by the data of high-performance liquid chromatography (HPLC). It is assumed that there is an outflow of ecdysterone to the root system, and then its redistribution occurs as the plant develops further with a partial discharge into the soil. Based on the data on the quantitative content of the target component by the HPLC method, it is recommended that for the preparation of the ecdysterone substance of many actoprotective phytopreparations and valuable WS, the preparation of the above-ground biomass of *Serratula coronata* L. should be carried out during the vegetation phase of this taxon.

Keywords: Serratula coronata L., aerial part, 20-hydroxyecdysone, high-performance liquid chromatography, extraction, ethyl alcohol, growth phase, vegetation.

Introduction

Serratula coronata L. of Asteraceae Dumort family is known in folk medicine as a remedy for treatment of inflammatory and infectious diseases (dyspepsia, pharyngitis, tonsillitis and others), as well as neuroses and mental illnesses [1, 2]. This plant attracted the attention of scientific medicine due the presence of phytoecdysteroids that are vegetable hormones [3].

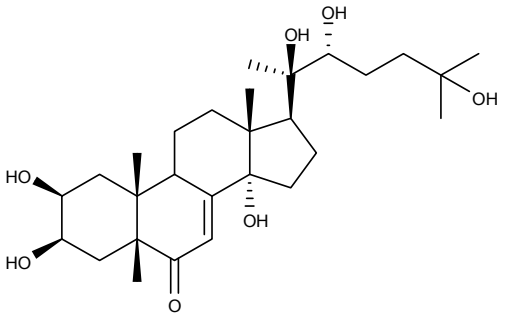


Figure 1. Structural formula of 20-hydroxyecdysone

The extensive chemical screening showed that *Serratula coronata* L. is worthy of special attention as a pharmacologically promising and industrially significant plant raw material for the isolation of adaptogens from the class of phytoecdysteroids. It was found that content of phytoecdysteroids, in particular, ecdysterone-2β, 3β, 14α, 20R, 22R, 25-hexahydroxy-5β (H) -cholest-7-en-6-one (20-hydroxyecdysone or 20E, Fig. 1) in the terrestrial organs of *Serratula coronata* L. is an order of magnitude higher than in the known medicinal plant *Rhaponticum carthamoides* (Willd) Iljin of Asteraceae Dumort family [4]. Leuzea (or maral root) is traditionally considered to be one of the strongest natural adaptogens. A substance containing

20E was isolated from the underground organs of the Leuzea at the end of the last century at the Institute of Plant Compounds Chemistry of the Academy of Sciences of the Uzbek SSR, on the basis of which the preparation «Ekdisten» was developed, which possesses specific adaptogenic properties. This drug was registered as a medicinal tonic [5].

At the present time, the composition of ecdysteroids of *Serratula coronata* L. is most thoroughly studied, which is used as the main pharmacological raw material for obtaining new adaptogenic, anabolic, and tonic agents [6]. So, on the basis of the above taxon, the preparation «Ecdyphyt» was developed in JSC «International Research and Production Holding» Phytochemistry» (IRPH «Phytochemistry»), which is the sum of extractives with the main active component 20E and the sum of ecdysteroids and flavonoids [7, 8].

«Serpisten» created by Volodin et al. [9,10] at the Institute of Biology of the Komiin the Science Center of the Ural Branch of the Russian Academy of Sciences is a mixture of ecdysteroids such as 20-E (80 %), 25S-inocosterone (11 %), α-ecdysone (5 %), and some other minor components isolated from the aerial part of *Serratula coronata* L. Biologically active compound «Serpisten» shows a high ergotropic, CNS-tonic, stress-protective, hematoprotective action as well as the action on the background of a slight anabolic effect.

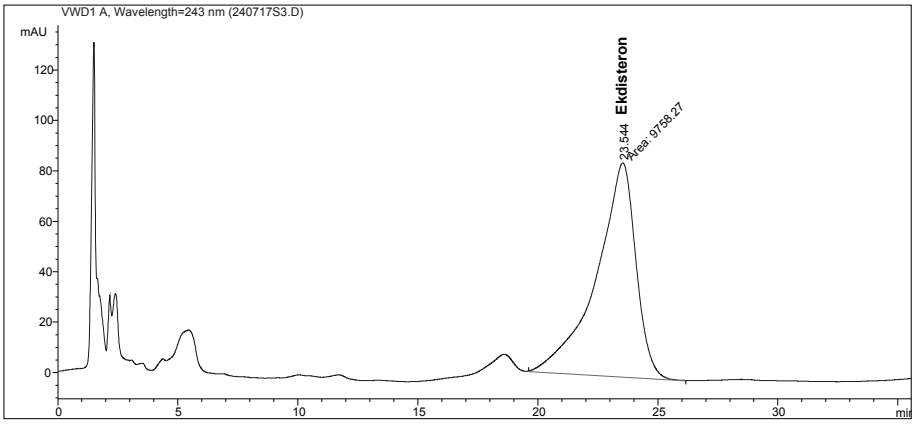
It is known that the complex processing of wild and cultivated medicinal plant material as a renewable material is one of the priority approaches in the chemical study of plants in terms of obtaining practically valuable substances and new working standards (WS) for the fast-growing pharmaceutical industry in the Republic of Kazakhstan. In the IRPH «Phytochemistry», there was recently carried out study of the ecdysteroid and flavonoid composition of the extract of the aboveground part of the freshly harvested plants of the plant *Serratula coronata* L. growing on the site of medicinal plants of IRPH «Phytochemistry» in order to develop an effective method for the separation of ecdysterone, the main component of the composition of ecdysteroids, and WS, as well as other minor phytoecdysteroids and flavonoids for their use as a new WS [11]. Plants grown in culture surpass natural specimens on the accumulation of biomass [7].

In this regard, the purpose of this work was to determine the most productive phase of growth, and the optimal method for extracting the aerial part of the *Serratula coronata* L., data that can be used in the future to produce the above preparations with a high content of the active main component of ecdysterone.

Experimental

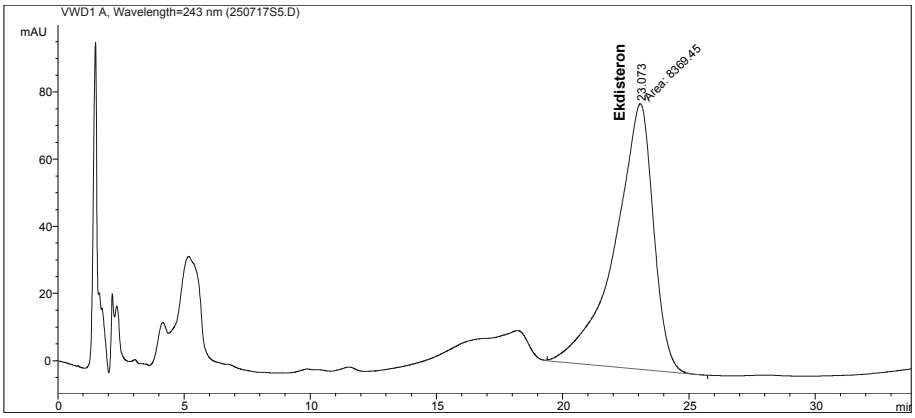
Plant raw material (the aerial part of the *Serratula coronata* L.) was harvested from the middle of May to the end of September 2017 in the collection plot of medicinal plants of the IRPH «Phytochemistry» (Karaganda) during the vegetation-withering phases.

The plant was dried to an air-dry state in a dry, cool place. The average sample of the raw material was ground to a particle size of 2–3 mm for the chemical screening of the plant. Each sample for extraction was 5 g. Various methods were used to extract 20-hydroxyecdysone, such as extraction with a water bath, maceration method, extraction using a Soxhlet apparatus using ethyl alcohol and its 70 % aqueous solution, isobutyl alcohol. These methods were applied in accordance with the earlier used ones. Each extraction method was carried out three times. The extracts were evaporated using a «BuchiRotavaporR-3» rotary evaporator. The resulting thickened samples weighing an average of 0.2 g were placed in a glass container and investigated using the HPLC method.



Isobutyl alcohol, yield of 20E is 13.86 %

Figure 2. Chromatogram of the extract of *Serratula coronata* L.



Maceration method, 96.2 % ethyl alcohol, yield of 20E is 12.03 %

Figure 3. Chromatogram of the extract of *Serratula coronata* L.

The extracts were analyzed by reversed-phase HPLC (HPLC, HEWLETT PACKARD Agilent 1100 Series, analytical column 4.6×150 mm, Zorbax SB-C18, mobile phase (MP): 10 % isopropyl alcohol, UV detection at wavelength 254 nm, column temperature 20 ºC, flow rate of the eluent 0.75 ml/min, sample volume 20 μl). The content of 20E was studied (Figs. 2, 3).

Results and discussion

It is known that the content of ecdysterone in a number of species of the genus *Serratula* L. strongly varies depending on the phenophase and their age status [7]. In this connection, a study was carried out to determine the distribution of ecdysterone in the cultivated plant of *Serratula coronata* L. at various stages of development such as vegetation, start of budding, budding, start of flowering, flowering, start of fruiting, fruiting, start of withering away and dying out.

The results of a quantitative analysis of the ecdysterone content depending on the extraction methods and on the species and concentration of the extractant in the studied taxon are presented in the Table below.

T a b l e

The content of ecdysterone in the aerial part of *Serratula coronata* L. depending on the method of extraction and the phase of plant development (in % of the weight of absolutely dry raw material) according to HPLC

Methods for extraction of 20E and the phase of development of the *Serratula coronata* L. plant	The quantitative content (20E) in %
1	2
Vegetation phase	
Extraction with 96.2 % ethyl alcohol	8.88
Extraction with 70 % ethyl alcohol	4.51
Extraction with isobutyl alcohol	13.86
Extraction with 96.2 % ethanol on a Soxhlet apparatus	11.03
Maceration with 96.2 % ethyl alcohol	12.03
Maceration with 70 % ethyl alcohol	5.94
Start of buddingphase	
Extraction with 96.2 % ethyl alcohol	5.51
Extraction with 70 % ethyl alcohol	3.51
Extraction with 96.2 % ethanol on a Soxhlet apparatus	4.11
Extraction with isobutyl alcohol	4.99
Maceration with 96.2 % ethyl alcohol	4.56
Maceration with 70 % ethyl alcohol	2.83
Budding phase	
Extraction with 96.2 % ethyl alcohol	2.56
Extraction with 70 % ethyl alcohol	1.89
Extraction with isobutyl alcohol	5.04
Extraction with 96.2 % ethanol on a Soxhlet apparatus	2.38
Maceration with 96.2 % ethyl alcohol	3.27

Continuation of Table

1	2
Maceration with 70 % ethyl alcohol	2.08
Start of flowering phase	
Extraction with 96.2 % ethyl alcohol	3.19
Extraction with 70 % ethyl alcohol	1.19
Extraction with isobutyl alcohol	1.83
Extraction with 96.2 % ethanol on a Soxhlet apparatus	2.81
Maceration with 96.2 % ethyl alcohol	2.52
Maceration with 70 % ethyl alcohol	1.89
Flowering phase	
Extraction with 96.2 % ethyl alcohol	2.35
Extraction with 70 % ethyl alcohol	1.91
Extraction with isobutyl alcohol	3.25
Extraction with 96.2 % ethanol on a Soxhlet apparatus	2.17
Maceration with 96.2 % ethyl alcohol	2.77
Maceration with 70 % ethyl alcohol	2.50
Start of fruiting phase	
Extraction with 96.2 % ethyl alcohol	2.83
Extraction with 70 % ethyl alcohol	2.87
Extraction with isobutyl alcohol	4.00
Extraction with 96.2 % ethanol on a Soxhlet apparatus	2.44
Maceration with 96.2 % ethyl alcohol	2.80
Maceration with 70 % ethyl alcohol	3.01
Fruitingphase	
Extraction with 96.2 % ethyl alcohol	1.77
Extraction with 70 % ethyl alcohol	2.01
Extraction with isobutyl alcohol	2.44
Extraction with 96.2 % ethanol on a Soxhlet apparatus	2.64
Maceration with 96.2 % ethyl alcohol	2.91
Maceration with 70 % ethyl alcohol	2.45
Start of withering away phase	
Extraction with 96.2 % ethyl alcohol	2.48
Extraction with 70 % ethyl alcohol	2.79
Extraction with isobutyl alcohol	1.35
Extraction with 96.2 % ethanol on a Soxhlet apparatus	3.85
Maceration with 96.2 % ethyl alcohol	1.84
Maceration with 70 % ethyl alcohol	2.08
Dying out phase	
Extraction with 96.2 % ethyl alcohol	2.09
Extraction with 70 % ethyl alcohol	1.12
Extraction with isobutyl alcohol	2.35
Extraction with 96.2 % ethanol on a Soxhlet apparatus	3.17
Maceration with 96.2 % ethyl alcohol	1.03
Maceration with 70 % ethyl alcohol	1.37

As can be seen from the table above, almost all 6 extracts of the vegetative phase found a high content of ecdysterone, which proves the high efficiency of using *Serratula coronata* L. as the main industrially significant source of the above-mentioned substance.

Thus, the study of seasonal dynamics of ecdysterone distribution under conditions of varying extraction methods has showed that its maximum accumulation is observed during the vegetative phase, and the optimal method in this phase is extraction with isobutyl alcohol, the extract of which contains 13.86 % of ecdysterone and maceration method with 96.2 % ethyl alcohol with a content of 20E 12.03 %, respectively. But, in our view, the maceration with 96.2 % ethanol is optimal, which fully complies with the international standards of good manufacturing practice (GMP) under pharmaceutical production conditions and excludes the use of toxic and expensive isobutyl alcohol solvent.

The relatively low yield of ecdysterone at other stages of development of the taxon under investigation suggests that there is an outflow of ecdysterone to the root system, and then its redistribution occurs as the plant develops further with a partial discharge into the soil.

Thus, we recommend the preparation of the aboveground biomass of *Serratula coronata* L. at the collection site of medicinal plants of the «IRPH «Phytochemistry» for obtaining the ecdysterone substance of many actoprotective phytopreparations and valuable WS based on the data provided by HPLC analysis. In general, a study of *Serratula coronata* L. in the conditions of culture, biological, phytochemical and technological features of plant material with the aim of creating an industrial base of the valuable taxon seems promising.

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Экстракция әдістері мен өсу фазаларын түрлендіру арқылы тәжді түймебас өсімдігінен экдистерон алу технологиясын оңтайландыру

«Фитохимия» ХҒӨХ» (Қарағанды қ.) дәрілік өсімдіктер коллекциялық учаскесінде дақылға енгізілген тәжді түймебас *Serratula coronata* L. өсімдігінің жер үсті бөлігіне, негізгі әсер етуші компонент экдистерон (20Е) мөлшерін әртүрлі өсу фазалары және оңтайлы әдістер арқылы бөліп алып аңықтау үшін кешенді зерттеу жүргізілді. Экдистеронды бөліп алу әдістерін түрлендіру жағдайында таралуының мезгілдік динамикасын зерттеу оның барынша жинақталуы вегетация фазасында байқалатынын, ал осы фазадағы оңтайлы әдісіне экстрактта 13,86 % экдистерон болатын изобутил спиртімен экстракция және 20Е мөлшері 12,03 % болатын 96,2 % этил спиртімен мацерациялау әдісі жататынын көрсетті. Технологиялық оңтайлы әдіс ретінде фармацевтикалық өндіріс жағдайында дұрыс өндірістік тәжірибенің (GMP) халықаралық стандарттарына толық сәйкес келетін және зарарлы әрі қымбат еріткіш изобутил спиртін қолдануды қажет етпейтін тәжді түймебасты 96,2 % этил спиртімен мацерациялау әдісі көрсетілді. Экдистерон мөлшерінің вегетация басынан соңғы фазасына дейін төмендейтіні жоғарыэффектілі сұйықтық хроматография (ЖЭСХ) әдісі арқылы дәлелденді. Экдистеронның тамырлық жүйеге қарай жылжуы, ал оның кейін өсімдіктің одан әрі даму кезеңінде біраз бөлігінің топыраққа өтуі арқылы қайта таралуы болжанды. ЖЭСХ әдісі арқылы мақсатты компоненттің сандық құрамы бойынша мәліметтер негізінде, экдистеронды көптеген актопротекторлы фитопрепараттар субстанциясын және бағалы стандарттық үлгіні алу үшін тәжді түймебастың жер үстілік биомассасын дайындауды осы таксонның вегетация фазасында жүргізілуі ұсынылды.

Кілт сөздер: *Serratula coronata* L., жер үсті бөлігі, 20-гидроксиэкдизон, жоғарытиімді сұйықтық хроматография, экстракция, этил спирті, өсу фазалары, вегетация.

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Оптимизация технологии получения экдистерона из серпухи венценосной варьированием методов экстракции и фаз произрастания

Проведено комплексное исследование надземной части серпухи венценосной *Serratula coronata* L., культивируемой на коллекционном участке лекарственных растений МНПХ «Фитохимия» (г. Караганда), в различных фазах произрастания и с применением наиболее оптимальных методов извлечения на содержание основного действующего компонента экдистерона (20Е). Изучение сезонной динамики распределения экдистерона в условиях варьирования методов извлечения показало, что максимальное его накопление наблюдается в фазу вегетации, а оптимальным способом в данной фазе являются экстракция изобутиловым спиртом, в экстракте которого содержится 13,86 % экдистерона, и метод мацерации 96,2 % этиловым спиртом с содержанием 20Е 12,03 % соответственно. Показано, что технологически оптимальным является метод мацерации серпухи венценосной 96,2 % этиловым спиртом, который полностью соответствует международным стандартам надлежащей производственной практики (GMP) в условиях фармацевтического производства и исключает использование токсичного и дорогостоящего растворителя — изобутилового спирта. Установлено, что содержание экдистерона от начала вегетации до завершающей фазы идет на спад, что подтверждается данными высокоэффективной жидкостной хроматографии (ВЭЖХ). Предполагается, что имеет место отток экдистерона в корневую систему, а затем происходит его перераспределение по мере дальнейшего развития растения с частичным сбросом в почву. На основе данных по количественному содержанию целевого компонента методом ВЭЖХ рекомендуется для получения экдистерона — субстанции многих актопротекторных фитопрепаратов и ценного РСО заготовку надземной биомассы серпухи венценосной вести в фазе вегетации указанного таксона.

Ключевые слова: Serratula coronata L., надземная часть, 20-гидроксиэкдизон, высокоэффективная жидкостная хроматография, экстракция, этиловый спирт, фазы произрастания, вегетация.

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