https://doi.org/10.18321/ectj766

Production Technologies of Pharmacologically Active Sesquiterpene Lactones

A.S. Kishkentayeva[1*], S.M. Adekenov[1], P.B. Drašar[2]

[1]JSC International Research and Production Holding "Phytochemistry", Gazalieva, 4 str., Karaganda, Kazakhstan
[2]University of Chemistry and Technology, 166 28 Prague, 6 – Dejvice, Czech Republic

Article info

Received:
13 April 2018

Received in revised form:
17 June 2018

Accepted:
21 August 2018

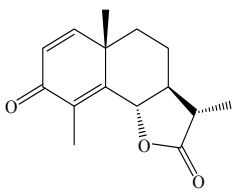
Keywords:
sesquiterpene lactone
α-santonin
alantolactone
isoalantolactone
tauremisin
arglabin
artemisinin
thapsigargin
trilobolide production technology

Abstract

Sesquiterpene lactones form a large group belonging to natural terpenoids series, generally found in plants of *Asteraceae family* and exhibiting anti-tumor, antiviral, immunostimulant, antifungal, antimicrobial, anti-inflammatory, antimutagenic, growth stimulating, antifeedant effects. Therefore, search for new compounds with a broad spectrum of pharmacological activity in this series provides the opportunities for effective and conceptually new drug design. The basis of the technology for the isolation of sesquiterpene lactones is the extraction of raw materials with various organic solvents, followed by chromatographic purification. Sesquiterpene lactones have no common properties that can be used in their isolation. Some of them are well soluble in non – polar solvents, others-only in polar, in this regard, the methods of isolation of sesquiterpene lactones are diverse. The greatest number of sesquiterpene lactones is isolated from leaves and flowers, slightly less – from roots and bark. Therefore, the development of methods for their isolation is associated with the selection of solvents and optimization of the extraction mode. Unfortunately, very few medicines based on sesquiterpene lactones are produced by the pharmaceutical companies today. Complexity of introduction of pharmacologically active sesquiterpene lactones technology into pharmaceutical production is in imperfection of their isolation methods from plant raw material, their purification and separation from obtained extracts. Production technologies of the patented medicines "Santonin", "Alanton" on the basis of sesquiterpene lactones are multiphase, labor-intensive, implying the use of many toxic organic solvents which is against the international GMP standards.

Introduction

The first sesquiterpene γ-lactone used in medical practice as original antihelminthic drug was α-santonin (1). The organization of pharmaceutical plant in the Southern Kazakhstan was caused firstly by source of santonin raw materials (1), namely the availability of industrial stocks of *Artemisia cina* Berg.



(1)

The technological process of santonin (1) production consists of 5 stages [1].

During the first stage anthodia of *Artemisia cina* Berg. are soaked in water and mixed with the lime containing at least 60% of calcium oxide. α-Santonin is dissolved in alkalis with the lactone ring opening and forms a salt of santonic acid.

During the second stage a sevenfold alkaline washing of santonic acid calcium salt by water occurs. The content of α-santonin in the extract varies from 0.7 to 1.4%.

During the third stage after completion of extraction and discharge of the extract into an extractor, sharp steam is applied and essential oil is distilled with water vapor. The received oil is settled and dried up over sodium sulfate. Once the distillation of essential oil is over, the extractor is

*Corresponding author. E-mail: phyto_pio@mail.ru

unloaded. The yield of α-santonin during the extraction stage is 95%.

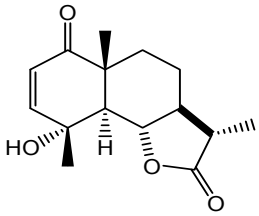
During the fourth stage the concentrated extract, which contains santonic acid calcium salt, pitch, and other extractives, is acidified by nitric acid. Thus, calcium nitrate and santonic acid are formed, latter of which gradually transforms into α-santonin. Raw α-santoninis tenfold washed out with water to receive a neutral reaction, then centrifuged, transferred to a drying cabinet, and dried at a temperature of 66–68 °C. The yield at this stage is about 80%.

The fifth production stage implies purification of technical α-santonin with the repeated crystallization from ethyl alcohol and further treatment on a custom designed filtering apparatus. After crystallization of α-santonin is centrifuged and washed out with the distilled water. The yield of pure α-santonin at this stage is 80–84%. Total yield of α-santonin depends on the content of target compound in the source raw materials and on the number of performed recrystallizations.

Thus, production technology of sesquiterpene lactone α-santonin is labor-intensive, using a multiple process for isolation. Due to the creation of comparative effective antihelminthic drugs α-santonin was withdrawn from production.

Another sesquiterpene of γ-lactone has used in practical medicine as cardiotonic agent has become eudesmanolide tauremisin (2) [2].

K.S. Rybalko with coauthors [2] suggested production method of tauremisin (2) from *Artemisia taurica* Willd.



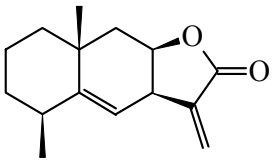
(2)

The crushed and air dry raw materials of *Artemisia taurica* Willd. are extracted with hot water at a temperature of 75–80 °C within 30 min. Then water extraction is treated with chloroform, then poured together and evaporated. Sulfuric ether is added to the received thick dark brown crystallizing substance till the setting of settlement stopped. The settlement was dissolved in chloroform, twice washed with 5% Na_2CO_3*$10H_2O$ solution to separate resinous substances of an acid nature, and twice with water. Chloroform is distilled, the residue is dissolved in alcohol, activated charcoal is added, and boiled within 5 min, then filtered. After cooling the crystals are sucked away and repeatedly recrystallized from alcohol. Tauremisin yield is 0.2% in calculation for air-dry raw materials.

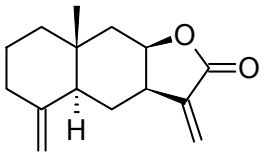
"Alanton" medicine, which contains in its content at least 95% of the total amount of sesquiterpene lactones alantolactone (3) and isoalantolactone (4) from *Inula helenium* L., is used forulcer treatment [3].

Sesquiterpene lactones alantolactone (3), isoalantolactone (4) are usually isolated from plant raw materials by extraction with various organic solvents, e.g. 85%-ethyl alcohol [3], acetone [4], chloroform, benzene, petroleum ether [5], ethanol and hexane mixture (1:4) [6], the ultrasound was also suggested when treating roots with an extracting agent [5, 7]. A column chromatography on Al_2O_3, silica gel, and silica gel additionally treated with 15%$AgNO_3$ is widely used for partitioning [5].

However, use of toxic solvents such as chloroform, benzene is against GMP standards.



(3)



(4)

Production of “Alanton” substance from the dry crushed roots and rhizomes of *Inula helenium* L. proceeds the following way [3]:

• extraction is carried out by percolation with 85% ethyl alcohol (with the ratio 1:10 of raw material to extracting agent);

• the received extract is evaporated under vacuum, then pour together the water residues. Terpenoid fraction is extracted from the water residue by methylene chloride. The methylene chloride extracts are discharged, poured together, dehydratedby the calcined sodium sulfate within 5–6 h, filtered and evaporated to receive 1/4 from the initial volume;

• the obtained solution is purified by column chromatography on aluminum oxide. Elution is done by methylene chloride, then the received eluate is evaporated till the solvent is fully removed. The residue is a thick dark yellow substance;

• as the final stage of Alanton production,10-fold of ethyl alcohol (96%) is added to the residue, mixed, cooled up to 0–5 °C, and allowed to remain for 24 h till the full sedimentation. Then it is

filtered and washed out by the quadruple amount of gasoline cooled up to 0–5 °C on the filter. The settlement is being dried during 10–12 h. Dried alanton is crushed in a ball mill, sifted and packed up. Alanton yield is 1.3% in terms of air dry raw materials. The content of sesquiterpene lactones in the drug should be not less than 95%. The disadvantages of this production method are its time consumption and low yield of a target product.

Babayev N.F. and Serkerov S.V. [6] offered an alternative method of Alanton substance production. The production technology to receive the sum of sesquiterpene lactones alantolactone (3) and isoalantolactone (4) from plant raw materials according to this method is carried out by 2-fold extraction from roots and rhizomes of *Inula helenium* L. by mixture of ethanol and hexane (1:4) when boiling with the backflow condenser, the weight ratio of roots and rhizomes with extracting agent is1:5 for 3 h. The obtained extracts are poured together, filtered from mechanical impurities, in doing so they precipitate out. The maximum amount of sesquiterpene lactones crystals is formed within 24 h at room temperature. They are yellowish crystals of the purified sum of sesquiterpene lactones alantolactone (3) and isoalantolactone (4) with a quantitative ratio 2:1 and amelting point of 86–88 °C. When using this method, isolation time of lactones is reduced from 192 to 58 h, whereas the yield increases up to 1.7–1.9%. Plekhanova N.V. et al. [4] suggested a production method of alantolactone from the roots of *Inula macrophylla* Kar. et Kir. (= *I. grandis Schrenk*) which includes the following stages:

• extraction by acetone within 4 h at room temperature (at the ratio 1:5 of raw material to extractant);

• evaporation of extract to receive a thick mass;

• treatment of the received thick extract with a mixture of petroleum ether: benzene (7:2.8) at a ratio of the sum of extractives and mixture (9:1). Solvents mixture is distilled up to 40% of the volume, and crystals are separated. Alantolactone yield from *Inula macrophylla* Kar. et Kir. is 1.12%.

Thus, the proposed production method of alantolactone (3) from *Inula macrophylla* Kar. et Kir. has a number of advantages, for example: considerable simplification of the process and short cutting some operations (water vapor distillation, using hot acid and alkali).

The disadvantage of the proposed method is the use of flammable solvents of acetone and petroleum ether, as well as toxic benzene.

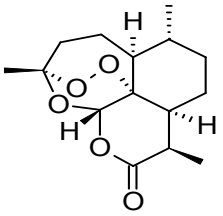
The production technology of alantolactone reference sample (3) has been developed which includes the following stages: extraction of essential oil from rhizomes and roots of *Inula helenium* L. by distillation with water vapor with simultaneous extraction in chloroform; isolation of the sum of sesquiterpene lactones by column chromatography on silica gel (eluent-mixture petroleum ether-ethyl acetate 9:1) [8].

Trenafilova A. et al. [7] conducted chemical studies of the roots of *Inula helenium* L. using the ultrasonic extraction. Based on the results of these experiments, the optimal parameters for a quantitative yield of alantolactone (3) and isoalantolactone were determined (4). 70% Ethyl alcohol was used as an extractant, ultrasonic treatment was done within 30 min at extraction temperature 25 °C. As a result, alantolactone (3) and isoalantolactone (4) were isolated from *Inula helenium* L. extract with the yield 18.04 and 12.77 mg/g, respectively.

In Kharkiv experimental plant GNCLS (Ukraine) technology of production of "Alanton" includes extraction of raw roots and rhizomes of *Inula helenium* L. by gasoline and infused with occasional stirring for 24 h. Then the gasoline extract (the first sink) is pumped into the container, and the raw material is re-filled with fresh solvent and insisted again, then the extract (the second sink) is combined with the previous one. This extraction operation is carried out three times, as a rule. Then, the solvent is distilled off, and the remaining evaporated extract (cube concentrate) is dissolved in a 4.5-fold volume of 95% alcohol, treated with activated carbon, and filtered. After filtering, the so-called alcoholic mother liquor is evaporated to half volume and the Alanton crystallizes at a temperature of 8–10 °C. The product is air dried and then washed with cold gasoline to remove yellow impurities, and a white or white crystalline substance with a yellowish hue [9] is obtained.

Summarizing the literature data on the methods for the isolation of alantolactone and isoalantholactone from the roots of *Inula helenium* L., it can be noted that the preferential method is an alcohol extraction using ultrasonic treatment, which makes it possible to avoid using costly organic solvents.

One of the medicines widely studied in a number of world scientific centers is an antimalarial "Qinghaosu" drug developed by the Chinese scientists based on sesquiterpene lactone artemisinin (5) which had been isolated from *Artemisia annua* L. [10].



(5)

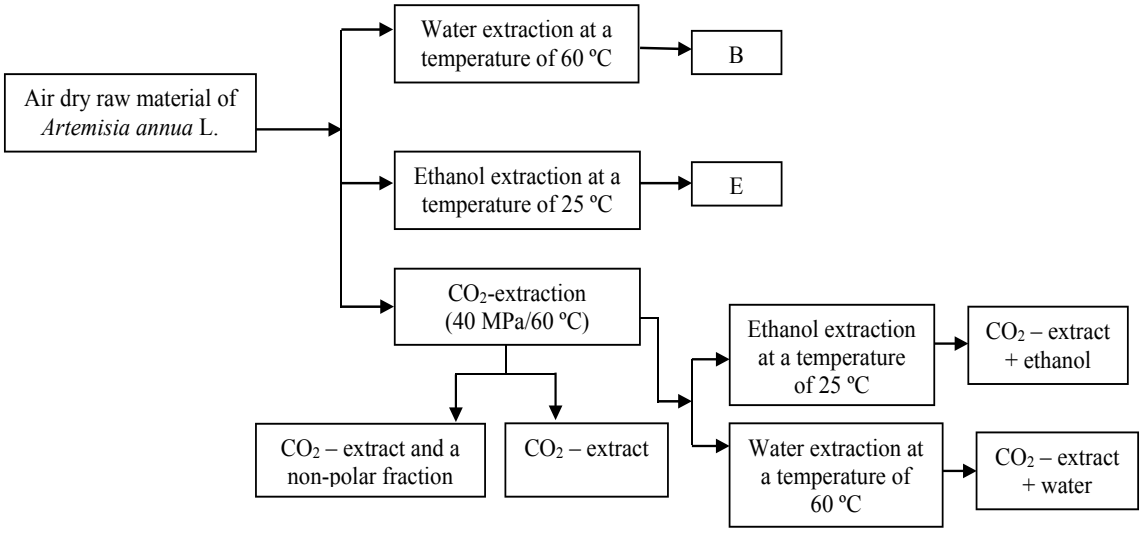
Martinez-Correa H.A. et al. [11] carried out extraction from the aerial parts of *Artemisia annua* L. using two methods. In the first case, various solvents were used: carbon dioxide CO_2 (40 MPa/60 °C), ethanol (25 °C) and water (60 °C). In the second case, two-phase extraction was carried out as is shown: at first carbon dioxide CO_2 extraction (40 MPa, 60 °C), and then extracted with the use of ethanol at 25 °C or water at 60 °C (second step) under atmospheric pressure (Scheme). Ethanol extracts were received the following way: 3 g of raw materials (thick extract after SFE) were dissolved in 10 ml ethanol at a temperature of 25 °C within 42 h and mixed in a shaker. Then the mixture was filtered and repeatedly extracted in 10 ml ethanol on the centrifuge at gravitational acceleration 2543, 25 °C within 5 min. Water extracts were received the following way: 3 g of raw materials (thick extract after SFE) dissolved in 60 ml of water. Mixture was stirred within 10 min at 60 °C, then on the centrifuge within 10 min at centrifuge at gravitational acceleration 10174. After vacuum filtration they received the extract. For supercritical process, 7 g of raw materials were used; extraction was carried out with the following parameters at 40 MPa, 60 °C and 4×10^{-5} kg/s of CO_2. Two fractions of supercritical extract were received: extract fraction and a non-polar fraction. The content of artemisinin in extracts and raw materials of *Artemisia annua* L. is given in Table 1.

Table 1
The content of artemisinin in extracts and *Artemisia annua* L. raw materials

Samples		Artemisinin	
		Content (mg/g in extract)	Content (mg/g in raw material)
Single-step extraction	CO_2 – extract	95.1	5.47
	CO_2 – extract + a non-polar fraction	-	-
	Aqueous extract (B)	-	-
	Ethanol extract (E)	95.6	5.49
Two-step extraction	CO_2 – extract + water	-	-
	CO_2 – extract + ethanol	-	-

It is clear from the data provided in Table 1 that the yield of artemisinin is comparable in the received CO^{2-}, and ethanol extracts.

Research of Martinez-Correa et al. [11] provides comparative data on extraction of artemisinin from *Artemisia annua* L. using the following methods: maceration, ultrasonic, supercritical, and microwave extraction by carbon dioxide (Table 2).



Scheme of extraction process from *Artemisia annua* L .plant raw materials.

Table 2
The content of artemisinin in *Artemisia annua* L. raw materials

Extraction type	Extracting agents	Extraction conditions	Yield of artemisinin (mg/g in raw materials)	References
1	2	3	4	5
InSoxhlet	Hexane	-	9.7 and 7.7	[12]
	n-hexane, petroleum ether, water, methanol, ethyl acetate, *n*-hexane and ethanol	60-80 °C, 2-20 h	6.0-6.2 1-12	[13-15]
Supercritical extraction	CO_2	300 bar, 50 °C	7	[13]
	CO_2	150 bar, 30 °C	6.2	[13]
	CO_2	17.3-31.1 MPa-40-60 °C	2.1-6.7	[12]
	CO_2	100 bar, 40 °C	9.5	[16]
	CO_2-ethanol (16.25%)	17.3- 31.3 MPa-40-60 °C	7.8-11.5	[15]
	CO_2-ethanol (1, 3, 5%)	150 bar, 50 °C	8	[17]
	CO_2-ethanol (20%)	31.3 MPa, 50 °C	6.7	[12]
	CO_2-hexane (16.25%)	7.0-20.8 MPa-30-50 °C	1.4-8.8	[12]
	CO_2-methanol (1, 3, 5 and 10%)	150 bar, 50 °C	6	[17]
	CO_2-toluene (1, 3, 5 and 10%)	150 bar, 50 °C	6.5	[17]
	CO_2-methanol-water (1, 3 and 5%)	150 bar, 50 °C	7	[17]
Ultrasonic extraction	Hexane	25-45 ºC/15-120 min	Not isolated*	[18]
	Petroleumether	30-60 ºC /120-300 W	4.2-7.4	[19]
Microwave extraction	Cyclohexane, hexane, petroleum ether, ethyl acetate, chloroform, acetone, methanol, acetonitrile	160W, 60 s	18.8-6.6	[14]

* – The content of artemisinin was defined according to HPLC analysis.

As it`s clear from Table 2, comparative quantitative yield of artemisinin is observed at extraction by hexane (in Soxhlet apparatus) and liquid carbon dioxide (CO_2).

Authors [18] proved that ultrasonic extraction from *Artemisia annua* L. raw materials by hexane at the following parameters: 40 kHz, 25 °C, 60 min, allowed them to receive a higher yield (by 58% higher, according to HPLC analysis) of artemisinin (5) in contrast with the usual soaking under the same conditions. It should be noted that if we increase time up to 120 min, there will be a decrease of artemisinin content.

Authors of the work [20] developed a method for the production of artemisinin (5) without column chromatography, for this purpose ultrasonic extraction of raw *Artemisia annua* L. with ethyl alcohol was performed. Extraction was carried out on an ultrasonic extractor from China Ningbo Zhenguo Pharmaceutical Equipment Manufacturing Co. model TCLX200, by the following technology: dry, crushed to the size of 60–80 mesh, the raw material is placed in an ultrasonic extractor, filled with 80% ethyl alcohol (hydromodule 1:17) and extracted at a frequency of 35 kHz, power at 1000 W, temperature at 40 °C for 30 min. The obtained extract was filtered, petroleum ether was added to the filtrate in a 5:1 ratio and treated at a radiation frequency of 30 kHz, a power of 1000 W, a temperature of 25 °C for 15 min. The ether layer was separated, passed through a column filled with activated carbon and concentrated under reduced pressure, the concentrate was poured into a crystallizer. The fallen artemisinin crystals are recrystallized from 80% ethyl alcohol. The degree of extraction of artemisinin from the raw material by this method is 97.25%.

Various methods for isolating artemisinin from *Artemisia annua* L. are given in the available patent and scientific and technical literature.

Number of companies [21–24] developed several methods for extracting artemisinin from raw materials using organic solvents, such as: hexane, mixtures of ethyl acetate: No. 6 Extraction Solvent Oil and petroleum ether: gasoline under vacuum, and other companies of China, Italy, and researchers from Germany [25–31] used as an extragent: butane, CO_2-gas, supercritical carbon dioxide and water, supercritical carbon dioxide in combination with microwave extraction, and also supercritical carbon dioxide with a modifier (ethyl alcohol). Scientists from Yunnan Normal University proposed a method for obtaining artemisinin without extraction [32]. Sanofi (France) has developed a longer-term method for producing artemisinin in a semisynthetic way from dihydroartemisinic acid [33].

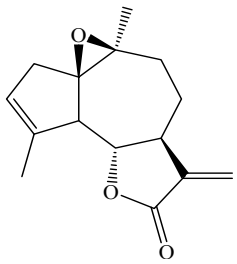
The main manufacturers of artemisininin China are: Novanat Bioresource Co Ltd., Guilin Pharmaceutical Co Ltd., Chongqing Kerui Pharmaceutical Ltd., Shanghai Natural Bio-Engineering Co Ltd. in China; Vedic Fanxipang Pharma Chemic Co Ltd., Mediplantex National Pharmaceutical Ltd., Saokim Pharmain Vietnam; Botanical Extracts EPZ Ltd. in Kenya, BIONEXX in Madagascar, Sanofi Aventisin France, Ajanta Pharma Ltd., Calyx Chemical and Pharmaceuticals Ltd. in India [10].

Thus, different effective methods are used for production of artemisinin from *Artemisia annua* L., in particular, the use of extraction by supercritical carbon dioxide combined with microwave treatment of 70% ethanol, and without the use of column chromatography of the target substance with supercritical carbon dioxide, followed by recrystallization from hot 75% ethanol.

Based on a sesquiterpene lactone arglabin (6), the original drug Arglabin was developed in the International Research and Production Holding "Phytochemistry" and is currently produced at Karaganda Pharmaceutical Plant. The production method of Arglabin was patented in 11 countries of the world, namely Japan, China, the USA, Great Britain, Germany, Switzerland, France, Austria, Italy, the Netherlands and Sweden [34].

In IRPH "Phytochemistry" an effective and environmentally-friendly technology of isolation and purification of the sesquiterpene lactone arglabin from *Artemisia glabella* Kar. et Kir was developed according to GMP requirements. It was experimentally determined that isolation technique of arglabin from carbon dioxide extract of *Artemisia glabella* Kar. et Kir. using a centrifugal partitioning chromatography is optimal for the preparative production and deployment in the manufacture of substance on its basis.

At the same time, the optimal parameters of the extraction of *Artemisia glabella* Kar. et Kir. raw material were determined using CO_2-gas in the supercritical state, providing a quantitative yield of arglabin (6).



(6)

The use of supercritical carbon dioxide extraction from *Artemisia glabella* Kar. et Kir. herb for extraction of arglabin has significant advantages in comparison with chloroform extraction (Table 3).

To increase the productivity, automatize, reduce the process duration, and exclude toxic solvents, a production technology of arglabin native substance has been developed (6) which involves the centrifugal partitioning chromatography [35].

Initially, arglabin was isolated from CO_2-extract of *Artemisia glabella* Kar. et Kir. with a centrifugal partitioning chromatography in two stages:

1. Purification using accelerated centrifugal chromatography of FCPC-5000 distribution.

2. Recrystallization of technical arglabin.

It has been experimentally determined that the method of arglabin extraction from CO_2 extract of *Artemisia glabella* Kar. et Kir. with the use of centrifugal chromatography of the distribution, which characterized by better productivity, full automation, shorter duration of the process in comparison with column chromatography. This method does not require the use of sorbents and high-purity solvents. Thus, the developed technology made it possible to ensure the yield of arglabin from the CO_2 extract to 30% and more than 2% of the plant material with a purity of the target substance of at least 99.0%.

The developed technology of isolation and purification of sesquiterpene lactone arglabin is introduced by the Karaganda pharmaceutical plant for the production of the original drug "Arglabin".

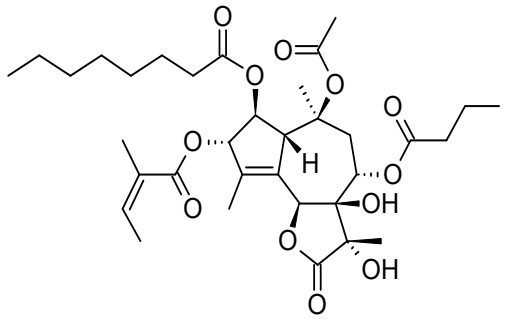
Thapsigargin (7) is sesquiterpene lactone isolated from *Thapsia garganica* L. [36, 37], the increasing interest in it arose with the discovery of its

Table 3
Comparative characteristics of extraction techniques of arglabin from *Artemisia glabella* Kar. et Kir. herb

Extraction technique	Extract yield and quantitative content of arglabin						
	Percentage of arglabin isolation,%	Extract yield		Content of arglabin in extract		Residual content of arglabin in a solvent cake	
		g	%	g	%	g	%
Carbon dioxide	92.4	45.6	4.6	13.8	30.2	0.08	0.09
Chloroform	78.0	150.0	15.0	11.6	7.8	2.28	0.26

ability to inhibit the sarco-endoplasmic reticulum calcium ATPase (SERCA) pump. The inhibition of this pump produces a high concentration of calcium in the cytosol, which leads to apoptosis. Several analogues of thapsigargin have been obtained, and a prodrug, thapsigargin peptide conjugate (Mipsagargin), has been designed. At the present, it undergoes clinical trials as antitumor agent.

Isolation and purification of thapsigargin are held using both classical and modern methods of extraction and chromatographic purification.



(7)

Appendino G. et al. [38] isolated sesquiterpene lactone thapsigargin from roots of *Thapsia garganica* L. by the following method: the powdered roots were extracted three times with acetone, the extracts were combined and evaporated. The thick extract was chromatographed on a column of silica gel with petroleum ether-ethyl acetate (7:3) and thapsigargin with a yield of 1.3% per the air-dry raw material was obtained.

Authorized system of the thapsigargin production from acetone extract of the aerial part of *Thapsia garganica* L. using the Speed-Extractor E-914 and centrifugal chromatography of the distribution was developed by the authors [39]. The development of thapsigargin is carried out in two ways, differing in the extraction stage, according to the following scheme:

a) Extraction by maceration: the air-dry raw material is extracted twice with acetone at room temperature for 12 h. The obtained extracts are combined and evaporated. The extract yield is 2.46% per air-dry raw materials.

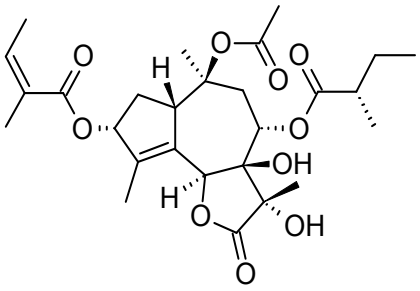
b) The air-dry raw material is being extracted twice with acetone in the accelerated extractor E-914 for 20 min at a pressure of 10 MPa. The obtained extract is evaporated. The yield of the extract is 2.64%.

Separation of extracts is carried out by liquid-liquid chromatography method, for this purpose the sample of extract is dissolved in mixture of mobile and stationary phases (1:1, concentration is 0.25 g/ml). Separation is carried out in solvents system composed of cyclohexan-ethylacetate-methanol-water (19:1:15:5), downwards, with the following parameters: elution rate from 5 to 13 ml/min, centrifuge at gravitational acceleration 140 to 358. When separating the extract obtained by the maceration method, thapsigargin is isolated with a yield of 1.67% per air-dried raw materials. Separation of the extract obtained using an accelerated extractor produces thapsigargin with a yield of 1.46% per air-dry raw materials.

Thus, methods using classical extraction and chromatographic separation methods that do not yet provide a quantitative yield of the target compound predominate in the isolation and purification of the sesquiterpene lactone thapsigargin.

Similarly, “brother” of thapsigargin, trilobolide (8) sesquiterpene lactone with similar biological properties, was prepared by classical extraction methods [40, 41] of the plant material (roots and seeds) by ethyl acetate, after prepurification of the material by petroleum ether. Alternatively, the patent [40] claims the extraction with supercritical carbon dioxide in fluid state doped with ethanol, performed in a specialized commercially available device. The SFE preparation with carbon dioxide doped with ethanol was later further developed using laboratory and semi-pilot plant

professional equipment [42]. After both methods of isolation the crude product is effectively purified by crystallisation from several solvent mixtures.



(8)

Conclusions

Most methods of isolation sesquiterpene lactones from plant extracts use traditional column chromatography on silica gel with the subsequent re-chromatography of the obtained fractions is used together with the preparative high performance liquid chromatography, what makes the cost of received substances more expensive.

Based on the conducted literature review, it is apparent that besides the efficiency and advantages of supercritical fluid, microwave and ultrasonic extraction techniques, their deployment definitely reduces the cost-effectiveness of sesquiterpene lactones production process.

Thus, the application of innovative methods to isolate and purify sesquiterpene lactones, such as supercritical fluid extraction, ultrasonic extraction, liquid-liquid chromatography, helps to simplify technological processes, reduce production costs, thereby to increase labor productivity and reduce the cost of the original drug.

References

[1]. S.A. Minina, I.E. Kaukhova Chemistry and technology of phytopreparations. Publishing house, Moscow, 559 p.

[2]. USSR Patent No.202468 Production method of tauremisin. K.S. Rybalko, A.I. Bankovsky, R.I. Evstratova, V.A. Burnt. Application 13.05.1963, issued 23.11.1967. Bulletin No. 195.

[3]. USSR Patent. No.577034. Production method of sesquiterpene lactones. P.P. Khvorost, D.G. Kolesnikov, N.F. Komisarenko, G.V. Oboleshcheva, A.I. Vidyukova, Ya.I. Khadzhai, M.M. Luchkova, V.P. Georgievsky, L.D. Degtyarov, V.V. Zinchenko. Application 15.04.1976, issued 25.10.1977. Bulletin No. 39.

[4]. USSR Patent No.727646 Production method of alantolactone. N.V. Plekhanova, S.A Lugovskaya, G.P. Fedorchenko. Application 02.02.1978, issued 15.04.1980. Bulletin No. 14.

[5]. I.A. Milman, *Chem. Nat. Compd.* 3 (1990) 251–262. DOI: 10.1007/BF00597842

[6]. USSR Patent No.1710062 Production method of sesquiterpene lactones. N.F. Babayev, S.V. Serkerov. Application 09.01.1990, issued 07.02.1992. Bulletin No. 5.

[7]. A. Trendafilova, Ch. Chanev, M. Todorova, *Pharmacogn. Mag.* 6 (2010) 234–237. DOI: 10.4103/0973-1296.66942

[8]. K.V. Belyakov, D.M. Popov, *Pharmacy* 1 (2004) 37–38.

[9]. V.P. Georgievsky. Technology and standardization of medicines. Kharkiv, Rireg, 1996, 749 p.

[10]. I.A. Khabarov, *Farmacevticheskij bjulleten'* [Pharmaceutical Bulletin] 1-2 (2016) 89–101 (in Russian).

[11]. Hugo A. Martinez-Correa, Raphaela G. Bitencourt, Anna Carolina A.V. Kayano, Pedro M. Magalhães, Fabio T.M. Costa, Fernando A. Cabral, *Ind. Crop. Prod.* 95 (2017) 535–542. DOI: 10.1016/j.indcrop.2016.11.007

[12]. Y.-L. Lin, C.-C.Yang, H.-K.Hsu, S.-L. Hsu, C.-M.J. Chang, J. Supercrit, *Fluids* 39 (2006) 48–53. DOI: 10.1016/j.supflu.2006.02.012

[13]. S. Quispe-Condori, D. Sanchez, M.A. Foglio, P.T.V. Rosa, C. Zetzl, G. Brunner, M.A.A. Meireles, *J. Supercrit. Fluids* 36 (2005) 40–48. DOI: 10.1016/j.supflu.2005.03.003

[14]. H. Misra, D. Mehta, B.K. Mehta, D.C. Jain, *Organic Chemistry International* (2013) Article ID 163028. DOI: 10.1155/2013/163028

[15]. T.C. Tzeng, Y.L. Lin, T.T. Jong, C.M.J. Chang, *Sep. Purif. Technol.* 56 (2007) 18–24. DOI: 10.1016/j.seppur.2007.01.010

[16]. G. Della Porta, E. Reverchon, A. Benakis Extraction and fractionation of antimalaric drugs by supercritical fluid, Ninth Meeting on Supercritical Fluids, 2004, p. 1-6.

[17]. M. Kohler, W. Haerdi, P. Christen, J.L. Veuthey, *J. Chromatogr. A* 785 (1997) 353–360. DOI: 10.1016/S0021-9673(97)00403-2

[18]. R. Briars, L. Paniwnyk, *Ind. Crop. Prod.* 42 (2013) 595–600. DOI: 10.1016/j.indcrop.2012.06.043

[19]. H. Zhang, L. Zhang, X. Hu, Y. Zhou, C. Ding, R. Yang, X. Wang, D. Li, *Sep. Sci. Technol.* 49 (2014) 673–681. DOI: 10.1080/01496395.2013.862545

[20]. CN Pat. 101205232A. Technical new method for extracting artemisinin from sweet wormwood plants by ultrasonic assistance. Guojun Wang, Xiao Ruan, Qiang Wang. Application 19.12.2006, issued 25.06.2008.

[21]. US Pat. 6685972A. Process for isolating

artemisinin from *Artemisia annua*. Kumar Sushil, Gupta Shiv Kumar, Singh Digvijay, Gupta Madan Mohan, Jain Dharam Chand, Kahol Atul Prakash, Khanuja Suman Preet, Singh Ram Govind. Application Number: 10/112070, publ. 02.03.2004.

[22]. CN Pat. 103694249A. Production technology for extracting artemisinin from *Artemisia annua*. Hu Canhua, Lei Yurong. Application 28.12.2013, issued 02.04.2014.

[23]. CN Pat. 102617591A. Method for producing artemisinin from *Artemisia annua* serving as Chinese herbal medicine. Xiao Yuan, Dongbin Zhou, Junfei Gao, Chujin Shu. Application 07.03.2012, publ. 01.08.2012.

[24]. CN Pat. 103664988A. Extraction and separation method for artemisinin. Li Zhipin. Application 25.12.2013, publ. 20.01.2016.

[25]. CN Pat. 102219790A. Green extraction process for artemisinin. Shengqun Huang, Wugou Liu, Zongyan Huang, Huaxing Tan, Xueping Kong. Application 05.05.2011, issued 19.10.2011.

[26]. CN Pat. 104628739A. Extraction technology of artemisinin. Tan Binfeng. Application 09.11.2013, publ. 20.05.2015.

[27]. CN Pat. 1931860A. Process of extracting, separating and purifying artemisinin from sweet wormwood herb. Gang Xu, Liang Deng, Yaping Zhao. Application 28.09.2006, issued 19.08.2009.

[28]. US Pat. 20100331553. Process for manufacturing artemisinin. Villanova Luciano, Villanova Azzurra, Cisale Felicia, Villanova Luigi. Application 25.09.2008, issued 30.12.2010.

[29]. CN Pat. 104140433A. Artemisinin preparation method. Lan He. Application 10.05.2013, issued 12.11.2014.

[30]. CN Pat. 103647480A. Method for extracting artemisinin and application of artemisinin. Nanjing Zelang Medical Technology Co. Ltd. Application 10.10.2013, issued 25.12.2013.

[31]. DE Pat. 10336056 A l. Extracting pharmacological agent from *Artemisia annua*, useful for treating cancer and AIDS in addition to malaria, comprises using carbon dioxide at relatively low temperature and pressure. Doebel Katrin, Sandau Petra, Franke Horst, Pulz Otto. Application 01.08.2003, issued 24.02.2005.

[32]. Pat. CN 103333178A. Method for preparing antimalarial active compound artemisinin through direct column chromatography. Chen Yegao, Ma Yanfang, Cui Guangxin. Application 29.07.2013, issued. 02.10.2013.

[33]. Pat. US 2015328617. Method and device for the synthesis of artemisinin. Seeberger Peter, Kopetzki Daniel, Lirvesque Francois. Application 29.08.2011, issued 19.11.2013.

[34]. Patent USA 6,242,617, B1, Jun.5.2001; Europian Patent № 0946565, 15.10.2003; Deutschen Patent № 697 2504.9-08, 23.10.03; Swiss Patent 97 947 981.3 (CH) EP 0946565; China Patent ZL 2006 8 0055852.4, 26.12.12. S.M. Adekenov Method and device for production of lyophilized hydrochloride-1(10)β-epoxy-13-dimethylamino-5,7α,6,11β(H)-guaia-3(4)-en-6,12-olide.

[35]. RK Patent No. 32482. Method of complex processing of raw wormwood smoothness. S.M. Adekenov, E.G. Tolokonnikov, Kh.I. Itzhanova, A.N. Zhabaeva, V.S. Korneev, I.A. Khabarov. Declared 03.12.2015, publ. 11.15.2017. Bul. No. 21.

[36]. U. Rasmussen, B. Christensen, F. Sandberg, *Acta Pharm Suec.* 15 (1978) 133–140. PMID: 79299

[37]. V.P. Sulsen, V.S. Martino, Sesquiterpene Lactones. Advances in their Chemistry and Biological Aspects, Springer, Cham. DOI: 10.1007/978-3-319-78274-4

[38]. G. Appendino, S. Prosperini, C. Valdivia, M. Ballero, G. Colombano, R.A. Billington, A.A. Genazzani, O. Sterner, *J. Nat. Prod.* 68 (2005) 1213–1217. DOI: 10.1021/np050115m

[39]. A. Ollivier, R. Grougnet, X. Cachet, D. Meriane, J. Ardisson, S. Boutefnouchet, B. Deguin, *J. Chromatogr. B* 926 (2013) 16–20. DOI: 10.1016/j.jchromb.2013.02.015

[40]. Pat. CZ 300806 Immunostimulation properties of trilobolide and a method of its preparation E. Kmoníčková, Z. Zídek, J. Harmatha, M. Buděšínský, K. Vokáč. Application 18.07.2007, issued 02. 07.2009. WO 2009/010021 A1.

[41]. Z. Smítalová Sesquiterpene lactones from Laserpitieae, PhD Thesis, Czechoslov. Acad,. Sci. Prague 1987.

[42]. J. Vyšohlíd Investigation of sesquiterpene derivatives, BSc. Thesis, UCT Prague 2016.