

Biopharmaceuticals of Plant Origine with Health Importance

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Abstract

Original Research Article

Natural biopharmaceutics of plant origin are of great importance for human health and their application is increased in different areas. Important plants with natural biopharmaceutics are: *Galanthus woronowi* Losinsk, *Galanthus nivalis* L., *Leucojum aestivum* L., *Lycoris aurea* L., *Lycoris radiata* L., *Narcissus tazetta* L., *Lactuca serriola*, *Eruca sativa*, *Heliotropium Bacciferum*, *Solanum glaucophyllum*, *Daucos carrota*, *Populus tremuloides*, *Cimicifuga racemosa*, *Trifolium pratense* subsp. *baeticum* (Boiss.) Vicioso, *Trifolium pratense* subsp. *kotulae* (Pawl.) Soják *Trifolium pratense* subsp. *pretense*. Galantamine is a natural alkaloid with an important role in treatment of Alzheimer's disease. Estradiol is used against osteoporosis. Herbal products with *Cimicifuga racemosa* are applied for treating menopausal symptoms and exert anticancer properties. *Trifolium pratense* is applied as expectorant, sedative, and anti-inflammatory agent. In accordance the multi-target therapy of Alzheimer's disease, the most important physical instrumental methods developed for the simultaneous determination of Galantamine hydrobromide and Pymadine in the model mixtures are HPLC method and TLC-densitometric method. The obtained from HPLC method data for limit of detection are $1.08 \cdot 10^{-4}$ g/ml; for Galantamine hydrobromide and $1.32 \cdot 10^{-4}$ g/ml for Pymadine and are lower in comparison with the obtained by TLC-densitometric method data for LOD as $1.87 \cdot 10^{-3}$ g/ml for Galantamine hydrobromide and $2.5 \cdot 10^{-3}$ g/ml for Pymadine. For the determination of Galantamine hydrobromide and Pymadine by HPLC and TLC and for assay of Estradiol valerate by TLC was applied method of calibration curve.

Keywords: Natural biopharmaceutics, Galantamine, Pymadine, Estradiol, HPLC, TLC.

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I. INTRODUCTION

The use of various substances of plant origin leads to the creation of biopharmaceutical products and food supplements, which significantly increase the quality of healthcare.

I. Galantamine in treatment of Alzheimer's disease.

Alzheimer's disease is a chronic neurodegenerative disorder with significant social importance. The dendritic spine loss and synaptic alterations are characteristics of the disease, which leads to the impairment of cognitive function [1]. Other characteristics of Alzheimer's disorder, which cause progressive memory loss include [2]:

- 1) development of senile plaques with high concentration of β -amyloid protein
- 2) neurofibrillary tangles of tau protein
- 3) mitochondrial dysfunction.
- 4) reactive oxygen species, which by the increased oxidative damage, induce the neurodegeneration of brain regions, important for cognitive performance [3].

Galantamine is a natural alkaloid, isolated from bulbs and aerial parts of plants from the family Amaryllidaceae: *Galanthus woronowi* Losinsk (isolated for the 1st time), *Galanthus nivalis* L. (Figure 1.),

Leucojum aestivum L., *Lycoris aurea* L., *Lycoris radiata* L., *Narcissus tazetta* L. [4].



Figure 1: *Galanthus nivalis* L

Synthetic Galantamine (Reminyl) (Figure 2.) was first registered for treatment of Alzheimer's disease

in Sweden in 2000 and is approved for this application in the European Union, USA. Canada and Japan.

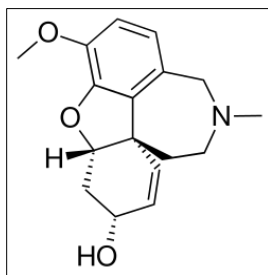


Figure 2: Chemical structure of Galantamine

The important strategy for treatment of Alzheimer's disease with natural compound Galantamine is based on its neuroprotective mechanisms which include:

1. blocking of the acetylcholinesterase [5]
2. increase of acetylcholine [5]
3. inhibition of γ -secretase [5]
4. scavenging of the free radicals [6]
5. stimulation of muscarinic receptors, which leads to the decreasing of A β -generation [5]
6. induction of amyloid- β -clearance by stimulation of microglial nicotinic acetylcholine receptors [7]
7. inhibition of β -amyloid aggregation [8].

It has been reported that the important applications of Galantamine include:

- 1) anesthesiology, physiotherapy, ophthalmology, gastroenterology [9]
- 2) neurology:
 - multiple sclerosis

- myasthenia gravis
 - cerebral paralysis
 - Thomsen's disease (myotonia congenita)
 - Werdnig-Hoffman disease (spinal muscular atrophy) [9]
- 3) mild to moderate Alzheimer's disease [10]
 - 4) Alzheimer's disease with cerebrovascular disease [11]
 - 5) vascular dementia [12]
 - 6) *augmentative therapy in autistic children* [13].

II. Estradiol in plants

Estradiol (Figure 3.) is an *steroid hormone* and is *agonist* of the *estrogen receptors*. Estradiol is involved in the regulation of female *reproductive cycles*. As a medication, primarily is used in hormone therapy for menopausal symptoms. Estrogen therapy [14] is used against osteoporosis [15]. Estradiol alone [16] and with Vitamin D exert a beneficial effect in prevention of osteoporosis [17].

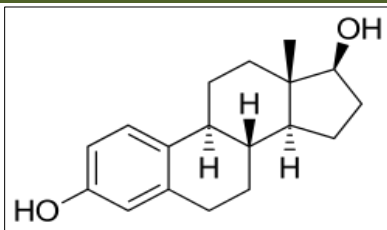


Figure 3: Chemical structure of Estradiol.

The seasonal variation in the 17β -Estradiol concentration in *Populus tremuloides* has been observed, as in winter tissues content is lower than in spring tissues, growing in more intense light [18].

17β -Estradiol has been found in *Lactuca serriola*, *Eruca sativa*, *Heliotropium Bacciferum* [19],

Solanum glaucophyllum [20], *Daucos carrota*, citrus, apple [21], kiwifruit [22].

II. *Cimicifuga racemosa*.

Cimicifuga racemosa (*Actaea racemosa*, black cohosh) (Figure 4.) is a species of the family *Ranunculaceae*. It is native to eastern North America [23].



Figure 4: *Cimicifuga racemosa*.

Extracts from the underground parts of the plant - *Cimicifugae racemosae* rhizoma and the root - *Cimicifugae racemosae* radix are used in traditional medicine [23].

In most European countries, China, Malaysia, Thailand, Argentina, black cohosh products have been approved by regulatory authorities.

In the USA and India a dietary supplement with *Cimicifugae racemosa* are marketed mainly to women for treating menopausal symptoms [24].

In most European countries, China, Malaysia, Thailand, Argentina and some other countries, black

cohosh products are available as herbal products which have been approved by regulatory authorities. In the USA and India a dietary supplement with *Cimicifugae racemosae* are marketed mainly to women for treating menopausal symptoms [24].

The rhizomes and roots contain various triterpene saponins and triterpene glycosides, such as actein and cimicifugoside, as well as cimicifugin, cimifugic acid, caffeic acid, phenolic acids [25].

It has been reported that extracts and the pure triterpene glycoside actein (Figure 5.), obtained from black cohosh inhibit growth of human breast cancer cells [26].

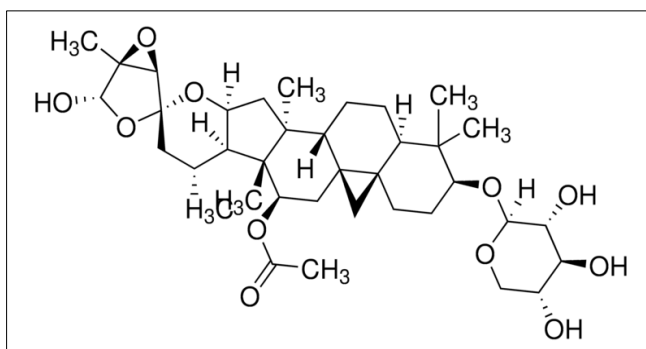


Figure 5: Chemical structure of actein

A triterpenoid Cimicifugoside (Figure 6.), isolated from *Cimicifuga simplex* is a specific nucleoside

transport inhibitor that displays synergistic potentiation of Methotrexate cytotoxicity [27].

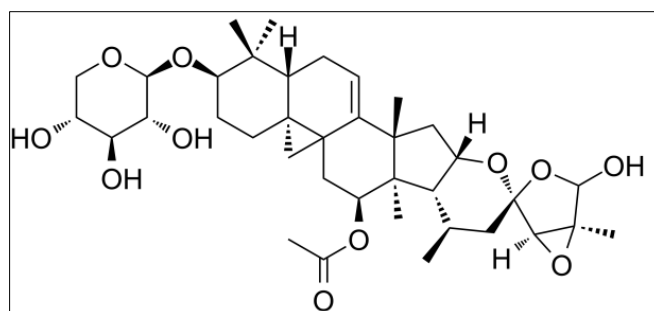
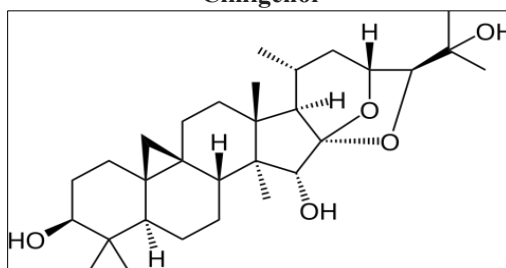


Figure 6: Chemical structure of Cimicifugoside

Cimigenol



Formononetin

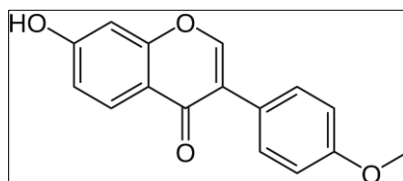


Figure 7. Chemical structures of Cimigenol and Formononetin

III. *Trifolium pratense*.

The genus *Trifolium* has 3 known subspecies:

- *Trifolium pratense* subsp. *baeticum* (Boiss.) Vicioso
- *Trifolium pratense* subsp. *kotulae* (Pawl.) Soják
- *Trifolium pratense* subsp. *pratense* (Figure 8.).



Figure 8: *Trifolium pratense*

Trifolium pratense is native to Europe, Western Asia, northwest Africa, North and South America. In the traditional medicine of India, *Trifolium pratense* is applied as antispasmodic, expectorant, sedative, anti-inflammatory and antidermatosis agent. In alternative medicine, red clover is used for treatment of symptoms

of menopause, coughs, disorders of the lymphatic system and a variety of cancers [28].

V, Instrumental methods developed for the determination of some biopharmaceuticals of plant origine with health importance

In accordance the multi-target therapy of Alzheimer's disease, the most important physical instrumental methods developed for the simultaneous determination of Galantamine hydrobromide and Pymadine in the model mixtures are HPLC method and TLC-densitometric method.

1. HPLC method for the simultaneous determination of Galantamine and Pymadine in the model mixtures [29].

It has been reported that a HPLC method for the simultaneous determination of Galantamine and Pymadine in the model mixtures has been reported.

In this method the HPLC conditions used include:

- 1) column RP C₁₈ ODC Spherisorb, column temperature: 25 °C
- 2) mobile phase: 50 mM disodium hydrogenphosphate: acetonitrile = 80 : 20 v/v
- 3) isocratic mode with flow rate: 1.5 ml/min.

4) UV-detection ($\lambda = 280$ nm).

The obtained data for the analytical parameter retention time, which is used for the identification of the compounds are:

$t_R = 3.179$ (Galantamine hydrobromide) and $t_R = 5.272$ (Pymadine).

The regression equations obtained demonstrated the linear relationship between the peak area and concentration:

$y = 1.1010 \cdot x + 387106$ (Galantamine hydrobromide)
 $y = 9.109 \cdot x + 1.106$ (Pymadine).

Chromatograms of model mixtures, containing Galantamine hydrobromide and Pymadine is illustrated in Figure 9.

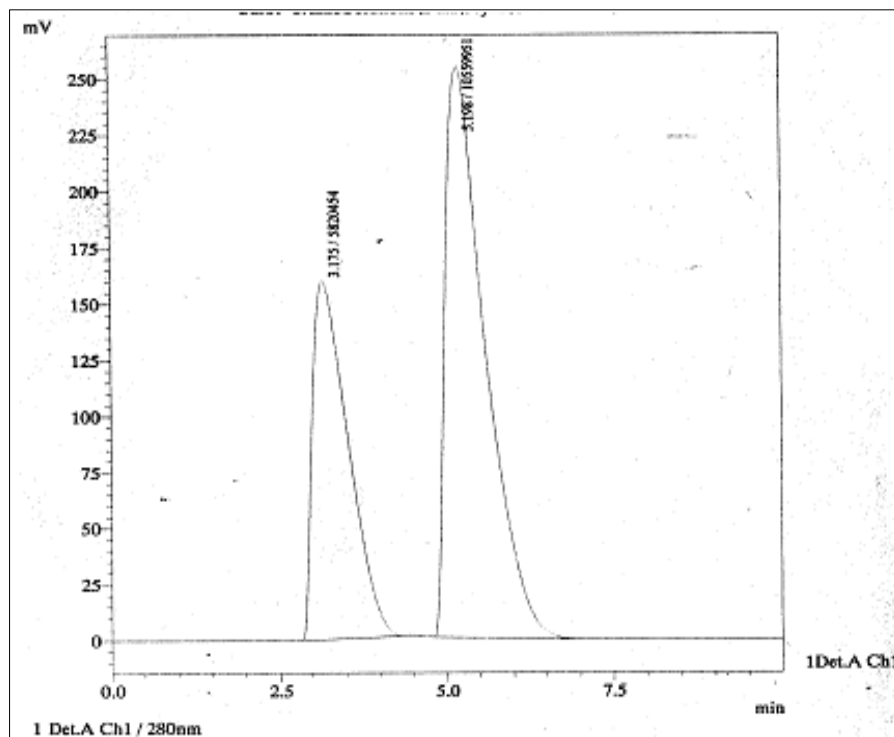


Figure 9: Chromatogram of model mixture, containing Galantamine hydrobromide and Pymadine at $\lambda = 280$ nm

2. TLC-densitometric method for the simultaneous determination of Galantamine and Pymadine [30]

TLC-densitometric method for the simultaneous determination of Galantamine and Pymadine in the model mixtures has been developed with a TLC Densitometer TR 541a, and the detection at $\lambda = 282$ nm.

In this method the chromatographic conditions used include:

- 1) Stationary phase: Silicagel G₆₀F₂₅₄
- 2) mobile phase: chloroform: acetone: ethylacetate: methanol = 20:10:5:5 v/v
- 3) detection at UV-lamp with $\lambda = 282$ nm

4) start-front distance: 150 mm.

The identification of the compounds was confirmed by the obtained data for R_f parameter of compounds and respective standards for Galantamine hydrobromide and for Pymadine.

For the identification of the compounds are used the obtained data for the analytical parameter R_f:

R_f = 0.663 for Galantamine hydrobromide
 R_f = 0.433 for Pymadine.

Linear relationship between concentration and the peak areas is shown from the respective regression equations:

$y = 2.107.x + 25364$ (Galantamine hydrobromide)

$y = 2.107.x + 65930$ (Pymadine) [30].

3) TLC-densitometric method for quality control of pharmaceutical dosage products, containing steroid component Estradiol valerate [31].

It has been described that a TLC-densitometric method for quality control of pharmaceutical dosage products, containing steroid component Estradiol valerate [31] has been developed with using a TLC Densitometer VILBER LOURMAT CN-15 LC., wavelength scan mode $\lambda = 254$ nm.

In this method the chromatographic conditions used include:

- 1) Stationary phase: TLC glass plates Silicagel G₆₀F₂₅₄
- 2) mobile phase: chloroform: acetone = 90: 10 v/v,
- 3) migration distance of mobile phase: 120 mm
- 4) detection at $\lambda = 254$ nm.

The amount of Estradiol valerate in Climen table. was determined by method of calibration curve by using of regression equation: $y = 28874286.x + 14290$ in concentration range: 5.10^{-4} g/ml ÷ 3.10^{-3} g/ml.

The data obtained by this method for limit of detection and for limit id quantitation in the chromatographic conditions used are:

LOD = $3.15.10^{-4}$ g/ml

LOQ = $9.54.10^{-3}$ g/ml.

From TLC-densitometric method for quality control of pharmaceutical dosage products, containing steroid component Estradiol valerate is confirmed that the content of Estradiol valerate in Climen CM1 correspond to the respective confidence interval: 1.81 mg ÷ 2.13 mg; SD = 0.09.

CONCLUSION

The study of natural products of plant origin in all areas of modern medicine, biology and other areas is increasingly relevant due to the great possibilities for application.

The advantage of HPLC method is that is more sensitive and limits of quantitation are $3.6.10^{-4}$ g/ml for Galantamine hydrobromide and $4.4.10^{-4}$ g/ml for Pymadine in comparison with the obtained by TLC-densitometric method data for LOD as $6.22.10^{-3}$ g/mlf or Galantamine hydrobromide and $8.35.10^{-3}$ g/ml for Pymadine.

Steroid component Estradiol valerate is in Climen CM1 correspond to the respective confidence interval: 1.81 mg ÷ 2.13 mg.

Additional information

Conflict of interest: The authors have declared that no competing interests exist.

Ethical statements: The authors declared that no clinical trials were used in the present study. The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalized human and animal cell lines were used in the present study

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Author contributions: All authors have contributed equally.

Data availability: All of the data that support the findings of this study are available in the main text or Supplementary Information.

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