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Research Article

STANDARDIZATION OF SNOWDROP (*GALANTHUS* L.) HERBAL PHARMACEUTICAL SUBSTANCES BY ULTRAVIOLET-SPECTROPHOTOMETRY

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ABSTRACT

Objective: The objective of the present study is to evaluate the electronic ultraviolet (UV) absorption spectra of herbal pharmaceutical substances (70% ethanol extracts) prepared from medicinal plant material of two snowdrop species – *Galanthus woronowii* Losinsk and *Galanthus nivalis* L.

Methods: The groups of biologically active substances were investigated by UV-spectrophotometry including special sample preparation for flavonoids and alkaloids.

Results: In the present study, data were obtained characterizing the composition of the main biologically active compounds of the genus *Galanthus* L., received its "spectrophotometric profiles." On the basis of spectral characteristics of *G. woronowii* and *G. nivalis* flavonoids and alkaloids, we confirm feasibility for using galantamine, lycorine, quercetin, and its glycosides (hyperoside *et al*.) state standard samples in standardization analysis.

Conclusion: It is shown that this method can be used to assess the quality of herbal pharmaceutical substances, serve to prove their identity in pharmacopoeial monograph.

Keywords: Ultraviolet-spectrophotometry, *Galanthus*, Herbal pharmaceutical substances, *Amaryllidaceae* alkaloids, Flavonoids.

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INTRODUCTION

Recently, there is a growing interest in relation to the homeopathic method of treatment, as well as a significant increase in the volume of registration of new homeopathic drugs (HDs) in Russian Federation. These trends have led to a new chapter in the domestic pharmacy – homeopathic pharmacy that study HD used in health care according to the principles of homeopathy. The main priorities of homeopathic pharmacy are development and improvement of the regulatory framework document, which regulates the production and quality control HD, increase HD range of domestic, as well as development and improvement of modern methods of analysis, quality control, and standardization [1,2].

HD quality assurance largely dependents on the use of standard homeopathic substances produced according to the good manufacturing practice. Homeopathic mother tinctures (HMT) are the feedstock for most HD. HMT is liquid hydroalcoholic extracts from raw materials of plant (or animal) origin. Most often HMT is produced by maceration (also percolation is possible). Quality control of HMT is to identify biologically active substances (BAS), the definition of authenticity, as well as other indicators that are defined privately normative documentation [3,4].

Our research was conducted at the Pharmaceutical and Natural Sciences Department (Pharmacy Institute) at the Sechenov First Moscow State Medical University. There normative documents for standardization of HMT and preparations developed purposefully over the last decades.

HDs of snowdrops are widely used abroad in the treatment of headaches, myocarditis, and diseases of the cardiovascular system. The raw material of common snowdrop (*Galanthus nivalis* L.) is used for the production of HMT [5], Voronov's snowdrop (*Galanthus woronowii* Losinsk) is also allowed for this purpose in Russia [6]. Appearance of snowdrops is shown at Fig. 1.

The relevance of this study is due primarily to the lack of national standardization methods of homeopathic pharmacy for HMT snowdrops derived from fresh flowering snowdrop plants of *G. woronowii* and *G. nivalis* (*Amaryllidaceae* J.St.-Hill.). *Amaryllidaceae* plants are often used in medicine for its unique properties [7-9]. In particular, the method of HMT preparation is unknown, qualitative reactions for determining the presence of *Amaryllidaceae* alkaloids [10,11] and flavonoids [12] (and other BAS groups) in HMT are not defined and there are no pharmacopoeial monographs for *G. woronowii* and *G. nivalis* homeopathic crude herbal drug (CHD) and HMT.

In pharmacognostic practice, ultraviolet (UV)-spectrophotometry method is most often used for the analysis of CHD and preparations based on it when it is necessary to study the total content of BAS. It should be noted that the UV-spectrophotometric analysis is quite easy to conduct, accurate, and economically cost effective, which accounts for its successful application to standardize [13]. It is applicable for the analysis of a number of com pounds having chromophoric groups (flavonoids, alkaloids, and other compounds). A spectrophotometric procedure was developed for the determination of total alkaloids content in *Tinospora cordifolia* M. with bromocresol green [14].

Flavonoid compounds contained in plant and pharmaceutical objects consist principally of a plurality of glycosides mixtures, which belong to different classes of polyphenols. It greatly complicates the selection methods of their determination and requires extra system studies of chemical composition. So far, a detailed spectrophotometric characterization of polyphenolic compounds found in snowdrops is not carried out [15]. Total flavonoids content is often determined by differential spectrophotometry with aluminum chloride in CHD [16-18].

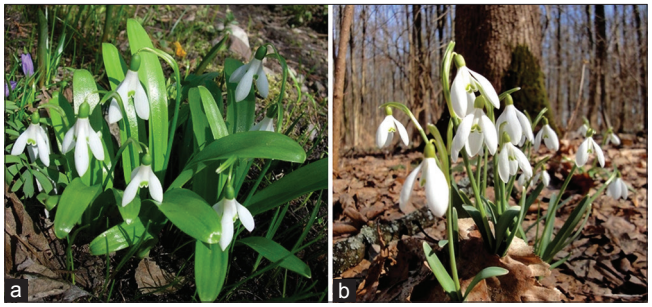


Fig. 1: (a) Voronov's snowdrop (*Galanthus woronowii* Losinsk); (b) common snowdrop (*Galanthus nivalis* L.)

METHODS

Chemicals

The following standard samples were used in the work: the standard sample of quercetin, CAS №. 117-39-5 (LLC "Phytopanacea" Russia, a series of 130,213, purity ≥99.5%), the standard samples and galantamine CAS №. 357-70-0 Lycorine CAS №. 476-28-8 (Nanjing Spring and Autumn Biological Engineering Co., Ltd, Jiang Su, China, high-performance liquid chromatography ≥98%).

Plant material

The objects of study were samples of Voronov's snowdrop (*G. woronowii* Losinsk) and common snowdrop (*G. nivalis* L.) harvested at the flowering stage at the Botanical Garden of Sechenov First Moscow State Medical University in Moscow (April-March 2016-2018).

HMT preparation

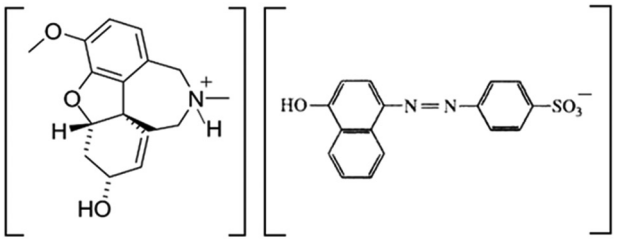
Pharmacopoeial HMTs were prepared according to the method 3a "HMT" [19] from the whole flowering plants (*Planta tota*). The choice of this method is based, primarily, due to the fact that plants of the *Galanthus* L. genus contain a substantial amount of mucus. It has also been prepared a series of infusions from underground parts (only bulbs with roots), without the aerial part for analytical purposes. The resulting infusion of the whole plant is a clear aqueous-alcohol extract of yellow-brown color with a greenish hue and a specific smell. HMT obtained from underground parts – transparent brownish liquid also has a specific smell.

Determination of BAS

An identification tests were carried out with HMT to determine the major BAS groups included in the GPh XI edition [20,21] and also that is widely used in scientific research.

To determine the *Amaryllidaceae* alkaloids, we use direct spectrophotometry – for HMT derived from underground parts; extraction-spectrophotometric method – for HMT obtained from whole plants. Scheme extraction-spectrometric method is as follows:

- Preparation of "Tropeolin 000-II – alkaloid". To aliquot of HMT, we added 1% hydrochloric acid solution that was stirred and placed in a separatory funnel, then we added 1% solution of tropeolin 000-II. The ion associate structure of the Tropeolin 000-II and galantamine can be represented as follows:



- Extraction of the resulting complex in the organic phase (extraction with chloroform three times) and measuring the optical density in obtained chloroform extract (orange-colored).

An acid hydrolysis of flavonoid glycosides in HMT was carried out for the analysis of flavonoids. 5 ml of HMT was evaporated on a water bath; the residue was dried in an oven at 105°C for 10 min, then dissolved in 15 ml of 10% sulfuric acid. Hydrolysis was carried out in a flask with ground joint volume of 100 ml, connected to a reflux condenser under heating in a boiling water bath for 2.5 h. Under these conditions, the hydrolysis of 3-O-glycosides advantageously takes place as 7-O-glucosides are more stable and are hydrolyzed under more severe conditions. Then, the flask was cooled to room temperature under a stream of cold water; the contents were filtered through filter paper. The precipitate remaining on the filter was washed with purified water and dissolved in 25 ml of hot 96% ethanol with the addition of 3 ml of a 2% solution of aluminum chloride, and the resulting solution was subjected to spectrophotometric analysis.

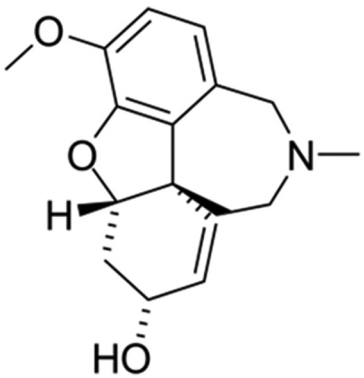
A study of the HMT electronic spectra was performed by the instrument "*Cary 50 Scan*" company *Agilent Technologies* (formerly - Varian, USA), followed by computer processing of the results of the program "Cary WinUV Analysis Pack ver. 3.1" for "Windows."

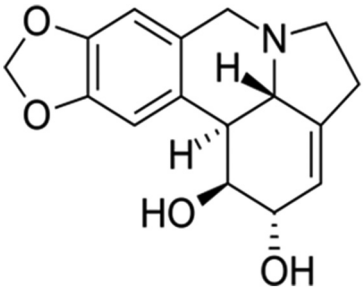
RESULTS AND DISCUSSIONS

As a result of identification tests in two snowdrops species HMT, we confirmed the presence of two BAS groups – flavonoids and alkaloids in HMT made from whole plants. As the biosynthesis of flavonoids and other polyphenol compounds nature occurs in aerial parts of plants, tinctures derived from underground parts lack of this BAS group. The results of qualitative reactions are shown in Table 1.

The analysis of the electronic spectra of standard samples of galantamine, lycorine, and snowdrops HMT (Fig. 2) obtained from the underground parts established the presence of two peaks approximately the same average width at 210 nm and 285 nm.

The first highest peak at 210±3 nm (2b) is characterized by a smooth left slope and a small bend on the right side, the second maximum at 285±3 nm is much smaller than the first. In general, the absorption maxima of the two compounds with the following structure coincide:





Galantamine, $C_{17}H_{21}NO_3$ Lycorine, $C_{16}H_{17}NO_4$

Table 1: The results of identification tests for BAS groups in HMT of *G. woronowii* and *G. nivalis*

Group of BAS	Reagent	HMT of *G. woronowii*		HMT of *G. nivalis*	
		Whole plant	Underground parts	Whole plant	Underground parts
Amaryllidaceae alkaloids	A solution of iodine in potassium iodide (Wagner-Bouchard reagent)	+	+	+	+
	A solution of potassium iodide, bismuth iodide (Dragendorff reagent)	+	+	+	+
	A solution of silicotungstic acid (reagent Bertrand)	+	+	+	+
	A solution of phosphomolybdic acid (reagent sonnenstein)	+	+	+	+
	A solution of picric acid	+	+	+	+
	A solution of tannin	+	+	+	+
Flavonoids	2% alcohol solution of $AlCl_3$	+	–	+	–
	Boric acid and citric acid anhydrous ethyl alcohol (Wilson reagent)	+	–	+	–
	Shinoda's test (Mg metal. in the presence of HCl conc.)	+	–	+	–

HMT: Homeopathic mother tinctures, BAS: Biologically active substances, *G. woronowii: Galanthus woronowii, G. nivalis: Galanthus nivalis*

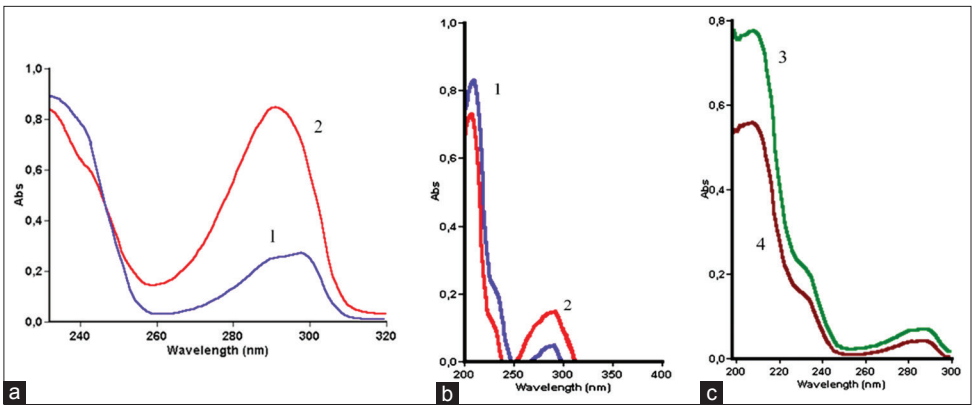


Fig. 2: The absorption spectra of standard samples of galanthamine (1) lycorine (2) 235-320 nm (a) and 200–400 nm (b) and homeopathic mother tinctures (c) of Galanthus woronowii (3) and the Galanthus nivalis (4) 200–300 nm

It can be assumed that the detected shape of the absorption spectral curves of lycorine and galantamine, related to the isoquinoline derivatives, is the consequence of the presence in their molecules of cyclic chromophores that are associated with the group = N-C.

Electronic absorption spectra of snowdrops HMT, derived from underground parts, have similar absorption maxima to analyzed standard samples (Fig. 2c).

HMT absorption spectra obtained from whole plants in the wavelength range have a plurality of absorption maxima in the range of 200–320 nm, due to the presence of a number of polyphenolic compounds and other impurities. Absorptions in the short-wave part of the spectrum are difficult to interpret because of the large number of absorption peaks overlapping each other to a large extent. This makes it difficult and makes it impossible to analyze the *Amaryllidaceae* alkaloids, so we used extraction-spectrophotometric method described in the standard documentation and the scientific literature. Complex formation galantamine with tropeolin 000-II is used for the quantitative determination of galantamine in the substance of galantamine hydrobromide [22] and in the pharmacopoeial monograph for *Ungernia victoris* Vved. CHD [23]. This technique allows to analyze HMT snowdrops derived from whole plants as selective extraction complex "tropeolin-alkaloid" allows you to get rid of unwanted impurities. Spectra of galantamine, lycorine, and HMT of *G. woronowii* and *G. nivalis* are shown in Fig. 3a. Absorption maximum at 480±3 nm is characteristic for galantamine and for lycorine. The total amount of these alkaloids is contained in HMTs. It should be considered in the standardization procedure of CHD and HMT. It is necessary to introduce new index: alkaloid total content in terms of galantamine or lycorine.

Taking into account the fact that HMT derived from whole plants contain many polyphenolic compounds, we have attempted to characterize some interpreted absorption maxima. One of these peaks observed at 330±5 nm, and there is a slight bend at 400 nm (the peak is not fixed); this characteristic can be associated with hydroxycinnamic acids [24]. Electronic absorption spectra of *G. woronowii* and *G. nivalis* HMT presented in Fig. 3b. It should be noted that the spectra are given in Fig. 3b may serve as a characteristic of officinal HMTs authenticity.

Quercetin was selected as standard sample according to the obtained intermediate experimental and published data. After the hydrolysis of HMT and subsequent treatment with 2% solution of aluminum chloride, we observed the absorption maximum at 430±5 nm. Obviously, during the hydrolysis of quercetin glycosides prevailing in *G. woronowii* and *G. nivalis* HMT, broken, releasing the free quercetin. Quercetin with 2% ethanolic solution of aluminum chloride forms a colored compound having a yellow-green fluorescence at a wavelength of about 430 nm (bathochromic shift) (Fig. 4).

Structural formulas of quercetin and its most frequently occurring glycosides are shown below:

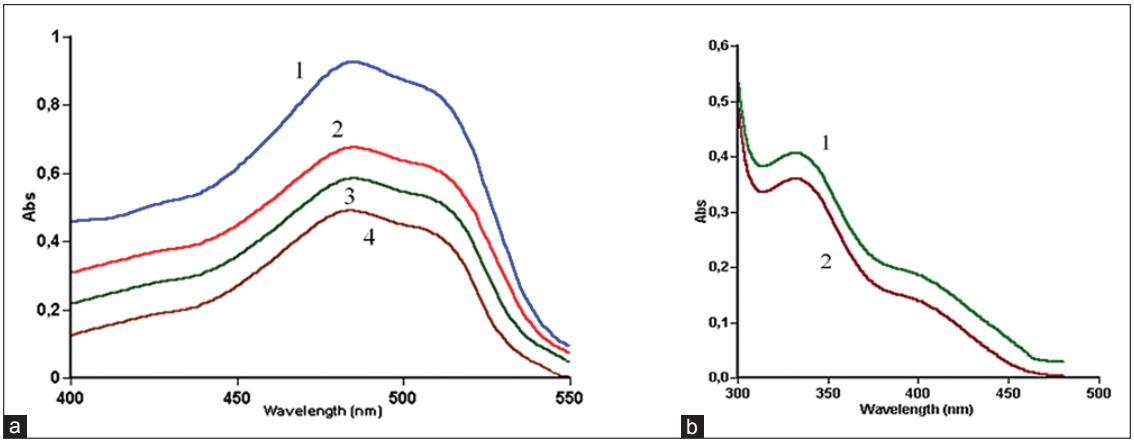


Fig. 3: The absorption spectra of the chloroform extract containing complex of tropeolin II-000 with standard samples of galanthamine (1), lycorine (2), homeopathic mother tinctures (HMT) of Galanthus woronowii (3) at 400–550 nm (a) and Galanthus nivalis (4) at 400–550 nm (a); individual absorption spectra HMT of G. woronowii (1) and G. nivalis (2), at 300–500 nm (b)

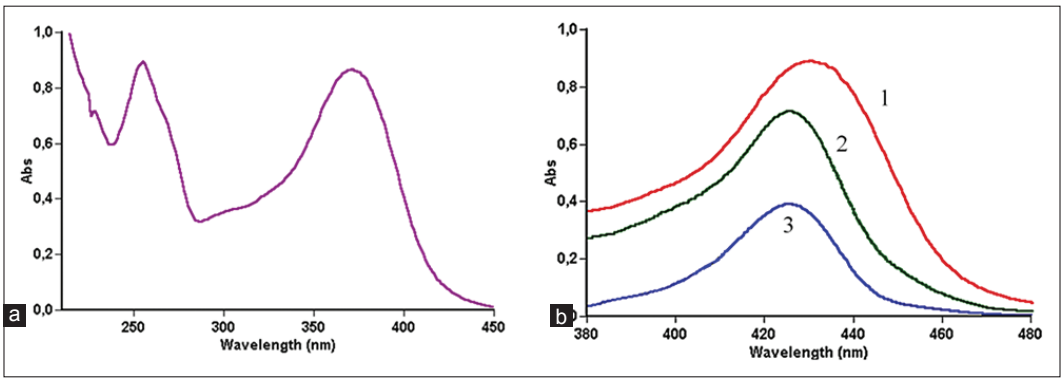
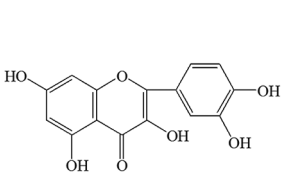
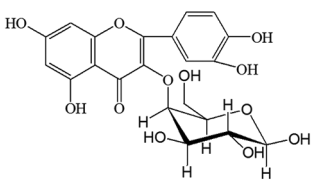


Fig. 4: The absorption spectrum of the quercetin standard sample at 200–450 nm (a); the absorption spectra of quercetin (1) and hydrolysis products homeopathic mother tinctures of *Galanthus woronowii* (2) and *Galanthus nivalis* (3) with a 2% solution of aluminum chloride at 380–480 nm (b)



Quercetin –3,5,7,3 ', 4'-pentoxide flavon



Quercetin glycoside – quercetin 3-*O*-*β*-D-galactoside (hyperoside)

Flavonoid compounds are secondary metabolite in plants; they show a wide spectrum of biological and pharmacological functions. Among the all flavonoids, quercetin gained special attention for its potential therapeutic activities (anti-inflammatory, antioxidant, antitoxic, anticancer, immunomodulatory, and others) [25]. Taking into account this aspect, we can assume that the snowdrops drugs will be in demand on the pharmaceutical market.

CONCLUSION

Qualitative analysis for the main BAS groups of *G. woronowii* and *G. nivalis* herbal pharmaceutical substances – HMT was conducted. We defined by UV-spectrophotometry that they are *Amaryllidaceae* alkaloids and flavonoids. On the basis of spectral characteristics of *G. woronowii* and *G. nivalis* HMT flavonoids and alkaloids, we confirm method feasibility for using galantamine, lycorine, quercetin, and its glycosides (hyperoside *et al.*) state standard samples in standardization analysis. Extraction-photometric method can be applied successfully for standardization and the determination of galantamine and lycorine in *G. woronowii* and *G. nivalis* HMT and CHD. The authenticity of HMT of *G. woronowii* and *G. nivalis* can be confirmed by electronic absorption spectrum with a maximum at 330±5 nm and bend at 400 nm.

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AUTHOR' CONTRIBUTIONS

I declare that this work was done by the author named in this article.

CONFLICTS OF INTEREST

None.

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