THE REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree
of Doctor of Philosophy

PHARMACOGNOSTIC STUDY OF SPECIES BELONG TO *PHLOMİS* GENUS

Speciality:	3400.02 - Pharmaceutical chemistry, pharmacognosy
Field of science:	Pharmacy
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GENERAL CHARACTERISTICS OF WORK

Relevance and developing level of the topic. Pharmacy is a field of science that is developing in various directions. One of the important and urgent tasks facing this field of science is to identify and to study natural sources, including biologically active substances in plants, and to create more effective medicines based on these substances. In this regard, it is important to study the chemical composition of plant species with sufficient raw material reserves, that widespread in the territory of the Republic of Azerbaijan.

Our republic has a colorful climate and a rich flora. Therefore, there is a great need to study the plants growing in the territory of repuplic of Azerbaijan, to select species with biologically active substances and to offer new drugs based on these plant species.The medicinal plants studied and the medicines offered based on them are important to meet the demand in various fields of health. At the same time, it is important to use plant resources efficiently, taking into account the fact that due to various reasons (environmental, industrial development, etc.) depletion of plant raw materials[1,2,3,4].

Biologically active substances synthesized in plants are successfully used in the treatment and prevention of a number of diseases with a therapeutic effect on the human body in the certain doses[5,6]. Natural source are important in modern medical practice

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[1] İsayev, C.İ. *Gentiana septemfida* L. (Yeddidilim acıçiçək) bitkisinin yayılma arealının və xammal ehtiyyatının öyrənilməsi / C.İ. İsayev , A.İ. Qədimli // Tibb və Elm jurnalı, -2020, №3 (21), -s. 48-52.

[2] Mövsumov, İ.S. Yunan qozunun (*Junglans regia* L.) meyvə yanlıqlarından yuqlonla zəginləşdirilmiş ekstraktın alınması. İxtira i2015 0042, Azərbaycan Respublikası / E.A. Qarayev, C.Y. Yusifova.

[3] Süleymanov, T.A. Azərbaycan florasından olan bəzi bitki növlərinin lipidlərinin tədqiqi / T.A. Süleymanov, Y.B. Kərimov //Azərbaycan Əczaçılıq jurnalı, -Bakı: -2002, № 1, -s. 20-22

[4] Абышев, А.З. Химия и фармакология природных кумаринов / А.З. Абышев, Э.М. Агаев, Ю.Б. Керимов // Баку: Caspian Supplies. – 2003. -112 с.

[5] Агаев, Э.М. Кумарины - производиные безопиран-2-она / Э.М. Агаев, А.З. Абышев // Азербайджанский фармацевтический журнал, -2002, № 1, - с. 31 - 41.

[6] Буданцев, А.Л. Ресурсоведение лекарственных растений / А.Л. Буданцев, Н.П. Харитонова // Санкт-петербург: -2003, -86 с.

due to the preference for the use of drugs based on plant raw materials[7,8].

The phytochemical and pharmacological raw materials of *Phlomis* L. species distributed in Azerbaijan are not studied. From these facts it can be concluded that It is important and relevant to study the pharmacognostic nature of some plants belonging to the genus *Phlomis* L., which is quite widespread in the flora of our republic.

Object and subject of the research. As the object of research *Ph. pungens* Willd. and *Ph. caucasica* Rech. *fil.* was used. *Ph. pungens* plant was collected from Shahbuz region of Nakhchivan, and *Ph. caucasica* from Khizi region of the Republic of Azerbaijan in 2014 during flowering stage.

The purpose and the objectives of the study. The purpose of the research is to identify important species of plants belonging to the genus *Phlomis* L. from the flora of Azerbaijan for pharmacognostic research, to obtain and identify biologically active substances from these plants, to prepare and approve normative documents on raw materials and medicines. The following tasks are planned to achieve this goal:

- Selection of important species for pharmacognostic research of the genus Phlomis L. widespread in Azerbaijan, determination of geobotanical regions of their distribution, identification of the annual supply of the raw materials and the shelf life;
- Detection of characteristic diagnostic features in the anatomical structure of the raw materials of the studied species;
- Phytochemical study of some species of the genus Phlomis L;
- Preparation of dosage forms on the basis of a total of biologically active substances obtained from the studied plant

[7] Велиева, М. Разработка технологии получения сухих духов на основе растительного сырья Азербайджана / М. Велиева, Н. Агаева, Мадатли Ф. // Sciences of Europe, -2017, изд. 20, № 20, -с. 37-42.

[8] Искендеров, Г. Способ получения диосцинина из якорцев стелющихся, № 026930, 2017, Евразийский патент / Г. Искендеров, К. Гусейнкулиева

species and determination of their pharmacological effects;

- Preparation and approval of project of pharmacopoeial articles on medicinal plant raw materials and dosage forms.

Research methods. Chromatographic and spectroscopic methods were used to analyze the plant species studied. The composition of the essential oils was identified by Gas Chromatography and Gas Chromatography / Mass Spectrophotometry - Agilent GC-MSD (Agilent Technologies Inc., Santa Clara, CA) LC/MS (Agilent, 6420A Triple Quadrupole) device; the NMR spectra of the obtained substances were recorded on a FAB-MS JEOL JMS-DX 300 spectrometer using the internal standard JEOL JNM-A 500 methanol-d4 tetramethylsylane (TMS). UV spectra were recorded on Shimadzu UV-160A spectrometer. IR spectra were taken on a Perkin Elmer FTIR 1720X spectrometer.

The main provisions of the defense

- *Ph. pungens* and *Ph. caucasica* species rich in biologically active substances are promising as a source of raw materials for the development of new medicines;
- The level of diagnostic features found in the morphological and anatomical structure of the raw materials of *Ph. pungens* and *Ph. caucasica* species allows to determine the identity of the raw material through macroscopic and microscopic analysis, and the annual supply of raw materials;
- The raw materials of *Ph. pungens* and *Ph. caucasica* are rich in chemical composition, therefore it is important to obtain and study iridoids, flavonoids, cinnamic acids, essential oils, and amino acids;
- The total flavonoids obtained from herbs of *Ph. pungens* and the antioxidant effect of "Flomexin" tablets based on it, as well as the fact that the alloxan model of plant extract has a hypoglycemic effect in diabetes, provides a basis for the development of a new drug;
- The approved Pharmacopoeia articles on herbs of *Ph. pungens* and on "Flomexin" tablets are sufficient to control the quality of raw materials and medicines.

The scientific novelty of the research

For the first time, as a result of the preliminary study of 6 species of the genus *Phlomis* L., which is widespread in the flora of Azerbaijan, *Ph. pungens* and *Ph. caucasica* (*Lamiaceae*) plants, which are important in terms of pharmacognostic studies and the development of new drugs, have been identified.

For the first time, annual supply of raw materials and characteristic diagnostic features in the anatomical structure of *Ph. pungens* and *Ph. caucasica* were identified.

As a result of phytochemical study of *Ph. pungens* and *Ph. caucasica* species, substances related to iridoids, flavonoids and phenylethanoids were obtained individually from raw materials and identified by chromatographic and spectroscopic methods.

An analytical normative document belong "Flomexin" (*Phlomexin*) tablets offered for the first time and *Ph. pungens* species, on the first type - Temporary Pharmacopoeia Articles were prepared and approved by the "Pharmacology and Pharmacopoeia" Commission of the Ministry of Health of the Republic of Azerbaijan.

Theoretical and practical significance of the research. *Ph. pungens* and *Ph. caucasica* species have been identified that are rich in biologically active substances such as iridoids, flavonoids, and phenylethanoids and have been considered important for pharmacognostic studies.

The amount of annual procurement of raw materials of *Ph. pungens* and *Ph. caucasica* species that can be supplied to ensure the raw material base, specific morphological and anatomical diagnostic features were identified in order to determine the shelf life and the identity of the raw material.

Approved Pharmacopoeia Articles on the raw material of *Ph. pungens* plant and on the new drug developed based on it – "Flomexin" tablets are sufficient to carry out quality control at the stages of production and use of the medicinal product.

The antioxidant effects of flavonoid compounds derived from the raw material of this plant, as well as the anti-diabetic effect of the aqueous extract will allow in the future to develop a new drug based on these raw materials.

The results obtained from the pharmacognostic study of plants of the genus Odotu - *Phlomis* L. are used in the teaching of "Analysis of the raw materials containing iridoids and essential oils" at the Department of Pharmacognosy of Azerbaijan Medical University.

Approbation and application. The main results of research work on the dissertation, in the final scientific conferences of the Azerbaijan Medical University «Təbabətin aktual problemləri» (2014-2015), at the symposium of *«International Symposium on Essential oils»* (2014, Turkey), Baku Science Festival (2014), II Azerbaijan Science Festival (2016), 3rd *İnternational Conference on Pharmaceutical Sciences* (2015, Georgia), 9th *Joint Natural Products Conference* (2016, Denmark),*"Разработка, исследование и маркетинг новой фармацевтической продукции"* (2016, Russia) and at the scientific-practical conference held at AMU on the occasion of the 120th anniversary of Aziz Aliyev (2017) was reported.

BFD 4.02 One-time Dissertation Council was held at AMU at the meeting of the scientific seminar (18.06.2021, protocol №1).

The results of the dissertation were published in 21 scientific works, as 12 articles and 9 thesis. A positive result was obtained from the Eurasian Patent Organization on the examination of 1 patent "On the readiness of the patent for issuance" (Appendix 3).

The organization name of the dissertation work performed.

The dissertation work was carried out at the Department of Pharmacognosy of Azerbaijan Medical University.

The volume and structure of the dissertation

The dissertation consists of 201 pages of computer writing, introductory part, 5 chapters, results, practical recommendations, list of references and appendices. 53 pictures and 28 tables are given in the dissertation. The list of used literature consists of 188 sources.

Dissertation work relevants to the subject of scientific research conducted at the department of Pharmacognosy (State registration number DQN: 01114104).

The volume of structural sections of the dissertation with separate signs, except for tables, pictures and the list of the used literature, introduction 1034, Chapter I 32890, Chapter II 27039, Chapter III 22221, Chapter IV 56770, Chapter V 31605, final part 27786, results 3740, practical recommendations 627 consists of signs. The total volume of the dissertation contains 203712 signs.

MATERIALS AND METHODS

For the purpose of research, the raw materials of species were used belong to *Phlomis* L. supplied from different geobotanical regions of the republic .

Several solvents, reactives and equipments were used to perform the tasks set in the dissertation. Aqueous solutions of different concentrations of ethanol, solvents such as methanol, chloroform, ethyl acetate, hexane and n-butanol were used to isolate biologically active substances from the studied plant raw materials.

Different sorbent and chromatographic methods were used in phytochemical analysis depending on the goals and duties. For chromatography, "*Filtrak*" chromatography paper, "*Silufol*" plaque, polyamide (ICN), silica gel 60H (230-400 mesh), silica gel 60 (0.063-0.200 mm) (Merck) and Sephadex LH-20 sorbents were used for column chromatography. For thin-layer chromatography (TLC), 60 F254 aluminum plates coated with silica gel (Merck) were used.

The plates were observed under UV rays. 1% solution of vanillin in solid sulfuric acid, 10% solution of sodium hydroxide and potassium hydroxide, 3% solution of aluminum chloride and ammonia vapor, as well as butanol-acetic acid-water (4:1:5), 15% acetic acid, chloroform-methanol (7:3, 8:2, 9:1), chloroform-methanol-water (7:2:1) solvent system were used as a detector.

Some sources and materials of the herbarium fund of the Institute of Botany of ANAS were used to determine the identity of the studied plant species.

A rotor evaporator (IKA, RV 8 V-C) was used under vacuum to release extracts from plant raw materials to dry residue.

The obtained fractions from plant raw materials were lyophized in (Christ, series № 21729, Alpha 1-2 LDplus) lyophysator.

Analyzes were performed on Gas Chromatography and Gas Chromatography / Mass Spectrophotometry Device - Agilent GC-MSD System (Agilent Technologies Inc., Santa Clara, CA) at Anadolu University, Eskişehir, Turkey, on LC / MS (Agilent, 6420A Triple Quadrupole) device at Hacettepe University in Ankara, Turkey. The NMR spectra of the obtained substances were measured on a FAB-MS JEOL JMS-DX 300 spectrometer using the internal standard JEOL JNM-A 500 methanol-d4 tetramethylsylane (TMS). UV spectra were recorded on Shimadzu UV-160A spectrometers. The IR spectra were taken on a Perkin Elmer FTIR 1720X spectrometer at Nagoya City University in Nagora, Japan.

Macro and microelement compositions of some extracts from raw materials and plant raw materials were studied with our participation at the Institute of Geology of the Azerbaijan National Academy of Sciences.

During microscopic analysis, micropreparations were prepared from raw materials. For this, the raw material is stored in a mixture of alcohol-glycerin-water (1: 1: 1), heated in a 3% solution of NaOH in alcohol for 1-2 minutes. Chloralhydrate solution was used to prepare the micropreparation.

The diagnostic signs were determined using a “MOTIC SFC-18 SERIES” microscope and an MBC-1 binocular. The micropreparations were taken with a SAMSUNG L74 WIDE digital camera.

RESULTS AND DISCUSSION OF RESEARCH

Species belonging to the genus *Phlomis* L. are of great interest both in terms of chemical composition and biological activity. The numerous compounds belonging to different groups of biologically active substances were found in the plant species (terpenoids, flavonoids, lignans, iridoids, etc.).

Resource science research of *Ph. pungens* and *Ph. caucasica* species were carried out and sufficient areas of mass distribution of these species were found in the flora of Azerbaijan. 9 directions were selected and found *Phlomis* L. species distribution areas, mass distribution areas in each direction. Widespread areas of plant species were found in Yardimli, Gazakh, Guba, Shabran, Khizi, Siyazan districts, as well as in Shahbuz, Babek and Julfa districts of Nakhchivan of the republic.

In the mentioned directions, as a result of the research to determine the annual supply of *Ph. pungens* and *Ph. caucasica* species raw materials, biological reserves is 372405 kg, of exploitable reserves is 232091 kg and the amount that can be supplied annually is 58027 kg of *Ph. pungens* raw materials, biological reserves is 41939 kg, of exploitable reserves is 27231 kg and the amount that can be supplied annually is 6809 kg of *Ph. caucasica* was determined.

As a result of microscopic studies carried out to determine the identity of *Ph. pungens* raw materials, characteristic diagnostic features of its anatomical structure were revealed.

As a result of the study of the anatomical structure of *Ph. pungens* raw materials, the following characteristic diagnostic features were revealed:

- the surface of the seeds is glossy and covered with simple hairs at the base; there are four oppositely arranged grooves.
- the surface of the bract is densely covered with hairs, and its apex ends with simple hairs.
- the calyx from the internal dorsal side is covered with multicellular simple hairs, and there are branched hairs outside.
- the corolla in the internal dorsal side is covered with simple hairs, and there are branched hairs at the bottom. Outside there are only branched hairs.
- the petiole is covered with a continuous layer of branched hairs.
- the dorsal side of the leaf blade is covered with a dense layer of hairs; there are glands along the edges of the leaf blade.

The bottom side of the leaf is also covered with hairs, especially the midrib.

The phytochemical composition of *Ph. pungens* and *Ph. caucasica* species belonging to the genus *Phlomis* L. That pharmacognostically significant and promising was studied. As a result of the qualitative reactions and chromatographic studies, it was found that the studied raw materials contained iridoids, flavonoids, cinnamic acids and other substances.

Phenilethanoid glycosides and flavonoids from *Ph. pungens* and *Ph. caucasica* species herb were obtained together on the basis of a single scheme. 70-80% ethanol was used for the extraction of raw materials.

Air-dried aerial parts of *Ph. pungens* (2.5 kg) were extracted (3x12.5 L, 48 h) with EtOH (80%) at room temperature. The combined extracts were evaporated in vacuo almost to an aqueous residue and separated into fractions by sequential extraction with extractants such as $CHCl_3$, EtOAc, and BuOH.The aqueous part is treated with chloroform to remove ballast substances, lipids and chlorophyll. It is then separated into appropriate fractions with ethyl acetate and n-butanol.

The BuOH extracts were evaporated *in vacuo* to a dry residue.

The BuOH extract (11 g) was chromatographed over a column (h = 45 cm, d = 3.3 cm) packed with polyamide (70 g) and eluted by H_2O–MeOH with increasing MeOH concentration.

Compound **1** was identified as 3-hydroxy-4-methoxy-*β*-phenylethoxy-O-[α-rhamnopyranosyl-(1→3)]-O-[*β*-apiofuranosyl-(1→6)]-4'-O-feruloyl-*β*-glucopyranoside (leucosceptoside B), $C_{36}H_{48}O_{19}$. The fraction eluted by $CHCl_3$–MeOH–H_2O (61:32:7) was rechromatographed over a column of *Sephadex LH*-20 using MeOH (100%) to obtain compound 2, forsitoside B, (3,4-dihydroxy-*β*-phenylethoxy-O-[α-rhamnopyranosyl-(1→3)]-O-[*β*-apiofuranosyl-(1→6)]-4'-O-caffeoyl-*β*-glucopyranoside).

The ^{1}H-NMR spectrum of compound **1** exhibited six aromatic protons, at δ_H 6.70-7.20 belonging to the acyl and aglycone moieties. Two *trans*- olefinic protons at δ_H 6.37 and δ_H 7.66 (d, J =15.6 Hz)

indicated that a trisubstituted cinnamic acide moiety should be present at the structure. On the other hand, a benzylic methylene (a multiplet at 2.83 ppm, β-CH_2 of aromatic side chain) and two nonequivalent protons at δ_H 3.75 and 4.02 (α, each 1H, m) were observed, they were attributed to a trisubstituted phenylethyl alcohol moiety. Additionally, the signals of two methoxyl groups were found at δ_H 3.82 and 3.89 (each 3H, s). Moreover, three doublets of the anomeric protons of **1** indicated its trisaccharidic structure. ^{1}H- and ^{13}C-NMR signals assigned to the sugar moiety showed that **2** should be composed of one hexose, one methylpentose and one pentose unites on the basis of its chemical shift and coupling constants. Chemical shifts of protons due to hexose as well as those of the methylpentose and pentose moieties were assigned unambiguously from the homonuclear ^{1}H, ^{1}H-correlation (COSY) and heteronuclear multiple quantum coherence (HMQC) experiment and, one β-glucose, one α- rhamnose and one β- apiose unit were determined.

The upfield shift of signals at δ_C 112.9 due to the C-5 of aglycon and δ_C 111.8 due to the C-2"" of acyl moieties indicated that methoxyl groups should be attached to C-4 of aglycon and C-3"" of acyl moieties. The connectivities between the acyl moiety, glucose, rhamnose, apiose, aglycon moiety and methoxyl groups were confirmed by the heteronuclear multiple bond correlation (HMBC) experiment which long range correlations were observed between the following protons and carbons:

Studied compound was identified as 3-hydroxy-4-methoxy-β-phenylethoxy-O-[α-rhamnopyranosyl-(1→3)]-O-[β-apiofuranosyl-(1→6)]-4'-O-feruloyl-β-glucopyranoside (leucosceptoside B), $C_{36}H_{48}O_{19}$ based on UV, IR, and NMR spectra. This was confirmed by a comparison with published data for leucosceptoside B.

The ^{1}H-NMR spectrum of compound **2** also exhibited six aromatic protons at δ_H 6.56-6.69 for aglycon and δ_H 6.77-7.06 for acyl moiety. Signals for two *trans*-olefinic protons appeared at δ_H 6.27 and δ_H 7.59 (d, J =15.6 Hz). Additionally, β-methylene at δ_H 2.80 (2H, m), and two nonequivalent proton signals at δ_H 4.08 and 3.72 (each 1H, m) of the side chain of the aglycone moiety were

observed. Three doublets of the anomeric protons of **2** indicated its trisaccharidic structure. ^{1}H-NMR signals assigned to the sugar moiety showed that **2** should be composed of one β-glucose, one α-rhamnose and one β-apiose unit on the basis of its chemical shift and coupling constants. All these signals were very similar to those of compound **1**, except two singlet signals arising from methoxyl groups at δ_H 3.82 and δ_H 3.89 for **1**. This finding suggested that compound **2** not possess methoxyl groups which was different from compound **2**. The ^{1}H-NMR spectral data of **2** were identical to those reported for 3,4-dihydroxy-β-phenylethoxy-*O*-[α- rhamnopyranosyl-(1→3)]-*O*-[β-apiofuranosyl-(1→6)]-4-*O*-caffeoyl-β-glucopyranoside (forsythoside B), $C_{34}H_{44}O_{19,}$ which was isolated from several *Phlomis* species previously.

The phenylethanoid glycosides leucosceptoside B and forsitoside B were isolated and identified for the first time from the aerial part of *Ph. pungens* from Azerbaijan.

The iridoid composition of the raw material was studied, as a continuation of scientific research on phytochemical study of *Ph. pungens* and *Ph. caucasica* raw materials belonging to the genus *Phlomis* L.

Butanol fraction of 80% alcohol has been studied isolated from raw materials. 11g n-butanol extract (h = 45 cm; d = 3.3 cm) contains the mixture of biologically active substances is chromatographed by in a 70g polyamide-filled column and and washed with H_2O / MeOH mixture until to obtain 8 fractions to obtain the main fractions of biologically active substances by starting with H_2O and gradually increasing the concentration of MeOH.

The various biologically active compounds are tested separately by thin-layer chromatography separately in polyamide sorbet in eluates from each fraction. Only in the 5-16th eluates of the 1st and 2nd fractions was found presence of iridoids. Iridoids from eluates 5-16th are chromatographed in silicagel to obtain the alleged individual compound.

As an eluent is used in a volume of 750 ml, in a ratio of 75:25 from a mixture of $CHCl_3$ / MeOH.

291 test eluate was obtained in a ratio of 1 to 8 of the solvent mixture during the chromatography process. Lamiide as an iridoid compound was found in obtained 11 trials (which are, 120-130 eluates).

This fraction (PP-1) with conventional name was identified by the NMR and HPLC-MS.

Substance (PP-1) is a lamiide ($C_{17}H_{26}O_{12}$) iridoid compound, a colorless, amorphous compound.

Its UV spectrum showed an absorption peak which was characteristic of an iridoid enol-ether system. On the other hand, UV λ_{max} in MeOH was found to be 232 nm. This finding indicated that a C-4 substituted iridoid structure. Molecular weight was found to be as 422,35 from LC-MS spectrum. The ^{1}H NMR spectrum (Table 1) displayed signals due to two tertiary methyls (δ_H, 1.09 s and 3.73 s), one methylene (δ_H, 2.25 dd and 2.36 dd), one methine (δ_H, 2.80 s), one oxymethine (δ_H, 3.53 dd), one acetal (δ_H, 5.82 s) and one olefinic (δ_H, 7.44 s) functions. Additionally, the anomeric proton resonance at δ_H, 4.60 (d, J=7.8 Hz) and the signals in the region 3.19-3.90 ppm indicated the presence of a β-hexose unit in the studied compound. Analysis of the ^{13}C-NMR spectrum of 1 (Table 1) revealed the presence of 17 carbon signals, six of which were assigned to hexose unit. The remaining 11 resonances, along with the corresponding ^{1}H NMR signals indicated of a C 10 iridoid skeleton having a carbomethoxy group at C-4 position. All of the ^{1}H and ^{13}C chemical shifts of the studied compound were determined on the basis of COSY, HSQC and HMBC experiments (Table 1). In the COSY spectrum of the studied compound, the oxymethine proton (δ_H, 3.53 dd, J=5.4/3.0) coupled with one of the methylene protons at δ_H, 2.36 dd (J=15/5.4), which were attributed to H-7 and H_2-6 of the cyclopentane ring of the aglycon, respectively. No other coupling was observed for H_2-6 and H-7 indicating that both C-5 (δ_C, 69.2) and C-8 (δ_C, 79.1) were totally substituted. The hexos unite was also determined by the examination of COSY spectrum as β-glucopyranose. The complete assignments were confirmed by the HMBC spectrum. The long-range correlations between H-3 (δ_H, 7.44 s) and C-11 (δ_C, 168.0) confirmed that the carbomethoxy group is

located to C-4. Furthermore, the cross-peak between H-10 (δ_H, 1.09 s) and C-8 (δ_C, 79.1) showed that the methyl group was at C-8 and the cross-peaks between H-1 (δ_H, 5.82 s) and C-1' (δ_C, 99.6) and vice versa, revealed that the β-glucopyranose is attached to C-1 (δ_C, 94.4).

Based on ^1H and ^{13}C NMR spectra, *COSY*, *HSQC* and *HMBC* experiments, the studies substance has been identified as a lamiide an iridoid compound.

In accordance with the plan of the dissertation, research was conducted on the extraction and research of flavonoids from raw materials. Due to the fact that flavonoids have a similar composition in the raw materials of *Ph. pungens* and *Ph. caucasica* species, the analysis was carried out on a sample of *Ph. pungens* raw materials.

2.5 kg of air-dried, crushed *Ph. pungens* herb is extracted three times at room temperature (with 24 hours of each extraction) with 80% 12.5 l ethanol. The combined extracts are evaporated under vacuum to an aqueous residue, then separated into fractions by extraction three times in a row with different extractants. The third fraction is extracted three times with 400 ml of n-butanol to obtain biologically active substances.

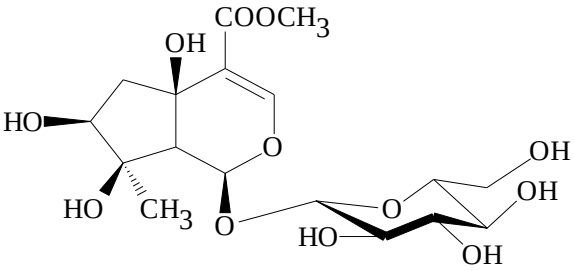
The extract obtained with N-butanol is evaporated under vacuum until a dry residue is obtained. The n-butanol dry residue is stored in the refrigerator for 1 day after the addition of 95% alcohol. A precipitate is observed in the n-butanol extract stored in the refrigerator. The precipitate is separated, evaporated to dryness under vacuum, and then dried in a thermostat.The dried precipitate was separated into individual substances by preparative chromatography on HPLC device.

The mixture was prepared fractionally using the HPLC device and analyzed on NMR device to determine the structural structure of the obtained substance.

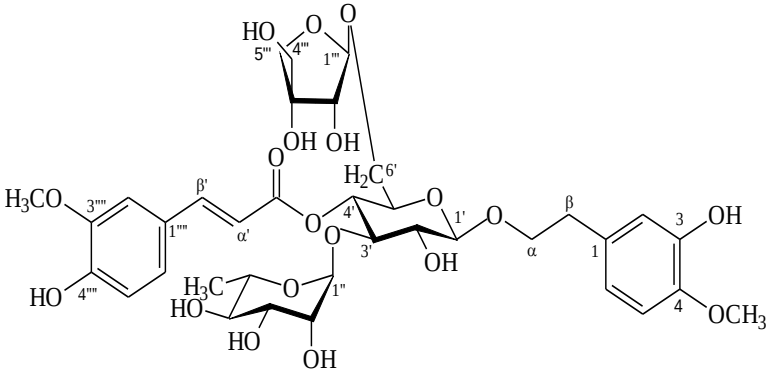
Based on the molecular weight of the substance-650.54, NMR-spectral indicators, it was determined that $C_{29}H_{30}O_7$ - luteolin 7-O-[4-O-acetyl- α -rhamnopyranosyl- (1 $\rightarrow$ 2)] - β - glucuronopyranoside.

The individual substance - luteolin 7-O- [4-O-acetyl- α - rhamnopyranosyl- (1 $\rightarrow$ 2)] - β -glucuronopyranoside flavonoid

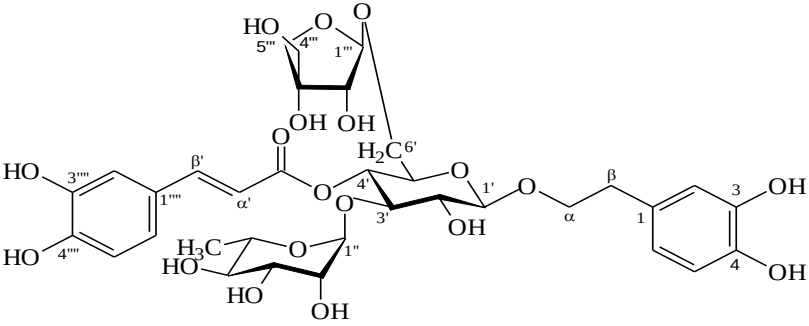
combination was firstly obtained and identified from a new type of local raw material using modern technological methods.



Lamiide



Leucoseptoside B



Forsythoside B

As mentioned earlier, 80% alcohol extract of the raw material was processed to dry residue, 95% ethyl alcohol was added to the remaining dry residue and a precipitate was formed after 1 day in the refrigerator, and the totality of the substances was separated from this precipitate and processed to dry residue.

The dried precipitation was cleaned and analyzed by LC-MS.

Chlorogenic acid, neo-chlorogenic acid, forsythoside B, quercetin-glucoside, quercetin-glucuronide, verbascoside, luteolin-glucoside, luteolin-glucuronide, luteolin 7-O-[2''-O-(4'''- O-acetyl-α-L-rhamnopyranosyl)]- β-D-glucuronopyranoside and apigenin-diglucoside from the aqueous residue of 80% alcohol extract made from *Ph. pungens* herb were obtained and identified.

Table

Rt	**M-H**	**MS^2**	**Result**
5.9	353	191, 179, 135	Chlorogenic acid
6.8	353	191, 179, 135	Neo-chlorogenic acid
8.0	755	593, 461, 161	Forsythoside B
8.5	463	301, 272, 228	Quercetin-glucoside
8.9	477	373, 343, 301,283	Quercetin-glucuronide
9.3	623	461, 315, 161	Verbascoside
9.9	447	285, 133	Luteolin-glucoside
10.2	461	285, 151, 133	Luteolin-glucuronide
12.7	649	515, 363, 285	Luteolin 7-O-[2''-O-(4'''- O-acetyl-α-L-rhamnopyranosyl)]- β-D-glucuronopyranoside
14.5	633	571, 363, 345, 269	Apigenin-diglucoside

It was determined by spectrophotometric method that 1.49±0.14% in the herb of *Ph. pungens* plant; *Ph. caucasica* herb contains 2.17±0.53% of flavonoids, which has sufficient raw material reserves in the flora of Azerbaijan.

The essential oil content of *Ph. pungens* raw material was studied. In order to obtain essential oil, 100 grams of *Ph. pungens* plant raw material leaves and herb were hydro-distilled for 4 hours using a Clevenger device and the oil was treated with anhydrous sodium sulfate and stored at 4-6 ° C.

Essential oils from raw materials have a pungent odor and light yellow color.

50 components were found, including germacran D (46,4%), (Z) -β-farnesene (9,2%) and hexahydro farnesyl acetone (7,6%) quantitatively predominant as a result of GC-MS study of essential oils.

Although palmitic acid, germacran D, (Z)-β-farnesene, β-bourbonene, hexahydro-farnesyl acetone, caryophyllene-oxide and phytol are the main groups of constituents in the oils of inflorescence, but individual differences are visible. For example, nonadecane, eicosane, salvial-4(14)-en-1-one, heneicosane and decanoic acid were found in the oil of leaves and inflorescence of plant samples collected from Galalti, but not in Altiagach and Nakhchivan samples.

In a similar manner, the oil of *Ph. pungens* leaves and inflorescence from Altiagach region contained (Z)-3-hexenal, α-bourbonene, cuparene, cubebol and elemol, mint sulphide, torreyol, hexadecanol and hexacosane.

It deems that altitude, soil composition, percentage of air moisture, climate changes, plant collection time, and development stage of the plant and genetically differences between populations of plant can affect the chemical composition of the essential oils and other secondary metabolites.

Research evidence suggests that the observed differences may be due to the existence of a new chemotype - sesquiterpene and fatty acid, which is provoked either by different climatic factors, or may originate by genetic differentiations.

Analysis of amino acids in the raw materials of the species belong to *Phlomis* L. genus was carried out by HPLC method.

Identification of amino acids is based on the retention period of the peak areas of standard samples.

Studies have shown that *Ph.pungens* and *Ph.caucasica* Rech.fil herb contain 23 amino acids, 9 of them are essential amino acids.

The amount of amino acid in *Ph.pungens* herb is higher than in *Ph.caucasica* Rech.fil. In addition, the amount of asparagic acid,

glutamic acid, arginine and alanine in *Ph.pungens* herb is significantly higher than the amount of these acids in *Ph.caucasica* Rech.fil. The amount of essential amino acids in *Ph.pungens* herb is practically higher than in *Ph.caucasica* Rech.fil herb. In our opinion, the difference in the amount of amino acids in these species depends on the ecological conditions of the region where they accumulate. Thus, the species used for research were collected from different geobotanical regions of Azerbaijan.

The elemental composition of *Ph. caucasica* and *Ph. pungens* was studied by atomic adsorption method and the relative amount of 18 elements in the raw material was determined. Potassium, calcium, sodium, silicon, magnesium from the macroelements, iron, zinc, manganese and copper from the microelements in the composition of raw materials are higher than other elements.

Based on the results of precautionary and phytochemical studies of perspective species (*Ph.pungens* and *Ph.caucasica*) of the genus *Phlomis* L., studies have been planned and carried out to determine the pharmacological effects of biologically active substances obtained from raw materials.

Antioxidant activity was studied in vitro using the *ABTS* method. Based on the results of this study, conclusions were obtained on which fractions of biologically active substances in *Ph.pungens* herb have the strongest antioxidant effect.

Thus, the butanol fraction of the plant collected from the Altyaghach region has a higher antioxidant effect and and it is promising to use the butanol fraction of the plant collected from the Altyaghach region for both individual substances acquisition and some other research in the future.

The studies on the antioxidant activity of total flavonoid obtained from *Ph.pungens* raw materials were carried out at the department of Pharmacology of Azerbaijan Medical University by the lipid peroxide method based on the *in vivo* model.

The experiments were carried out on 70 white rats of both sexes weighing 170-200 g after 14 days of quarantine, grown in the vivarium of the Scientific-Research Center of the Azerbaijan Medical University. After the animals were divided into 7 groups,

first group control group, in the other 6 groups, the effective dose was determined against the background of the effect of increasing the dose of the total flavonoids of *Ph. pungens* on the amount of lipid peroxide products. Disorders of the enzymatic system in the body in many diseases lead to an increase in the amount of products of combustion of lipids by peroxide.

The most effective antioxidant dose of total flavonoid obtained from *Ph.pungens* is 400 mg/kg.

The total flavonoid obtained from *Ph.pungens* has a strong antioxidant effect with 400 mg/kg by reducing the amount of lipid peroxide products in the brain structures and blood of white rats.

The anti-diabetic effect of *Ph.pungens* extract was performed at the Department of Pathological Physiology of the Azerbaijan Medical University.

The test sample (*Ph.pungens* extract) was injected intravenously at a dose of 25 and 50 mg/kg once daily for three weeks prior to the simulation of experimental diabetes, and the experiment was performed for 30 days. The experimental animals were given water in the same mode. The comparison drug was used as a dose of metformin 100 mg/kg.

As a result of injecting *Ph.pungens* Willd extract in both doses and metformin in a prophylactic dose, compared with the comparative pathology of basal glycemia prevents growth.

39.6% at a dose of 25 mg and 45.3% at a dose of 50 mg of *Ph.pungens* Willd extract, also confirms the decrease of the sugar in the blood for 20 minutes, this fact is observed in animals.

Thus, *Ph.pungens* Willd extract at doses of 25 mg/kg was found by significant hypoglycemic effect in alloxan model diabetes. *Ph.pungens* Willd extract lowers basal glycemia levels and is superior to metformin for this activity at doses of 50 mg/kg.

As shown in the previous chapters of the dissertation, as a result of pharmacognostic study of species belonging to the genus *Phlomis* L. *Ph. pungens* and *Ph. caucasica* were selected as promising species due to raw material reserves and rich chemical composition, phytochemical study of these raw materials was carried out and pharmacological effects of extracts were determined. 80%

alcohol extract has antioxidant and anti-inflammatory effects made from *Ph. pungens* herb, a tablet drug form based on the solid extract has been prepared.

According to the latin name of the raw material, the tablets are called "*Phlomexin*".

As a result of comparative research, as follows, the optimal composition and amount of the excipients have been determined, used for the preparation of "Flomexin " tablets.

Ingredients of one tablet of "Flomexin":

	gr	%
Ph. pungens (solid extract)	0,2 qr	45.46
Lactose Ph. Eur., 1047900, [5989-81-1]	0,2 qr	45.46
Starch Ph. Eur., 1085100, [9005-84-9]	0,022 qr	5
Methylcellulose USP., 9004-67-5	0,004 qr	0.91
Aerosil Ph.Eur., 01/ 2002: 0434	0,006 qr	1.36
Talk Ph. Eur., 1087000, [14807-96-6]	0,006 qr	1.36
Calcium stearate USP, 1592-23-0	0,002 qr	0.45

The average weight of a tablet is 0.44 grams 0,44 qr

Preparation of "Flomexin" tablet (for 300 tablets) consists of the following steps.

Preparation of "Flomexin" tablets consists of the following steps:

- preparation of a mixture of lactose, methylcellulose and solid extract;
- preparation of starch paste
- transformation of the mixture with starch paste into a dough-like mass;
- granulation;

- drying of wet granules;
- mixing of dry granules with starch residue, aerosil, talc and calcium stearate;
- purchase of tablets from the obtained granule mass in a tablet machine "Bonapase CPR-6";

After phytochemical study of species (*Ph. pungens* and *Ph. caucasica*) belonging to the genus *Phlomis* L., as well as the study of the pharmacological effects of extracts from raw materials, research was conducted on the preparation of project of Pharmacopoeia Articles and the normative documents on the studied raw material and the proposed drug form.

Research has been conducted on the preparation of drafts of articles.

It should be noted that it is important to develop a normative document that contains a set of relevant requirements for quality control, both in the production and use of medicinal plant raw materials and dosage forms.

The new drug "Flomexin" tablets, offered for the first time as a result of the research, as well as for the production of the drug used as a raw material belonging to a *Ph. pungens* species, Pharmacopoeia Article was prepared and approved by the Pharmacology and Pharmacopoeia Commission of the Ministry of Health.

These analytical normative documents were prepared for the first time.

RESULTS

1. For the first time, as a result of the preliminary study of 6 species of the genus *Phlomis* L., which is widespread in the flora of Azerbaijan, *Ph. pungens* and *Ph. caucasica* (Lamiaceae) plants, which are important in terms of pharmacognostic studies and the development of new drugs, have been identified [1, 2, 3].
2. The level of diagnostic features found in the morphological and anatomical structure of the raw materials of *Ph. pungens* and *Ph. caucasica* species allows to determine the identity of the raw

material through macroscopic and microscopic analysis, and the annual supply of raw materials. In the mentioned directions, as a result of the research to determine the annual supply of *Ph. pungens* and *Ph. caucasica* species raw materials, biological reserves is 372405 kg, of exploitable reserves is 232091 kg and the amount that can be supplied annually is 58027 kg of *Ph. pungens* raw materials, biological reserves is 41939 kg, of exploitable reserves is 27231 kg and the amount that can be supplied annually is 6809 kg of *Ph. caucasica* was determined [21].

3. As a result of the study of the anatomical structure of *Ph. pungens* raw materials, the following characteristic diagnostic features were revealed:
   - the surface of the seeds is glossy and covered with simple hairs at the base; there are four oppositely arranged grooves.
   - the surface of the bract is densely covered with hairs, and its apex ends with simple hairs.
   - the calyx from the internal dorsal side is covered with multicellular simple hairs, and there are branched hairs outside.
   - the corolla in the internal dorsal side is covered with simple hairs, and there are branched hairs at the bottom. Outside there are only branched hairs.
   - the petiole is covered with a continuous layer of branched hairs.
   - the dorsal side of the leaf blade is covered with a dense layer of hairs; there are glands along the edges of the leaf blade. The bottom side of the leaf is also covered with hairs, especially the midrib [6, 8].
4. The phenylethanoid glycosides leucosceptoside B and forsitoside B were isolated and identified for the first time from the aerial part of *Ph. pungens* from Azerbaijan [13, 14, 19].

Chlorogenic acid, neo-chlorogenic acid, forsythoside B, quercetin-glucoside, quercetin-glucuronide, verbascoside, luteolin-glucoside, luteolin-glucuronide, luteolin 7-O-[2’’-O-(4’’’- O-acetyl-α-L-rhamnopyranosyl)]- β-D-glucuronopyrano-

side and apigenin-diglucoside from the extracts made from *Ph. pungens* and *Ph. caucasica* herb were obtained and identified [9, 17, 18]

It was determined by spectrophotometric method that 1.49±0.14% in the herb of *Ph. pungens* plant; *Ph. caucasica* herb contains 2.17±0.53% of flavonoids, which has sufficient raw material reserves in the flora of Azerbaijan [10, 12].

5. The essential oils, amino acids and elements of the selected raw materials were studied. 50 components were found, including germacran D (46,4%), (Z) -β-farnesene (9,2%) and hexahydro farnesyl acetone (7,6%) quantitatively predominant as a result of GC-MS study of essential oils [4, 16].

   Studies have shown that *Ph.pungens* and *Ph.caucasica* Rech.fil herb contain 23 amino acids, 9 of them are essential amino acids [7]. The elemental composition of *Ph. caucasica* and *Ph. pungens* was studied by atomic adsorption method and the relative amount of 18 elements in the raw material was determined. Potassium, calcium, sodium, silicon, magnesium from the macroelements, iron, zinc, manganese and copper from the microelements in the composition of raw materials are higher than other elements [5].
6. The antioxidant effects of flavonoid compounds derived from the raw material of *Ph.pungens*, as well as the anti-diabetic effect of the aqueous extract was found [11]. *Ph.pungens* extract at doses of 25 mg/kg was found by significant hypoglycemic effect in alloxan model diabetes. *Ph.pungens* Willd extract lowers basal glycemia levels and is superior to metformin for this activity at doses of 50 mg/kg [15].

   An analytical normative document belong “Flomexin” (*Phlomexin*) tablets offered for the first time and *Ph. pungens* species, on the first type - Temporary Pharmacopoeia Articles were prepared and approved by the "Pharmacology and Pharmacopoeia" Commission of the Ministry of Health of the Republic of Azerbaijan [20].

PRACTICAL RECOMMENDATIONS

1. It is important to study *Ph. tuberosa* L. and *Ph. cancellata* Bunge.species, which are widespread in the flora of Azerbaijan and have sufficient raw material reserves, from a pharmacognostic point of view for the preparation of new medicines.
2. The hyperglycemic activity of extracts of *Ph. caucasica* and *Ph. pungens* raw materials provides a basis for future research into the development of anti-diabetic drugs.
3. *Ph. caucasica* and *Ph. pungens* species identified in different geobotanical regions of the Republic, distribution areas and prepared maps can be used in the preparation of the "Atlas of Medicinal Plants" of Azerbaijan.

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LIST OF ABBREVIATIONS AND SYMBOLS

COSY – Correlation Spectroscopy
DMSO – Dimethyl sulfoxide
FAB-MS – Fast atom bombardment -Mass spectrometry
GC-MS – Gas chromatography–Mass spectrometry
GC-MSD – Gas Chromatography-Mass Selective Detector
HMBC – Heteronuclear Multiple Bond Correlation
HPLC-UV/MS – High-performance liquid chromatography - Ultraviolet - Mass spectrometry
HSQC – Heteronuclear Single Quantum Coherence
IR – Infrared
LC-MS – Liquid chromatography- Mass spectrometry
MeOD (CD_3OD) – Deuterated methanol
MS – Mass spectrometry
nm – nanometer
ppm – parts per million
UV – Ultraviolet

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