REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

MYCOBIOTA AND FUNGICIDAL PROPERTIES OF LICORICE (*GLYCYRRHIZA GLABRA* L.) GROWING UNDER DIFFERENT ECOLOGICAL CONDITIONS

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INTRODUCTION

The research relevance and completion degree. As is well known, *"the Republic of Azerbaijan, despite its small size, has a diverse and colorful flora, including over 5000 plant species"*[1]. They differ from each other in terms of distribution areals, natural resources, interactions with other living things over many years, "*phytocomponent composition, and other characteristics they carry*"[2]. At the same time, these plants differ from each other in terms of their purpose, as they are also characterized as *"medicine, dye, decorative, oil, fodder, fruit, etc"*.[3,4,5] and in their study, these indicators are taken as a basis in several cases.

On the other hand, plants, which are one of the important groups of biodiversity y on earth, *"both wild and cultivated, play an important role in the life activity of all living beings"*[6], and today there is no alternative for their use in fodder, food, medical and technical purposes and there is no doubt that plants will retain this status for a long time. As a result, people's use of plants is expanding, and they are starting to use them in new fields and for other purposes that arise in response to modern-day needs. Although this has practical implications, it does not always end with a positive value for the plants themselves.

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[1] Mehdiyeva, N.P. Azərbaycanın dərman florasının biomüxtəlifliyi /N.P.Mehdiyeva. -Bakı: "Letterpress", -2011, -186 s.

[2] Мамедова, Ш.М.,Новрузов, Э.Н. Содержание и качественный состав каротиноидов плодов некоторых форм облепихи (Hippophae rhamnoides L.), произрастающих в Северном Азербайджане// Вестник МГОУ, серия «Естественные науки», -2016. №3, -с.33-41.

[3] [3]Abbasova, T.S. Abşeronun quru subtropik meyvələrinin mikobiotası:/b.ü.f.d. dissertasiyasının avtorefaratı/-Bakı, 2007, -22s

[4] Əliyev, N. Azərbaycanın dərman bitkiləri və fitoterapiya/N.Əliyev. -Bakı: "Elm" nəşriyyatı, -1998, -343 s.

[5] Qurbanov, E.M. Dərman bitkiləri/E.M.Qurbanov. -Bakı:BDU, -2009, 360 s

[6] Abubakar, B.U. Plant: a Necessity of Life /B.U.Abubakar, S.Abubakar, Ch.Alaku [et al.]//International Letters of Natural Sciences, -2014, v.15(2), -p.151-159

Intensive use leads to the narrowing of the areal of many plants. Plants also face diseases *"caused"*[7] by various organisms, *"primarily fungi"*[8,9,10]. All this makes the development of methods and approaches aimed at using plants according to the principles of sustainable development one of the current research directions of the modern era.

At the initial stage of the research conducted in this direction, it is necessary to solve the problem, that is, the exact identification of the "participants" of the problem that causes the decrease in the biological activity of plants, which primarily includes their identification of species. Plants are also systematized based on the properties they are used for or carry. A large group of plants are systematized according to their medicinal value, and *"about 1,547 species of plants included in the flora of Azerbaijan have these characteristics"*[1]. These plants are studied in "botanical, pharmacological as well as mycological aspects" [11,12,13].

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[7]Velásquez, A.C., Castroverde, C.D.M., He, S.Y. Plant-Pathogen Warfare under Changing Climate Conditions//Curr Biol., -2018. v.21, 28(10). -p.R619-R634.

[8] Гаджиева, Н.Ш. Грибы на эфиромасличных растениях, входящих во флору Азербайджана/ Н.Ш.Гаджиева, К.Ф.Бахшалиева, Н.Р.Намазов [и др.] //Вестник Московского Государственного Областного Университета, серия "Естественные науки", -2012, № 2, -с.24-27

[9] Переведенцева Л. Г. Микология: грибы и грибоподобные организмы/ Л.Г.Переведенцева. - СПб.: Издательство "Лань", -2012, -272с.

[10] Саенко, Г.М., Шуваева, Т.П., Гайтотина, И.В. Болезни мяты (Mentha L.), лаванды узколистной (Lavandula angustifolia Mill.) и шалфея мускатного (Salvia sclarea L.) в коллекции ВНИИМК (обзор) // Масличные культуры. - 2019. вып. 4 (180). - с. 179-188.

[11] Ələsgərova, A.N. Azərbaycan florasının yovşan (Artemisia) növləri və onların xemotaksonomiyası/A.N.Ələsgərova. -Bakı: "Elm" nəşriyyatı, -2019. -452s.

[12] İsmayilova, G.E. Azərbaycan florasına daxil olan bəzi bitkilərdən alınan efir yağlarının ağ naftalan yağı ilə kompozisiyasının antimikrob aktivliy:/ b.ü.f.d. dissertasiyasının avtoreferratı/-Bakı, 2018, -24s.

Nevertheless, today many medicinal plants included in the flora of Azerbaijan are superficially studied in various aspects of research or are generally named in the list of sampled plants. As a result, it would be incorrect to say that medicinal plants included in the flora of Azerbaijan have been thoroughly studied. This thought manifests itself more clearly in the study of those plants in ecological and mycological aspects. In addition, medicinal plants contain various components, due to which the activities of organisms that cause various pathologies in plants are either limited or they are completely destroyed. Their pharmacological activit y has not been comprehensively studied, although they are already used for practical purposes.

Licorice (*Glycyrrhiza glabra* L.) is one of such plants that are medicinally important. It has not been extensively studied in Azerbaijan, but its roots are used. Although this plant, "which is spread worldwide"[14], including all the *"ecologically different areas of the Republic of Azerbaijan"*[15], has large resources. Certain studies have been conducted on the study of this plant in botanical and pharmacological aspects, and several issues have been clarified in this direction. Even in October 2019, the Licorice Industry Park, which would be engaged in the s upply, processing, and production of the licorice plant, started operating in the Agdash district of the Republic of Azerbaijan. Despite this, there has been almost no research on the interactions of licorice with other living entities, particularly those that cause disease.

Th e purpose and tasks of the research. The purpose of the research is to evaluate the *Glycyrrhiza glabra L*. plant according to its resources, species composition of mycobiota, and specific gravity

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[13] Hacıyeva, N.Ş. Azərbaycanın dərman bitkilərinin mikobiotasının növ tərkibi, ekobioloji xüsusiyyıtləri və onlardan istifadənin mikoloji təhülkəsizlik prinsipləri:/b.e.d. dissertasiyasının avtoreferatı/-Bakı, 2017, -41s.

[14] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7173723/

[15] Qurbanov, E.M. Dərman bitkiləri/E.M.Qurbanov. -Bakı:BDU, -2009, 360s.

of pathogens, as well as fungicidal properties of the components obtained from them.

To achieve this purpose, the following tasks were considered important in the research:

- Determination of the general characteristics, some physico-chemical properties, and resource potential of the *G. glabra* plant that spread in ecologically different areas of Azerbaijan;
- Evaluation of fungi involved in the formation of the mycobiota of the *G.glabra* plant spread in the studied areas based on their species and number;
- Characterization of the fungal biota and pathogenic species of the *G.glabra* plant according to ecologically and trophic relationships, frequency of occurrence, and manifestations of plant-fungus relationship;
- Comparative evaluation of some constituent components of *G.glabra* plant spread under different environmental conditions based on their physicochemical and fungicidal properties.

Research methods The cenotic population of *G. glabra*, which grows naturally in regions, was taken as the research unit. The planned route and the method of selecting permanent areas (1m x1m) were used for sampling. The underground parts (up to a depth of 50 cm) of licorice growing in permanent areas were collected and weighed o n an electronic scale (wet weight). In addition, in the course of the research, samples were also taken from the aboveground parts of the licorice and a number of their morphometric indicators were determined.

During the study of the *G.glabra* plant's mycobiota, samples were collected from aboveground and underground parts that were likely to show signs of the fungus or the disease caused by it, and they were analyzed using mycological and phytopathological methods and approaches. All the experiments in the research were repeated at least four times, and the results were statistically processed. During statistical processing, it was $S_x/X \leq 0.05$ (S_x – standard deviation, X-mean value), the purity of the reagents used and the accuracy of the devices were at the required level in similar analyses.

The main provisions of the defense.

- The formation of the morphological appearance of the *G.glabra* plant, the number of individuals per unit surface and the quantitative indicator of the biomass of the underground part are closely related to the ecological conditions of the areas where it is distributed.
- The *G.glabra* plant is not only a place where true fungi (Mycota) live but also favorable for their pathological activities so that species new to the nature of Azerbaijan and species with substrate specificity are involved in both the formation of mycobiota and the occurrence of pathologies.
- Environmental conditions, especially the moisture factor, have a more significant effect on the uneven distribution of the species involved In the formation of the mycobiota of *G.glabra* across the study areas, as well as whether it is far or near according to Sørensen–Dice coefficient.
- The *G.glabra* plant also contains phytocomponents that slow down or completely stop the growth of fungi and bacteria, and the biological characteristics of the test cultures are among the factors that influence the formation of their effect.

The scientific novelty of the research. As a result of the conducted research, the *G.glabra* plant, spread in the areas of Azerbaijan with different ecological conditions, was comprehensively studied according to the morphometric characteristics of the above-ground part, the weight of the underground part, the species of its mycobiota, the prevalence of the observed pathologies, and the antimicrobial activity of various components obtained from the plant.

It became obvious s that, depending on the environmental conditions, the morphometric measurements of the aboveground part of the *G.glabra* plant are characterized by different indicators according to the number of individuals per unit surface and the weight of the underground part, the number of individuals per 1 m^2 area was 8-26, the weight of the underground part varied between 5.4-20.7 kg.

It was found that the natural resources of the G. glabra plant in Azerbaijan are sufficient in all areas where it grows wild, and although 95% of it is used, 12.5% is kept as a resource.

The *G.glabra* L. plant is defined as both a habitat for fungi and a site where diseases are induced by them. Among them, a species such as *Ascochyta glycyrrhizae* Vasag, which is new for the mycobiota typical of Azerbaijani nature, also substrate-specific (true stenotrophs) *Phyllosticta glycyrrhizae* (Hollos) Y.S.Paul & L.N.Bhardwai, *Puccinia glycyrrhizae* F.L.Tai and *Uromyces glycyrrhizae* (Rabenh) Magnus occur.

According to the frequency of occurrence (FO) of the fungi found in the plant, 11.1% belong to dominant species (FO=52.6-58.7%), 40.7% to common species (FO=12.5-43.5%), 48.2% to random and rare species (FO=0.01-9.6%), according to areal classification, 51.9% was boreal, 44.4% was cosmopolitan and 3.7% xerophytic.

For the first time in the conducted studies, the concordance of the mycobiota of the wild-growing *G.glabra L.* plant according to the Sørensen–Dice coefficient was determined for different regions.

It was found that the *G.glabra L.* plant contains components with antimicrobial activity, regardless of the method of isolation, and the formation of a quantitative indicator of this activity is significantly influenced by the place of collection of the plant and the biological characteristics of the test culture itself.

Theoretical and practical significance of the research. The obtained results are actual materials useful in terms of specifying the resource potential of *G.glabra* growing wild in Azerbaijan, their mycobiota and the conditions required to o btain remedies with fungicidal activity.

The obtained results showed the necessity of the preparation of normative documents that include the mycological safety principles of the use of *G.glabra* plant, and the determination of the species of fungi that are evaluated as indicators of the mycological safety of plant-based raw materials and finished products, the specific name of mycotoxins that are not allowed to be present, and the concentration indicator that is allowed which will be useful in the preparation of

normative documents regulating the use of medicinal plants and in the improvement of existing documents.

Th e use o f the remedies obtained from the *G.glabra* plant, first of all, the use of essential oil in the form of a composition with White Naphthalan oil, allows to obtain more effective preparations.

Publication, approbation and application of the dissertation. 14 scientific works related to the topic of the dissertation were published and the materials of the dissertation were presented at the scientific conference of young scientists and students on "Innovations in biology and agriculture to solve global challenges" (Baku, 2018), at the jubilee conference of the National Academy of Mycology on "Mycology and microbiology" (Russia F., Moscow, 2018), at the Republican scientific conference on "Modern problems of biology" (Sumgait, 2018), at the XI nternational scientific conference on "Scientific achievements and challenges in biology" (Baku, 2021), II Republican scientific conference entitled "Ecology and soil sciences in the XXI century" (Baku, 2021).

The name of the organization where the dissertation work was performed. The main part of the dissertation was carried out at the Faculty of Ecology and Soil Science of Baku State University, and other parts related to the determination of fungi were completed at the Institute of Microbiology of the Ministry of Science and Education.

The structure and volume of the dissertation. The total volume of the dissertation consists of 2153004 characters including the introduction, literature review, materials and methods, experimental part, discussion, results, a list of references and a list of abbreviations.

CHAPTER I
BIORESOURCE POTENTIAL OF LICORICE (*GLYCYRRHIZA GLABRA* L.), ECOLOGICAL CHARACTERISTICS OF SPREAD, MYCOBIOTA AND FUNGICIDAL PROPERTIES OF ITS COMPONENTS

This part of the dissertation begins with general information about the ecological situation of the Earth. Then the information about the medicinal plants included in the flora of Azerbaijan is analyzed, their characteristics are given, and finally, the results of the research conducted on the ecology of the pl ant *Glycyrrhiza glabra L.*, which was selected as the research object, are summarized and the tasks that are important to solve are touched upon.

CHAPTER II
MATERIALS AND METHODS

2.1. General characteristics of the research areas

The studies were conducted in the Kur-Araz lowland, the Greater Caucasus, the Lesser Caucasus, and the Lankaran-Astara region. The territories of the mentioned geomorphological units differ from each other in terms of area, flora and fauna, and natural soil and climatic conditions.

2.2. Sampling and the methods used in the analysis

The research object was the cenotic population of *G.glabra L.* plants growing wild in the selected regions. Permanent areas (1x1) were selected for productivity determination. The underground organs of the licorice in those plots (up to 50 cm depth) were collected and weighed on an electronic scale (wet weight). The obtained results showed the output of raw phytomass of *G.glabra L.* per unit area.

In addition, in the course of the research, samples were taken from the above-ground parts of the licorice and a number of their morphometric indicators (the number of leaves, length and width of compound leaves, number of flowers, etc.) were determined. In this case, the number of plants in 1 m^2 was taken as an indicator of their density per unit surface. “Methods and approaches”[16,17] of different authors were used in the work to determine the productivity of the underground part of the plant per unit area , the number of plants and the morphometric measurements of the aboveground parts.

To assess the viability of cenopopulations, the IVC vitality index was used:

$$IVC = \frac{\sum_{i=1}^{n} X_i/X_o}{N}$$

Where Xi - is the average value of the trait in the cenopopulation, Xo - is the average value of the trait for all cenopopulations (when monitoring one cenopopulation, the average value for all years of observations)

N – is the number of traits.

During the sampling of the mycobiota of licorice, the traditional route method was used, and at the same time, samples were taken from the permanent areas. Samples were collected from above-ground and underground organs that maintain the plant's vitality and are visually likely to contain fungi. Sampling, on-site passportization

[16] Недилько, О. В. Изменчивость солодки голой по морфологическим, биохимическим и ресурсным признакам в природных популяциях Волгоградской области//Экология: факты, гипотезы, модели: материалы конф. Молодых ученых. - Волгоград, -2018. -с.95-98.

[17] Недилько, О.В. Эколого-биологические и ресурсныеособенности Glycyrrhiza glabra L. в природных условиях Волгоградской области /О.В. Недилько, Н.В.Овсянкина, К.М.Холод [и др.]//Грани познания, -2019. №6 (65), -с.102-105.

and preparing them for laboratory studies were carried out according to accepted *"methods and approaches"*[18,19] in mycology.

During the analysis of the fungal biota of the collected samples according to the species and number, Malt Extract Agar (MEA), Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), Rice Extract Agar (REA) were used as nutrient media, and their preparation and sterilization, transfer of samples to nutrient media, obtaining pure culture were carried out according "*to accepted methods and approaches in microbiology"*[18,19]. Species affiliation of pure cultures was carried out using known determinants based on cultural-morphological signs of fungi, as well as signs of diseases caused by them (true biotrophs). "*The official website*"[20] of the International Mycological Association (BMA) was used for the systematization and naming of fungi.

In the course of research, the following formula was used during the characterization (N) of the mycobiota of the *G. glabra* plant according to its number (CFU/g):

$$N=abc/d$$

Where, a – the number of colonies formed in a Petri dish (colonies), b – the amount of dilution used when transferring the fungi to the nutrient medium, c- the number of droplets in 1 ml of suspension when transferred to nutrient medium (droplets) and d – the amount of material taken for analysis expressed in g.

The frequency of occurrence (FO) and the distribution (D) of the fungi samples taken from the *G. glabra* plant were determined based on the following formula:

$$FO(D) = (n/N)x100$$

[18] Методы экспериментальной микологии./Под. ред. Билай В.И. Киев: Наукова думка, -1982, -500с.

[19] Нетрусов, А.И. Практикум по микробиологии./ А.И.Нетрусов,., М.А.Егорова, Л.М.Захарчук -М.:Издательский центр «Академия», -2005, -608с

[20] http://www.mycobank.org/MycoTaxo.aspx

Here, N is the total number of plants sampled during the calculation of both FO and D, and n is the number of plants in which the fungus was detected (with disease symptoms).

Aqueous extract (AE) and essential oil (EO) obtained from the underground part of the plant were used to isolate constituent components from the *G.glabra* plant, and their isolation was carried out according to the methods and approaches used in the research of different authors.

During the study of the bactericidal and fungicidal activity of the constituent components of the *G.glabra* plant, more precisely, the essential oil and aqueous extract obtained from it, visually healthy underground and aboveground parts were used. Their activity was studied with the application of well-known methods. In general, during the research, about 900 samples were taken from the underground and aboveground parts of the *G.glabra* plant and analyzed according to the purpose of the work.

All experiments were repeated at least four times and the obtained results "*were processed statistically*"[21]. From the obtained results, the ratio of the mean squared deviation to the average value of the trait for all cenopopulations (X_0) was ≤ 0.05, and the results were considered reliable.

[21] Кобзарь, А. И. Прикладная математическая статистика./ А. И. Кобзарь. М.: ФИЗМАТЛИТ, -2006, -816 с.

CHAPTER III
EVALUATION OF RESOURCES AND MYCOBIOTA OF THE *GLYCYRRHİZA GLABRA L.* PLANT DISTRIBUTED IN AZERBAIJAN

3.1. Morphometric evaluation of the aboveground parts and evaluation by weight of the underground parts of *Glycyrrhiza glabra* L. distributed in Azerbaijan

G.glabra L., one of the medicinal plants included in the flora of Azerbaijan, differs from other plants with similar properties in terms of both its resources and distribution, as well as its use. The study of G.glabra, which is widely distributed in the Republic of Azerbaijan, is not at the required level, primarily in eco-mycological aspects. For this reason, it was considered appropriate to first clarify some issues related to the resource potential of licorice, which is distributed in ecologically different areas of Azerbaijan.

From the obtained results, it became clear that the *G.glabra* plant is characterized by a different quantitative indicator according to its morphological images, the number of individuals per unit surface, and the weight of the underground part (Table1).

The change of these indicators is also influenced by the meteorological indicators of the area. Thus, the numbers are slightly higher in areas with high precipitation (LA), and slightly lower in areas with low precipitation (GC). Calculation of the vitality index of cenopoulation also showed that conditions are favorable for *G.glabra* L. in LA. Thus, during the calculation of the IVC as a result of 3-year monitoring, it became clear that IVC=1.3 for the *G.glabra* plant growing in the LA zone, while

Table 1

General characterization of morphometric indicators and resources of *G. glabra* plant

Traits	GC	LC	KA	LA	Average trait value by country
Plant height (cm)	90.4	103.7	110.5	118.5	105.8
Number of compound leaves (leaf)	21.1	24.2	26.0	28.2	24.9
Length of compound leaves (cm)	10.8	12.1	12.5	12.9	11.2
Number of flowers (flower)	18.5	23.6	27.1	31.5	25.2
Number of fruits (fruit)	19.9	21.4	23.2	25.1	22.4
Number of plants per 1 m^2 (plant)	8-14	11-17	12-23	12-26	11-20
Weight of the underground part of the plant per1 m^2 area (wet weight, kg)	5.5-10.7	7.3-14.6	7.8-17.4	8.0-20.7	7.2-15.9

a similar indicator was 0.89 for GC (Absheron ER). Based on this fact, it can be noted that the moisture factor plays a limiting role in the harvesting of the *G.glabra* plant under Azerbaijan conditions.

Based on the obtained results, the biological resources of the *G.glabra* plant, the amount that can be exploited and prepared for use, were calculated according to known methods and approaches. It became clear that 95% of the known biological resource is characterized a s a resource to be exploited, and 12.5% of it is a resource to be prepared for use.

3.2. Evaluation of the mycobiota of *Glycyrrhiza glabra L*. based on species composition, and diseases caused by pathogenic species involved in the formation of mycobiota

Concerning the research of mycobiota, in the studies conducted since 2017 in the largest geomorphological units of the Republic of Azerbaijan, it was found that 27 species of fungi were involved in the formation of the mycobiota of *G.glabra L*. (table 2). 78.3% of recorded fungi belong to sac fungi, of which more than half (15 species) are anamorphs, and 6 species are telemorphs.

Among the observed fungi, most of the phytopathogens belong to anamorphs, and in general, anamorphic fungi cause 9 diseases in *G.glabra* (spotting, rotting, fusarium wilt, wilting, rust, cercosporosis, powdery mildew, nigrospora sphaerica and Phyllactinia). Even if all telemorphs participate in the formation of the pathogenic mycobiota of *G glabra*, the prevalence of diseases caused by them (2.7-7.5%) is lower than that of anamorphs (0.01%-58.7%) and they cause 4 diseases.

Table 2

Quantitative characterization of taxonomic classification of fungi found on the *G.glabra L.* plant

Phylum	Class	Order	Family	Genus/Species
Mucormycota	1	1	1	2/3
Ascomycota	4	10	13	19/21
Basidiomycota	2	2	2	3/3
Total	7	13	16	24/27

The recorded species of Basidiomycota also contain pathogens, the prevalence rate of diseases caused by them ranges between 1.7% and 7.8% and they cause 2 pathogens (rust and rhizoctinosis or root rot).

As mentioned, 3 species of fungi belonging to the Phylum of *Mucormyceta (=Zygomycota)* of 2 genera are involved in the formation of the mycobiota of the *G.glabra* plant, but their causing any pathology has not been confirmed either in our research or literature data. Their frequency is not so high (1.5-2.6%).

It is interesting that among the fungi found on the *G.glabra* plant, there is a species such as *Ascochyta glycyrrhizae*, which is new to the mycobiota of Azerbaijan, as well as *Nigrospora sphaerica*, *Puccinia glycyrrhizae* and *Stagonosporopsis cucurbitacearum*, which have substrate specificity.

The fact that the ecological conditions of the studied areas are different is also reflected in the mycobiota of the plants growing under those conditions. Thus, the mycobiota of the licorice spread in the Aran economi c region (Kur-Araz lowland) is relati vely rich, as well as the number of recorded diseases ranges between 17-23.

During the assessment of the mycobiota of separate economic regions according to the Sørensen–Dice coefficient, it became clear that Aran and Absheron are relatively close to each other (75.0%), and Absheron and Lankaran-Astara are the most distant (63.2%). When the mycobiota of the *G.glabra* plant distributed in different zones were studied according to their number, it became clear that the number of the mycobiota of the *G.glabra* plant depends on both ecological conditions and the seasons of the year. Thus, the highest

İndicator ($2.7x10^3$, autumn) was recorded in the Kur-Araz lowland and the lowest indicator ($1.5x10^2$, winter) in the Greater Caucasus. The numerical composition according to seasons was observed in autumn or summer.

3.3. Evaluation of the fungi involved in the formation of the mycobiota of *Glycyrrhiza glabra L.* according to the areal classification

As stated, 27 true fungal species (Mycota or Fungi) play a role in the formation of the mycobiota of the *G.glabra* plant. When these fungi were evaluated according to their areal classification, it was revealed that most of the recorded fungi belong to cosmopolitan and boreal types. Thus, 44.4% of the observed fungi (*Alternaria alternata, Aspergillus ustus, Botrytis cinerea, Cladosporium herbarum, Erysiphe communis, Fusarium moniliforme, F.oxysporium, Microsphaera diffusa, Mucor plumbeus, M.rasemous, Penicillium chrysosporium* and *Rhizopus stolonifer*) belongs to cosmopolitans, and 51.9% (*Ascochyta glycyrrhizae, Cercospora glycyrrhizae-echinatae, Gliocladium nigrum, Leveillula taurica, Nigrospora sphaerica, Phyllosticta glycyrrhizae, Pleospora herbarum, Puccinia glycyrrhizae, Rhizoctonia solani, Sclerotinia sclerotiorum, Stagonosporopsis cucurbitacearum, Thelaviopsis bazicola, Trichothecium roseum* and *Uromyces glycyrrhizae*) belongs to boreal type. The xerophytic type is represented by 1 species (*Verticillium dahile*), which accounts for 3.7% of recorded fungi.

3.4. An annotated list of species of fungi involved in the formation of the mycobiota of *Glycyrrhiza glabra* L. and evaluation of their relationship with the host plant

In the compilation of the annotated list of species determined to be involved in the formation of mycobiota of the licorice plant during research, the list of fungi (Mycota or Fungi) is given according to

separate divisions and the closeness of the systematic relationship of the species for each division.

In the studies, an annotated list of 27 species of fungi found in *G.glabra* shows that those recorded species are characterized by many aspects and wide diversity.

The quantitative expression of the diversity of fungi involved in the formation of the mycobiota of the *G.glabra* plant revealed that most of the fungi are anamorphs of the sac fungi (55.6% of the total fungi). In terms of ecotrophic relationships, 18.5% of recorded fungi belong to true biotrophs (*Erysiphe communis, Leveillula taurica, Microsphaera diffusa, Puccinia glycyrrhizae and Uromyces glycyrrhizae*), 74.1% to facultatives or polytrophs, and 7.4% belongs to saprotrophs (*Mucor plumbeus, M. racemous*). According to their distribution in the substrate, 81.5% of the fungi involved in the formation of the mycobiota of *G. Glabra* plant are eurytrophs, 7.4% are conditional stenotrophs (*Erysiphe communis and Microsphaera diffusa*) and 11.1% are true stenotrophs (*Phyllosticta glycyrrhizae, Puccinia glycyrrhizae and Uromyces glycyrrhizae*).

The assessment of the fungi found in the *G. glabra* plant according to the frequency of occurence (FO) revealed that only 3 species (*Alternaria alternata Ascochyta glycyrrhizae and Pleospora herbarum*) were dominant in the formation of the mycobiota of the *G. glabra* plant (FO=52.6-58.7 %). The number of common (widespread) fungi s pecies was 11 (FO=12.5-43.5%), and the number of random and rare species was 13 (FO=0.01-9.6%).

If we evaluate the mycobiota of the *G.glabra* plant according to this indicator, it can be noted that the mycobiota of the plant is not characterized by indicators that are very positive from the phytosanitary and phytopathological point of view. Thus, among the dominant and common species of fungi involved in the formation of mycobiota, many species cause dangerous plant diseases. Thus, at least 2 of the mentioned fungi (*B. cinerea* and *F. oxysporium*) are included in the list of 10 most dangerous fungi that cause diseases in plants. This fact allow s us to highlight the need to follow mycological safety principles when using the *G.glabra* plant for various, especially medical purposes.

CHAPTER IV
EVALUATION OF THE CONSTITUENT COMPONENTS OF THE *GLYCYRRHIZA GLABRA* PLANT INCLUDED IN THE FLORA OF AZERBAIJAN ACCORDING TO ANTIMICROBIAL ACTIVITY

4.1. Evaluation of the *Glycyrrhiza glabra* L. plant distributed in different areas of Azerbaijan according to some physicochemical parameters

The *G.glabra* plant used to obtain constituent components was collected from different geomorphological units of Azerbaijan, depending on which there are certain differences in the morphometric indicators and resources, which was also clear from the information given at the beginning of the work. This affects the physicochemical parameters of the isolated substances, that is, the aqueous extract (AE) and the essential oil (EO), as well as the fungicidal properties and therefore, at this stage of the research, the moisture content, optical density and pH of the substances obtained from licorice collected from different zones, were determined first. The obtained results revealed that although h the de termined indicators of the licorice collected from different zones were close to each other, certain differences were also observed, which mainly manifested themselves in the moisture index and optical density of the isolated substances (Table 3). A s can be seen, the optical density of EO obtained from *G.glabra* L. collected from Greater Caucasus is high compared to others, and low in *G.glabra* L. collected from Lankaran-Astara. Comparing this with the initial moisture content of the samples taken, it is clear that higher initial moisture content is accompanied by lower optical density.

Table 3

Some physicochemical parameters of AE and EO obtained from licorice collected from different areas

Areas where the research was conducted	The initial moisture content of the sample (%)	pH		Optical density (656 nm)	
		AE	EO	AE	EO
Kur-Araz lowland (Central Aran + Karabakh)	65	6.1	6.8	0.703	1.221
Greater Caucasus (Absheron)	63	6.1	6.7	0.725	1.253
Lesser Caucasus (Gazakh-Tovuz + Ganja-Dashkasan)	68	6.1	6.8	0.682	1.142
Lankaran-Astara	71	6.0	6.7	0.556	1.116

4.2. Antimicrobial activity of substances isolated from the plant *Glycyrrhiza glabra*

During the study, two remedies (AE and EO) obtained from *G.glabra* L. were used, and both classical (*Bacillus subtilus, Stafilococcus aureus, Pseudomonas aureginoza Echericha coli* and *Candida alpicans*) and targeted (*Aspergillus niger, A.ochraseus, Fuzarium oxysporium, Penicillium citrinum* and *P.cuclopium*) test cultures were used during their evaluation for antimicrobial activity. For this purpose, fungi such as were used. Antimicrobial activity was determined by both biomass yield and diameter of the lysis zone

obtained according to the disk diffusion method. Evaluation of the activity of plant-derived agents in relation to both fungi and bacteria was graded using a 3-point scale (weak - up to 20 mm, medium - 20-29 mm, and strong - more than 29 mm) of the diameter of the lysis zone.

The study of the fungicidal properties of the obtained materials revealed that both AE and EO isolated from the *G. glabra* plant have antimicrobial activity, but the quantitative indicator of that activity is different depending on both the substance obtained from the plant and the biological characteristics of the microorganisms used as test cultures. Thus, increasing the concentration of AE added to the medium leads to an increase in fungicidal activity (compared to the control, the biomass yield decreases from 89.6% to 2.9%), but in no case does the growth stop completely, that is, fungistatic activity (significant weakening of growth) is observed, rather than fungicidal.

When EO obtained from the *G.glabra L.* plant is added to the medium, a complete stop of growth is also observed, and this occurs at certain concentrations of EO (Table 4). Increasing the amount of EO added to the medium to 0.1% caused no growt h at all in fungi such as *C. herbarium, Fusarium moniliforme, F. oxysporium* and *Penicillium cyslopium,* i.e. fungicidal activity was observed. Fungi species such as *A. flavus, A. ochraceus, C. alpicans and P. citrinum* showed little growth, but the amount of biomass varied between 0.2 and 1.4% compared to the control. Since this indicator is extremely small, th e amount of 0.1% of EO obtained from the *G.glabra* L. plant can be characterized as the amount that realizes the fungicidal activity indicator in relation to all test cultures.

Depending on the areas from which licorice is collected, there are certain differences in th e physicochemical indicators of AE and EO obtained from them. The study of t he effect of these differences on the fungicidal properties of the mentioned substances revealed that certain differences occurred in this case as well, and the high optical density led to a relatively high fungicidal activity, which manifested itself more clearly

Table 4

Effect of EO obtained from the licorice plant on the growth of test cultures (according to biomass yield, g/l)

Test cultures	Control (Czapek medium)	Concentration of added EO (%)			Activity (based on residual weight compared to control(%)		
		0,01	0,05	0,1	0,01	0,05	0,1
A.flavus	5,23	0,97	0,18	0,01	18,5	3,4	0,2
A.ochraceus	5,62	1,30	0,53	0,08	23,1	9,4	1,4
Candida alpicans	3,35	0,65	0,32	0,02	19,4	9,6	0,6
C.herbarum	3,71	0,90	0,27	0	24,3	7,3	0
F.moniliforme	3,74	0,54	0,23	0	14,4	6,1	0
F.F.oxysporium	3,96	0,32	0,17	0	8,1	4,3	0
P.citrinum	3,82	0,72	0,20	0,02	18,8	5,2	0,52
P.P.cyclopium	4,12	0,54	0,28	0	13,1	6,8	0

at hig h concentrations. This situation shows itself in relation to both AE and EO. So, when using both AE and EO of the licorice collected from Absheron-Khizi ER, the fungicidal activity was higher compared to others.

The moisture factor is important for the intensive development of fungi. Therefore, the role of the moistur e fac tor during the reservation of the collected plant materials was clarified. For this purpose, the collected root of the *G.glabra* L. plant was dried for different periods under the same conditions to obtain 3 products with different initial moisture (A- initial moisture ≤ 10%, B – 11 > initial moisture ≤ 20%, C- initial moisture > 21%) and after being kept under the same conditions for 1 month, it was analyzed according to

the number composition of its mycobiota and antimicrobial activity. According to the obtained results, the initial moisture above 10% leads to an increase in the number of fun gi involved in the formation of th e mycobiota o f the materials belonging to the *G. glabra* L. plant, and on the contrary, to a decrease in the antimicrobial activity of AE obtained from it. This is also the basis for emphasizing the importance of taking th e moisture factor into account when using plant-based materials.

Plants are important as a source of natural drugs in human treatment, but some plant-derived substances, including essential oils, have also ne gative effects because they are toxic, burning, tanning, etc., and it is not convenient to use them in a pure form. For this reason, it is considered convenient for their use in the form of a composition. Accordingly, we also used W hite Naphthalan oil (WNO) obtained from Naphthalan oil based on high purification technology. For this purpose, a composition with essential oils obtained from the *G.glabra* L. plant was prepared and tested. The obtained results allowed us to note that this approach is relatively favorable. Mo re specifically , the antimicrobial activity of the obtained composition is higher than that of both EO and WNO at the same amount, and the optimal ratio was 0.2/1. In this case, the effect increases between 11% and 17%, which allows increasing both the effect and the efficiency of the use of resources due to the fact that a smaller amount of substances is taken in the preparation of the composition.

FINAL ANALYSIS OF RESEARCH RESULTS

Research on medicinal plants in various aspects, primarily botanical, pharmacological, biochemical, mycological, and dietological, is being c onducted and is still ongoing both globally and in Azerbaijan. Nevertheless, the number of plants studied from the aforementioned aspects, as well as from an ecological standpoint, is significantly lower than the number of known species, and among the plants that have not been studied from this perspective, there are even

plants that have been widely used for many years for a variety of purposes. One them is a perennial herb *G.glabra* L., which is known to be distributed in most parts of the world, studied in various aspects and widely used for practical purposes, distributed in all large geomorphological units of Azerbaijan, such as GC, LC, KA and TM, and whose root is more suitable for use from a practical point of view, widely used in the food industry, folk medicine, clinical medicine, phytotherapy, and perfumery. Nevertheless, the work dedicated to the study of this plant in mycological and ecological (more precisely, eco-mycological) aspects has either not been carried out at all, or has been poorly carried out under specific ecological conditions. Lik e many plants, *G.glabra* L. can be valued as an open and interesting object for systematic research in these aspects. For this reason, the plant *G.glabra* L. was chosen as an object in the presented work and the study of this plant, distributed in the ecologically different areas of Azerbaijan, according to the species composition of its micobiota, the specific weight of the pathogens involved in the formation of the mycobiota, and the fungicidal properties of some compounds obtained from the plant, was set as a goal. According to the experimental work performed to achieve this goal, the results were expressed in the form of 5 final results and 3 practical recommendations.

CONCLUSIONS

1. It has been established that the *Glycyrrhiza glabra* L. plant spread in the territory of the geomorphological units of the Republic of Azerbaijan with different ecological conditions is characterized by a different quantitative indicator according to its morphological appearance, the number of individuals per unit surface, and the weight of the underground part, so, the number of licorice plants per 1 m^2 area varies between 8 and 26 depending on the area, and the weight of the underground parts of licorice collected from 1 m^2 area varies between 5.4 and 20.7 kg[12, 14].

2. According to the obtained results, 95% of t he biological resources of the *Glycyrrhiza glabra* L. plant, which is widespread in Azerbaijan, have the characteristic to be exploited, and 12.5% of it is a resource to be prepared for use[12, 14].
3. The conducted study shows that the *Glycyrrhiza glabra* plant is characterized as one of the habitats of fungi, and 27 species of fungi are involved in the formation of its mycobiota, of which 21 species belong to Ascomycota, 3 species to Basidiomycota and 3 species to Zygomycota. Recorded fungi cause diseases such as alternaria, spotting, fusarium, rust, white and pink rot, root rot, wilting and species such as *Ascochyta glycyrrhizae* Vasyaq, which is new to the mycobiota of Azerbaijani nature, and species with substrate specificity (true stenotrophs) *Phyllosticta glycyrrhizae* (Hollos) Y.S.Paul & L.N.Bhardwai, *Puccinia glycyrrhizae* and Uromyces glycyrrhizae (Rabenh) Magnus are among them[3, 5, 7, 13].
4. The observed fungi were found to differ from each other according to the general frequency of occurrence in the plant and the areal classification of distribution. Thus, according to the frequency of fungi found in the plant, 3 species were dominant (FO=52.6-58.7%), 11 species were common (FO=12.5-43.5%), and 13 species were random and rare (0.01-9.6%). According to the areal classification, 51.9% was boreal, 44.4%-cosmopolitan, and 3.7%-xerophytic[6, 10-11].
5. It was found that due to the different ecological conditions, the distribution of fungi in the *Glycyrrhiza glabra* L. plant was uneven, and the mycobiota of the licorice growing in the Kur-Araz lowland was richer in terms of species composition, while that of the on e growing in the Greater Caucasus (Absheron ER) was the poorest. Comparing the mycobiota of different geomorphological units according to The Sørensen–Dice coefficient showed that the Kur-Araz lowland and the Great Caucasus (Absheron) were closer to each other (75.0%), while Absheron and Lankaran-Astara were more distant (63.2%.)[10-11].

6. It has been established that *Glycyrrhiza glabra* L. contains components with fungicidal activity and it is possible to obtain them from the plant by both water extraction and hydrodistillation. The formation of fungicidal activity of the components obtained in both ways is influenced by the place of collection of the plant, the method of extraction of the component and the biological characteristics of the test culture itself. The use of plant-derived agents in the form of compositions with White Naphthalan Oil leads to an increase in antimicrobial activity, which creates additional opportunities for more efficient use of resources[1-2, 4, 8-9].

PRACTICAL RECOMMENDATIONS

1. For the obtaining of preparations with fungicidal activity, the initial moisture content of the collected plant samples should be taken into account, and in this regard, it is more convenient to collect them from the Greater Caucasus, more precisely from the territory of Absheron ER.
2. It is more convenient to use the aqueous extract and essential oil obtained from licorice in the form of a composition with White Naphthalan Oil, and it is recommended to use 1 ml White Naphthalan Oil, and 0.2 ml EO.
3. It is necessary to prepare regulatory documents containing the mycological safety principles of the use of medicinal plants, including *G.glabra*, as well as the determination of the species composition of fungi evaluated as indicators of the mycological safety of plant-based raw materials and finished products, to indicate the specific name of the unacceptable mycotoxins and the concentration indicators of the permitted ones.

THE LIST OF PUBLICATIONS ON THE TOPIC OF THE DISSERTATION

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