

Micological Safety Principles Of Some Medicinal Essential Plants Growing In Azerbaijan

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Summary. Most of the plants spread in Azerbaijan, including medicinal ones, have become the subject of various studies, and they have been studied to some extent in various aspects (botanical, pharmacological, etc.). However species of medicinal plants belonging both to the flora of Azerbaijan and being introduced have not been the subject of systematic mycological, even phytopathological research and only in some research it is also possible to come across the names of such plants when indicate the habitats of the fungi. Although as a result of fungal diseases the productivity of some species reduce each year, most of them are destroyed and in the population the number of individuals decreases. In order to prevent them, i.e. elimination of the observed adverse effects, identification complex measures, a comprehensive study of the mycobiota of medicinal plants, especially its pathogenic representatives (species composition, ecological-trophic relationships, frequency of occurrence, metabolic activity, etc.), clarification of the form of the relationship between the fungus-host plant are one of the most important and topical issues.

The main argument for the urgency of the issue is that most of the medicinal plants utilized in folk medicine are used without heat treatment, and their cultivation, collection and preparation for use is carried out in an open system [1]. This inevitably characterizes them as an open system for the collection of various microorganisms, including the fungi themselves and their metabolites. Studies have shown that among the fungi contained in medicinal plants, there are enough toxins, allergens and pathogens, especially common pathogens, and these plants are also

enriched with fungal mycotoxins, as well as the fact that substances taken by people for different purposes (food and medical) are also one of the sources of transmission of various diseases [3, 4].

Keywords: Medicinal plant mycobiota, pharmacological activity, mycological safety

Introduction.

As it is known, medicinal plants are one of the largest groups of higher plants and their vegetative or generative organs are utilized as raw materials for the using tools in medicine, folk medicine, as well as veterinary practice, including those with pharmacological activity. From the beginning of mankind, plants have been used not only as a source of food for humans, but also in the treatment of any disease. Despite the fact that science currently knows about 500,000 plant species, only a small part of them are often used in medicine [2].

Azerbaijan, home to 9 out of 12 known types of climate, is rich in vegetation including plants of great economic importance and is also a source of a number of substances that are a constant component of the human diet, including medicinal plants. 1547 (34.3%) species out of 4745 wild plant species growing in the flora of Azerbaijan are medicinal plants. As a result of the research, it was determined that 272 species of medicinal plants included in the scientific pharmacopoeias of different countries from the world flora grow wild in the flora of Azerbaijan. 77 species of medicinal plants included in the scientific pharmacopoeia of the Azerbaijani flora are Caucasian endemic, 6 species are Azerbaijani endemic and 44 species are relict plants.

Nowadays among the medicinal plants found in Azerbaijan- pumpkin (*Cucurbita pepo* L.), thymes (*Thymus collinus* Bieb.), common fennel (*Foeniculum vulgare* Mill.), marshmallow plant (*Althaea officinalis* L.), cumin (*Cuminum cuminum* L.), fig (*Ficus carica* L.), laurel (*Laurus nobilis* L.), olive (*Olea europaea* L.), Japanese saffron (*Sophora japonica* L.), Common wormwood (*Artemisia absinthium* L.), fennel (*Acorus celamus* L.), mint (*Mentha piperita* L.), corn (*Zea mays* L.), common pine (*Pinus sylvestris* L.), chamomile (*Matricaria chamomilla* L.), valerian (*Valeriana officinalis* L.), innab (*Zizyphus* Mill.), Rosemary (*Albizzia julibrissin* Durazz.), barberry (*Berberis vulgaris* L.), peony (*Paeonia* L.), medicinal rosemary (*Rozmarin officinalis* L.), hawthorn (*Crataegus pentagyna* L), etc. are considered to be relatively widespread.

Medicinal plants draw attention mainly due to their biological, primarily pharmacological activity. Pharmacologically active substances differ in their chemical composition and currently are widely used in their systematization. However, these types of plants currently used in folk medicine either do not have normative documents regulating the number of microorganisms, including fungi, as well as the amount of their harmful metabolites, or the existing documents do not reflect these issues in accordance with modern requirements [5, 6].

The purpose of the study. It is the preparation of basic data for the organization of mycological safety of medicinal plants, the study of the ecobiology of fungi inhabiting medicinal plants and the study of the species composition and distribution patterns of a number of medicinal plants included in the flora of Azerbaijan, especially those relatively widely used in folk medicine.

The object of research is some herbal products of medicinal importance: Dock (*Rumex L*), chamomilla (*Matricaria chamomilla*), Yarrows (*Achillea millefolium L*), Sea buckthorn (*Hippophae rhamnoides L.*), Sage (*Salvia officinalis L.*) Pumpkin (*Salvia officinalis L.*), Jasmine (*Jasminum officinale*), Ordinary rue (*Peganum harmala*).

The methodology of the research is based on theoretical data and experimental research was the process of thinning colonies according to the rules of microbiology, obtaining a pure culture, identification by appropriate determinants based on cultural-morphological and physiological characteristics.

MATERIALS AND DISCUSSIONS

Medicinal plants grown in different parts of Azerbaijan were selected as the object for the planned mycological research, and more than 200 species of plants with these characteristics were sampled during the research. The taxonomic relevance of the sampled plants is summarized in Table 1. Different areas of the Republic of Azerbaijan were selected for sampling which were the Great Caucasus, Little Caucasus, Kur-Araz lowland and Lankaran-Astara zone. In accordance with the purpose of the study samples were taken from the vegetative and generative organs of wild and cultivated medicinal plants in the mentioned areas, which are likely to be fungi. In this case, the selection of permanent sites for planned routes and stationary observations, which are widely used in the course of mycological research, etc. methods were utilized. It should be noted that samples of plant materials prepared for use and sold to the public in various trade facilities were also taken during the research. In total, more than 3,500 samples were taken and analyzed for the purpose of the study.

Due to the purpose of the work modern mycological and phytopathological methods and approaches were used in the analysis of the collected samples [7, 8].

Additives of malted juice (AMC), rice (RA), starch (SA) and potato (PA), additives, added Capek and Chapek-Dox were used as nutrient media for the plants of fungi living in the studied plants. The conditions were prepared, sterilized and poured into Petri dishes according to known methods [6]. Based on the results obtained in the laboratory, as well as the results of field observations, the identification of the fungus itself or the pathology it causes is carried out. Currently, BMA and CBC baseline data and modifiers that allow identification to be performed on cultivative-morphological and biological traits, have been used.

In the course of the research, their growth coefficient (GC) was also used to determine whether the fungi differed in their growth. The following formula was used to calculate the GC:

$$GC = \frac{DHS}{T} (1)$$

Here, D is the diameter of the colony (mm), H is the height of the colony (mm), S is the density of the colony determined by visual inspection (from 1 to 5), T is the cultivation period (days).

The frequency of occurrence of fungi on samples, as well as the prevalence of diseases caused by pathogenic plants was determined by the following formula:

$$P = \frac{n}{N} 100 (2)$$

Here, P is the frequency of fungi in the samples (or the prevalence of the disease caused by the pathogen – in%), n - the number of detected fungi (number of infected plant individuals in the studied area, in numbers), N - the total number of samples (total number of plant species in the studied area).

When determining the enzymatic activity of fungus, a liquid Capek medium was used for their cultivation, the composition of which was as follows (g / l): Cultivation was carried out at 26°C for 15 days and enzyme activity was determined in plant solution every 5 days according to appropriate methods[3]. In the course of the research, experiments were performed in at least 5 repetitions and statistical processing of the obtained results was carried out, in which the Gauze method was used. In all cases, the information corresponding to the formula $m / M = P \leq 0,05$ was considered correct and only they were included in the articles.

Research results

Now about the results obtained from medicinal plants and based on these methods:

As a result of the samples analysis taken from wild and cultivated medicinal plants in different areas of Azerbaijan, it was determined that a total of 168 species are involved in the formation of their mycobiota and information on their taxonomic structure is summarized in Table 1. As it is known, most of the recorded fungi are true fungi and a small number are fungal-like organisms. Among them, the predominant species of indeterminate fungi, more precisely, anamorphic fungi. Among them the species of indeterminate fungi, more precisely, anamorphic fungi predominate. Thus, 83.3% of the fungi registered in the study fall into this group. Representatives of basidiomycetes and oomycetes take the second place - 5.2%. The share of telemorphs of teliomycetes, zygomycetes and ascomycetes contains 4.6%, 4.0% and 2.9%,

respectively.

Table 1. Taxonomic structure of isolated fungi in the course of research

Condition	Section	Class	Row	Family	Gender	Type
Mycota	Zygomycota	1	1	2	3	9
	Ascomycota	5	10	17	29	114
	Bazidiomycota	2	6	8	15	33
Chromista	Oomycota	1	2	2	3	12

Among the registered fungi, the most common species are representatives of the genus *Colletotrichum*. Thus, as a result of research of this genus, 14 species (8.3% of the total species) of medicinal plants are widespread in Azerbaijan. The species *Ascochyta*, *Phoma*, *Fuzarium*, *Septoria* and *Penicillium* can also be considered numerous, and their number varies from 10 to 12 species (Table 2).

Table 2. Distribution of species of fungi found in medicinal plants by species

Names of genders	Number of species
<i>Colletotrichum</i>	17
<i>Phoma</i>	14
<i>Ascochyta</i>	13
<i>Fuzarium</i>	12/1
<i>Septoria</i>	12
<i>Penicillium</i>	11
<i>Alternaria</i> , <i>Phyllosticta</i>	7-8
<i>Aspergillus</i> , <i>Diplodina</i> , <i>Mucor</i> , <i>Phytophthora</i> , <i>Verticillium</i>	5-6
<i>Botrytis</i> , <i>Cephalosporium</i> , <i>Cercospora</i> , <i>Cerrena</i> , <i>Cladosporium</i> , <i>Cylindrosporium</i> , <i>Dicoccum</i> , <i>Eryshiphe</i> , <i>Fomitopsis</i> , <i>Ganoderma</i> , <i>Hormiscium</i> , <i>Inonotus</i> , <i>Laetiporus</i> , <i>Macrophoma</i> , <i>Macrosporium</i> , <i>Monilia</i> , <i>Peronospora</i> , <i>Pestotia</i> , <i>Phellinus</i> , <i>Phomopsis</i> , <i>Plasmopara</i> , <i>Plectosphaerella</i> , <i>Puccinia</i> , <i>Rhisopus</i> , <i>Sclerotina</i> , <i>Spongospora</i> , <i>Sporotrichum</i> , <i>Stagonospora</i> , <i>Stemphylium</i> , <i>Trichothecium</i> , <i>Trametes</i> , <i>Trichoderma</i> , <i>Urocystis</i> , <i>Uromyces</i> , <i>Ustilago</i>	1-4

Comparing the results with those obtained in other studies conducted in Azerbaijan, it is clear that many of them have been recorded in Azerbaijan in one or another biotope. However, in our studies, the distribution

of a number of species recorded in the nature of Azerbaijan has not yet been found and the number of those that meet this characteristic is 15 (Table 3).

Table 3. Taxonomic relevance of fungal species, which was recorded for the first time in the nature of Azerbaijan

Zygomycota	Mucor corticola Hagem, M. plumbeus Bon.,
Ascomycota	Ascochyta anethicola Sacc., Asc. pinodes (Berk. et. Blox) Jones., Asc. pseudopinodella Bond – Mont et. Xassi, Diplodina lactucae (Oudem) Sacc., Dicoccum asperum (Corda) Saccardo, Penicillium stoloniferum Thorn., P. puberulum Bainier, Verticellium pulverulentum Couwenteg., V. lateritium Berk., Phoma roumii Fron., Ph. minulella Sacc et. Penz., Septoria petroselini Desm., S. sojae Thuern
Bazidiomycota	Phellinus chrysoloma (Fr.) Donk

The fact that the rich vegetation of any area provides a wide variety of species of other living things, especially fungi, is not a matter of debate among the scientific community. If we consider the rich nature of Azerbaijan as a known fact, the same landscape should be observed in Azerbaijan. However, the results of research conducted in Azerbaijan in this area, as noted above, can not be considered satisfactory. Such a situation can be attributed to the geographical regularities of the distribution of fungi inherent in the nature of Azerbaijan. Thus, in the research conducted in this area, fungi or a specific group of fungi (antimony, rust, etc. fungi) distributed in a specific zone of Azerbaijan (for example, Nakhchivan AR), as well as fungi distributed in a specific substrate type (for example, in the main forest species of Azerbaijan) have been characterized in this aspect. All this can not be considered sufficient to characterize the mycobiota inherent in the nature of Azerbaijan in this aspect, and therefore it is a task to characterize the fungi recorded in the course of research in this aspect.

Although this division is not considered ideal, it is one of the most widely used today. However, a number of authors, referring to this division, describe the fungi using the terms "real", "optional" or "polytrophic" and consider it important to overcome the shortage of this division. On the other hand, in order to fully characterize true biotrophy, as well as saprotrophy, some researchers also consider the ecological or physiological nature of biotrophy [8, 9].

In our opinion, in accordance with the current level of development of mycological science, it is more expedient to use this section together with the proposed polytrophic term, and it was considered expedient to characterize the fungi registered in the course of research according to this division.

During the characterization of the registered fungi in tropical relationships in medicinal plants of Azerbaijan, it became clear that although the number of biotrophs has a relative advantage among the registered fungi, the number of true biotrophs among them is not so high and symbiotrophs are not found among them. As can be seen, although 44.8% of the total fungi belong to saprotrophs and 55.2% to biotrophs, fungi with biotrophic and saprotrophic polytrophic or facultative nature make up 80.5% of the total fungi [10, 11].

In the course of the research, when clarifying the distribution of fungi registered in the medicinal plant growing zones of Azerbaijan according to their ecological-trophic relations, it became clear that the general indicators registered in Azerbaijan are confirmed in the zones with a small difference. Thus, 53.4% of fungi registered in the Great Caucasus belong to biotrophs, and 46.6% to saprotrophs.

Table 4. Distribution of fungi by plants

Plants	Taxonomic relevance of fungal species				
	Oomycota	Zygomycota	Ascomycota	Bazidsiomycota	Total
Japanese sophora	0	0	8	8	16
Pear (<i>Pyrus communis</i>)	0	1	6	7	14
Almonds (<i>Amygdalus communis</i> L)	0	2	12	10	24
Tomato	3	0	31	0	34
Yarrows (<i>Achillea millefolium</i> L.)	0	0	7	1	8
Sea buckthorn (<i>Hippophae rhamnoides</i> L.)	1	0	10	1	12
Aubergine (<i>Solanum melongena</i> L)	2	2	18	0	22
Whistle (<i>Verbascum thapsiforme</i> Schrad.)	1	0	12	0	13
Sage (<i>Salvia officinalis</i> L.)	2	5	22	0	29
Watermelon (<i>Citrullus vulgaris</i> L)	2	1	21	0	24

Melon	2	1	30	0	33
Pumpkin(<i>Pepocucurbita</i> L)	1	2	12	1	16
Hips (<i>Rosa</i> L)	0	0	19	1	20
Jasmine (<i>Jasminum officinale</i>)	1	0	26	3	30
Ordinary rue(<i>Peganum harmala</i>)	0	0	5	0	5
Chamomile (<i>Matricaria chamomilla</i>)	1	0	8	4	13
Dock (<i>Rumex</i> L)	1	1	5	1	8
Others	7	8	43	16	75

Discussion of the results of the study

When characterizing the mycobiota of any plant species, the frequency of occurrence of the fungus in that plant is an important indicator and now for the characterization of mycobiota specific to this or that plant, ie which species of mycobiota are dominant and which are random species. For this reason, it was considered expedient to characterize the mycobiota of medicinal plants in this aspect[12, 13]. During the analysis of samples taken from this or that plant, it became clear that the distribution of registered fungi in the natural zones of Azerbaijan is characterized by different quantitative indicators both in terms of the number of species and the frequency of their occurrence.

As can be seen, the increase in the number of fungi also leads to a decrease in the antifungal activity of extracts from medicinal plants, but in this case the quantitative indicator of the decrease is higher than the antibacterial activity. For example, the antibacterial activity of an extract from the plant *Foeniculum vulgare* Mill decreased by 17.6% in relation to *St. aureus* when the number of fungi varied from ≤ 103 to ≥ 105 , while it was 33.3% in relation to *Candida albicans*. Similar rates were found in *Bac. subtilis*, *Ps. aeruginosa*, and *Esc. coli* at 21.1%, 13.3, and 23.8%, respectively. Thus, the quantitative composition of fungal biota has a stronger effect on the antifungal activity of medicinal plants. A similar situation is observed in extracts from other plants. In our opinion, the reason for this should be sought in the intensification of the struggle for survival among close taxonomic groups.

Conclusions

1. Research has shown that the mycobiota of medicinal plants in Azerbaijan includes 168 species. According to their taxonomic relevance, these fungus belong to 39 genders of 13 families of 9 rows of 5

classes of 4 subdivisions (sections)(Zigomycota, Ascomycota, Bazidiomycota və Deyteromycota) of the real fungi department (Euomycota) of the world of fungi(Mycota).

2. 15 species of fungi (*Mucor corticola* Hagem, *M.plumbeus* Bon., *Penicillium stoloniferum* Thorn., *P.puberulum* Bainier, *P.griscolum* Smith., *P.stoloniferum* Thom., *P.sartorii* Zikai., *Aspergillus melleus* Lukavva, *Verticellium pulverulentum* Couwenteg., *V. lateritium* Berk., *V.terrestre* (Link) Lindau, *Dicoccum asperum*(Corda) Saccardo, *Fuzarium sporotrichiella* Bilai., *F.argillaceum*.(Fr) Sacc., *Phoma subvelata* Sacc., *Ph. roumii* Fron., *Ph. minulella* Sacc et. Penz., *Phomopsis dauci*Arx, *Ascochyta anethicola*Sacc., *Ascochyta anethicola*Sacc., *Asc. pinodes* (Berk.et. Blox) Jones., *Asc. pseudopinodella* Bond – Mont et. Xassi, *Hormiscium stilbosporum* (Corda) Saccardo, *Diplodina lactucae* (Oudem) Sacc., *Septoria petroselini* Desm., *S. sojina* Thuern, *Phellinus chrysoloma*(Fr.) Donk presenting in the formation of the mycobiota of the researched medicinal plants are new to the mycobiota peculiar to the nature of Azerbaijan.

3. 44.8% of the registered fungi in medicinal plants of Azerbaijan belong to saprotrophs due to ecological-trophic relations, and 55.2% to biotrophs, but the biotrophy and saprotrophy of 80.5% of the total fungi are not real. In addition, 25.7% of fungi registered in medicinal plants are allergenic and 28.3% are toxigenic.

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