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Mycobiota and Antifungal Activity of the Components of Some Medicinal Plants Spread in the Different Ecological Conditions

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Abstract

In the conducted studies, some medicinal plants included in the flora of Azerbaijan were studied by the mycobiota and the antifungal activity of the constituent components. It became clear that in the formation of the mycobiota of the studied plants are involved both fungi and fungi-like organisms and in the formation of the mycobiota of individual plants, in addition specific species are also involved universal species. The number of specific species varies from 1 to 5, and the number

of universal species varies from 6 to 16. Aqueous extracts and essential oils obtained from medicinal plants differ from each other in terms of their constituent elements and to the quantitative indicator of antifungal activity. The antifungal activity indicatsors of the components obtained from plants with a relatively high specific weight of universal species in the formation of mycobiota is low, which allows to note that universal species affect the component composition of plants in a wider range.

Keywords: medicinal plants, mycobiota, universal and specific species, component composition, antifungal activity

Introductıon

Identification and practical use of medicinal potential of plants is a growing trend worldwide [18]. Because currently, according to the data of the World Health Organization, about 80%[16.] of the world's population uses various remedies obtained from plants, and the demand for these types of remedies is increasing. Nevertheless, the number of species of this type of plants is extremely small compared to what is known to science, and in general, the status of many plants from this point of view is unclear. So that, a total of 10% of about 0.35-0.50 million species are used for this purpose[18]. For this reason, one of the current research directions of recent times is the determination of the potential of unstudied or poorly studied plants in this direction. It should be noted that even widely studied plants can change their qualities according to changing environmental conditions. Also, ecological factors of the environment in which it spreads play a role in the formation of this or that feature and this can also be characterized as an open object for research. For this reason, a comprehensive study of a plant that spreads in a specific area does not mean that it is not an open object for research, more precisely, the principle of "a specific approach to a plant growing in a specific soil-climate condition" is still relevant.

Plants, animals, fungi, and bacteria spread in the territory of Azerbaijan have a rich and colorful variety that, which manifests itself clearly in its natural soil-climate conditions. It should only be noted that 9 out of 11 climate types are found in the not so large territory of the Republic of Azerbaijan [7]. The territory of Azerbaijan is formed by the some of ecologically different parts, which has made it necessary to conduct various types of research here, and probably this situation will continue for a long time.

The diversity inherent in the nature of Azerbaijan has not escaped its flora either, so the number of species of plants growing in its territory is around 5000. These plants are also characterized by wide diversity according to their vital forms,

resources, and purposes. Plants that are considered medicinally important makes up 1/3 of the flora [11] Plants that are considered medicinally important makes up 1/3 of the flora, which some of them are officially registered.

Various (botanical, phytochemical, as well as mycological and microbiological) researches are conducted in Azerbaijan regarding the study of medicinal plants[3, 6.], and work is being done on the preparation of the scientific and practical bases of their use. Some interesting results were obtained in the research conducted in this direction, and in general, the number of medicinal plants sampled in this aspect was around 200 species which up to 10% have been studied for antifungal activity. Nevertheless, the results of research conducted in this field today cannot be considered satisfactory. So far, with the exception of Alhagi mororum Medik and Glycyrrhiza glabra L. [2], neither the mycobiota of other plants, and the change of the antifungal activity of the constituent components of plants depending on the environmental condition has been studied.

Therefore, the purpose of the presented work was dedicated to the study of the mycobiota of some medicinal plants belonging to the flora of Azerbaijan and distributed in ecologically different areas, and the antifungal activity of some materials obtained from them.

Material and methods

Researches was conducted in 2015-2022 in the northeast of the ecologically diverse Greater Caucasus (Shahdag National Park), Talish Mountains (Hirkan National Park), Lesser Caucasus (Goy-Gol Reserve), Kura-Araz Plain (Shirvan State Reserve) of the Republic of Azerbaijan. As research objectwere used plants such as *Capsella bursa-pastoris Medic., Cichorium intybus L., Cucurbita pero L., Hypericum perforatum L., Lamium album L., Melissa oficinalis L., Melilotus officinalis L., Mentha piperitae L., Polygonum hydropiper L., Plants such as Sambucus nigra L., Symphytum asperum Lepech., Tussilago farfara L.* which spread in all the researched areas.

Sampling of medicinal plants selected as the object of the research was carried out on various routes selected by area, as well as on selected permanent of areasSampling was carried out mainly according to the phenological phases of plants (beginning of growth, flowering, fruit formation and drying of the aerial body). In total, more than 2000 samples of vegetative and generative organs of plants were taken during the years of research, and analyzed according to the purpose of the work.

A leaf taken as a sample for the identification of fungi on the surface and spread on plants were pressed onto the surface of the agar medium, then removed, and the infected medium was placed in a thermostat [15].

To take out the fungi obtained as a result of research into pure culture culture were used media such as agarized malt juice (MA), starch agar (SA), potato (PA) and rice (RA) agars, which are widely used in the cultivation of fungi. The preparation, sterilization and pouring of the media into Petri dishes were carried out according to the known methods [12, 15] accepted in microbiology and mycology, at the same time, it was prepared under the appropriate atmospheric pressure within the required time frame, following the technical regulations precisely.

The identification of the fungi was carried out using the determinants prepared according to similar indicators based on the cultural-morphological characteristics of the fungi, which were first cultivated in agarized media and removed to pure culture [4, 10, 17, 19-20] and finally for the purpose of clarification was used, the base data of the official website of the International Mycological Association (İMA) [9] and İndexfungorum[8].

At the next stage of the research, were taken samples from the vegetative and generative organs of the studied plant, and prepare aqueous extract and essential oil, which were obtained according to the brewing and hydrodistillation methods, respectively. During the study of antifungal activity, fungi were cultivated in a liquid nutrient medium with the addition of a certain amount of materials obtained from plants, the composition of which was as follows (in g/l): glucose – 10; NH_4NO_3 - 1,5; NaCl - 0,5; K_2HPO_4 – 0,5; $MgSO_4 \cdot 7H_2O$ - 0,5; $FeSO_4$ - 0,01. After sterilization, the acidity of the medium is 6.2. Cultivation was carried out for 5 days at 28^0C, and then the resulting biomass was filtered from the culture solution and after filtration, the biomass was dried until a constant weight was obtained. Activity according to biomass yield was expressed in %.

Were used test culture taken in pure culture and having strong phytotoxic activity[2-3] as a *Alternaria alternata, Aspergillus flavus, A.parasiticus, Fusarium moniliforme, F.oxysporium* and *Verticillium dahliae*, extracted from different areas of the Republic of Azerbaijan

Results and their discussion

Fungi, as a group of biodiversity, have the ability to spread wherever there is organic matter, even in places where the conditions for living are not entirely favorable. One of the most common places where they are found is plants, where they use plants as both a place of residence and feeding. One of the manifestations of their relationship is the occurrence of various pathologies. As a result of this, both the productivity of the plants and the effect of the constituent components decrease. For this reason, clarifying the mushroom-plant relationship, studying the species composition of the mycobiota of the plant, the eco-trophic relationships of the fungi involved in the formation of the mycobiota, as well as the manifestations of their eco-trophic specialization (toxigenicity, conditional pathogenicity, allergenicity) in order

to use them effectively is important. At the same time, this issue is important for the preparation of normative documents for ensuring the microbiological safety of the use of plants for food, fodder and medical purposes.

For clarification of the above firstly, the mycobiota of plants was studied according to species composition. Based on the results of the research conducted in this regard, became clear that fungi were found in the all samples taken from the studied plants, and the total number of species was 60 (tab. 1). As seen, fungi are represented in different numbers in the formation of mycobiota of plants, and this number manifests itself differently in areas with different environmental conditionsThus, in the formation of the mycobiota of Mentha piperitae in Hirkan National Park (HNP) participate 18 , in Shahdag National Park 16, in Shirvan State Reserve 15, and in Goygol Reserve 16 species. The reason for this, in our opinion, is due to the fact that the chemical composition of plants and the ecological conditions of the areas are different. For example, the area where the HMP is located is the area with the most precipitation (1200-1400 mm) and but the area where the Shirvan State Reserve is located is the part with the least precipitation (200-400 mm)[7].

According to the distribution of the fungi involved in the formation of the mycobiota of the studied plants on individual plants, there are more eurytrophs, that is, those that do not have substrate specificity. Thus, 43 species of recorded fungi have this characteristic, and they are recorded in at least on 5 species of plants. Species such as *Alternaria alternata, A. solani, Botrytis cinerea, Fusarium oxysporium, F. solani, Rhisopus stolonifer, Rhizoctonia solani* and *Verticellium dahile* have the ability to spread in almost 10-12 plant species and cause various pathologies in them.

In the next stage of research, was considered appropriate to study the antifungal activity of WE and EO obtained from plants.

The importance of the research conducted in this direction is due to the fact that the number of the world's population is increasing within a fixed area, and accordingly, the expansion of urbanization is inevitable. As a result, the utilization status of the territories used for different purposes (for example, for rural and urban farms), as well as those that are not yet used for one reason or another, changes. One of the consequences of this is the narrowing of the range of plants, which include medicinal plants. For this reason, increasing the efficiency of their use, preparation cientific and practical basis for obtaining components with higher effect by using less plants is one of the necessary tasks to be solved by the modern era. In addition, the anthropogenic impact on the environment is increasing, and as a result, the number and nature of diseases is also changing. If we take into account the increasing demand for preparations obtained from natural sources in order to have preventive effects in order to prevent them, no additional argument will be needed to confirm what has been said.

Table 1. Quantitative general characteristics of the fungi recorded in the studied plants

Plant	Taxonomic affiliation of recorded plants																Total (No)
	Chromista				Mucormycota				Ascomycota				Bazidiomycota				
	1*	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1
Capsella bursa-pastoris Medic	-	-	1	-	1	2	1	1	10	9	8	8	-	1	1	-	11
Cichorium intybus L.	1	-	-	-	1	2	2	2	8	10	9	10	1	1	-	-	13
Cucurbita pero L.	1	2	2	1	2	3	2	2	14	16	14	13	1	1	1	1	22
Hypericum perforatum L.	-	-	-	-	2	2	2	2	11	12	12	11	1	0	1	1	14
Lamium album L.	-	-	-	-	1	2	1	1	9	10	9	10	1	1	1	-	13
Melissa oficinalis L.	1	-	-	-	1	2	2	2	10	9	8	9	-	1	1	-	12
Melilotus officinalis L.	-	-	1	1	1	1	1	1	8	10	9	9	1	2	2	1	13
Mentha piperitae L.	1	1	1	1	2	3	2	2	11	13	11	12	2	1		1	18
Polygonum hydropiper L.	-	-	-	-	1	1	1	1	12	14	13	13	1	2	1	1	17
Sambucus nigra L.	-	-	-	-	2	2	2	2	9	10	8	9	1	1	1	1	13
Symphytum asperum Lepech.	1	1	1	1	1	2	2	1	11	12	11	10	1	1	1	1	16
Tussilago farfara L.	1	1	1	1	2	2	2	2	12	13	11	11	1	1	1	1	17
Total	4	5	5	4	4	5	4	4	37	40	36	35	9	10	8	9	60

Note : *- 1- Goygol Reserve, 2- Hirkan National Park and 3- Shirvan State Reserve and 4- Shahdag National Park

It should be noted, that toxigenic fungi were used as a test culture during the determination of antifungal activity of tools obtained from plants. Thus, toxigens are a wide and heterogeneous group of fungi that differ according to their morphological features, nutrition, reproduction, habitats, development cycle, and synthetic metabolites' effect on living organisms. Secondary metabolites, more precisely, mycotoxins, which they synthesize as a result of life activities has a carcinogenic, mutagenic, tetragenic, embryotoxic, allergenic, immunosuppressive effect [1, 5]. For this reason, the partial or complete cessation of the growth of fungi corresponding to this characteristic is one of the current research directions of the modern era.

During the study of the fungicidal properties of the components obtained from the investigated medicinal plants, it became clear that both WE and EO have a negative effect on growth of fungal, and this is confirmed by the data obtained and summarized in the sample of WE and EO from plants distributed in the studied areas (tab. 2 and 3). As can be seen, that although the amount of WE added to the medium is 10 times higher than that of EO, the EO obtained from plants shows higher antifungal activity than WE in all cases, and in some cases this is also characterized as fungicidal activity, so, in general, none of the fungi have the ability to grow.

Different antifungal activity of WE and EO obtained from plants has been confirmed in other studies [13-14].This is due to the fact that during water extraction, a number of components of the plant, primarily those contained in the EO and which are the basis of antifungal activity, do not pass into the solution.

The reason for the different activity of WE and EO obtained from plants is due to the difference in the chemical composition of plants and the natural soil-climate conditions of the area where the plant is spread. This is also confirmed by the comparison of the antifungal activity of the remedies obtained from plants taken from different research areas. So that, from the data in Tables 2 and 3, it is clear that in all cases, the maximum activity indicator is observed in the plant collected from the Shirvan State Reserve, and the lowest indicator is observed mainly in the plants collected from the HNP. Others are characterized by intermediate indicators, which is related to the proximity and distance of the ecological conditions of the mentioned areas. 3-4 times less precipitation and relatively high average annual temperature in the Shirvan State Reserve compared to the HNP are also characterized as factors that positively affect activity.

It would be appropriate to touch on one point from the findings regarding the antimicrobial activity of WE and EO obtained from plants collected from different areas of Azerbaijan (tab 2 and 3), which is related to whether there is a dependence between the mycobiota of plants and its antifungal activity. As mentioned, the effects of WE and EO from plants are different, so, the highest activity of the effects of both means is observed when using components obtained from Capsella bursa-pastoris, and the lowest when using components obtained from Cucurbita pero plants. The indicators for others lie between the specific to these two plants. When compare this result based on the number of species involved in the formation of mycobiota of plants, it is clear that there is an inverse relationship between the number of species and the antifungal activity, that is, the antifungal activity is relatively reduced as the number of species increases. On the

other side, as the number of species involved in the formation of mycobiota increases, the specific weight of species that are considered universal pathogens and do not have substrate specificity also increases.

Table 2. Effect of WE obtained from studied plants on the growth of test cultures (1.0%, 7 days, in % of residual biomass)

Plant	*Alternaria alternata*	*Aspergillus flavus*	*A.parasiticus*	*Fusarium moniliforme*	*F.oxysporium*	*Verticillium dahliae*
C.bursa-pastoris	13.5-15.5	12.7-16.1	14.3-15.1	13.2-14.4	12.6-13.9	13.0-13.7
Cintybus	14.2-16.6	13.6-16.7	15.4-17.0	14.1-15.0	13.2-14.8	14.0-15.3
C.pero	15.7-18.4	17.6-18.9	18.0-20,2	16.7-17.5	16.5-18.1	16.9-20.1
H.perforatum	14.5-17.0	16.3-17.1	16.6-18.0	14.0-15.1	13.9-15.2	14.1-16.7
L.album	14.1-16.5	13.7-16.8	15.3-17.1	14.2-15.3	13.3-14.6	13.5-14.7
M.oficinalis	13.9-16.2	13.1-16.4	14.9-15.9	13.8-14.9	12.9-14.3	13.1-13.9
M.officinalis	14.0-16.5	13.5-16.8	15.6-17.2	14.0-16.0	13.6-14.9	14.1-15.9
M.piperitae	15.5-18.0	17.1-18.0	17.6-19.3	13.0-14.1	12.0-13.2	16.0-19,0
P.hydropiper	15.4-18.1	17.0-17.7	17.3-19.0	12.8-13.8	11.9-13.0	14.2-17.9
S.nigra	14.0-16.6	13.0-16.8	14.3-15.1	13.2-14.4	12.6-13.9	13.6-14.8
S.asperum	15.0-17.7	16.5-17.4	17.0-18.6	12.5-13.5	11.7-12.8	15.0-18.3
T.farfara	15.2-18.0	16.7-17.5	17.2-18.9	12.6-13.9	11.9-13.0	14.5-17.5

Table 3. Effect of EO obtained from studied plants on the growth of test cultures (1.0%, 7 days, in % of residual biomass)

Plant	*Alternaria alternata*	*Aspergillus flavus*	*A.parasiticus*	*Fusarium moniliforme*	*F.oxysporium*	*Verticillium dahliae*
Capsella bursa-pastoris	0	0	0	0	0	0
Cichorium intybus	0.1	0	0	0	0	0
Cucurbita pero	1.9-5.7	2.1-6.4	1.9-6.3	2.1-6.8	2.3-7.3	2.0-6.3
Hypericum perforatum	0.2-1.0	0.4-1.5	0.5-1.9	0.3-1.3	0.5-1.8	0.6.2.0
Lamium album	0	0,1	0	0	0,1	0
Melissa oficinalis	0	0	0	0	0	0
Melilotus officinalis	0	0	0,1	0	0	0
Mentha piperitae	1.6-3.7	1.7-4.4	1.7-4.3	1.8-4.7	1.9-5.1	1.7-5.2
Polygonum hydropiper	0.8-2.2	0.7-2.3	0.8-2.4	0.9-2.8	1.0-4.0	0.9-2.7
Sambucus nigra	0	0	0	0	0	0
Symphytum asperum	0.5-1.9	0.6-2.0	0.7-2-1	0.6-2.3	0.4-2.1	0.6-2.2
Tussilago farfara	0.9-2.3	1.1-4.4	1.0-4.3	1.1-4.8	1.3-5.3	1.1-4.1

Conclusion

Fom the results was clear that, despite the fact that the studied medicinal plants contain components with antifungal activity, they are also one of the habitats and feeding places of fungi, and 11-22 species participate in the formation of their mycobiota. The ecological conditions of the area also affect the number of fungal species involved in the formation of the mycobiota of this or that plant. Various means obtained from the studied plants (WE and EY) show antifungal activity against toxigenic fungi, and the natural climatic conditions of the areas where the plants are taken also play a role in the formation of this level of activity. On the other side, the large number of species involved in the formation of mycobiota and do not have substrate specificity can also be characterized as a factor limiting the antifungal activity of the components obtained from plants

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