

BIOLOGICAL SCIENCES

MYCOBIOTA OF SELECTED MEDICINAL PLANTS IN THE GADABAY REGION (AZERBAIJAN): TAXONOMIC STRUCTURE AND ECOLOTROPHIC CHARACTERISTICS

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Abstract

Medicinal plants constitute an essential component of traditional and modern medicine; however, their therapeutic value may be significantly compromised by microbial and fungal contamination. Although numerous studies worldwide address mycobiota associated with medicinal plants, region-specific investigations in Azerbaijan remain scarce. This study aimed to assess the diversity, taxonomic structure, and ecolotrophic roles of microscopic fungi colonizing selected medicinal plants-*Urtica dioica* L., *Stellaria media* L., and *Cephalaria gigantea* (Ledeb.) Bobrov-naturally growing in the Gadabay region. Mycological analyses were conducted using standard cultivation, microscopic, and statistical methods. More than 15 species of micromycetes were isolated and identified. Fungi belonging to the phylum **Ascomycota** dominated the mycobiota (66%), while **Zygomycota** accounted for 34%. The most frequently encountered genera included *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Mucor*, and *Rhizopus*. Plant-fungal interactions were classified into parasitic, saprotrophic, and occasionally mutualistic relationships. Pathogenic species were predominantly associated with senescent, stressed, or mechanically damaged plant tissues. The findings emphasize the necessity of mycological monitoring to ensure the hygienic quality and biological safety of medicinal plant raw materials.

Keywords: medicinal plants; mycobiota; micromycetes; fungal contamination; plant-fungal interactions

1. Introduction

Medicinal plants have been widely used for centuries as natural remedies due to their rich content of biologically active compounds and relatively low toxicity compared with synthetic pharmaceuticals. In recent decades, global interest in herbal medicine has increased substantially, driven by a growing preference for natural products and sustainable healthcare solutions. Azerbaijan, particularly its mountainous regions, possesses significant floristic diversity and a long tradition of medicinal plant use (Aslam et al., 2025; Ahmadov, 1984; Ibadullayeva 2001; Gasimov, Aliyev, 1998).

The Gadabay region, located in the Lesser Caucasus, is characterized by diverse ecological conditions that support numerous medicinal plant species. Despite their therapeutic potential, medicinal plants are highly susceptible to microbial contamination during growth, harvesting, drying, storage, and processing. Among microorganisms, microscopic fungi represent one of the most critical groups affecting plant quality, safety, and pharmacological efficacy.

The objects of these investigations are microscopic fungal species (mycoflora) distributing on some medicinal plants (*Urtica dioica* L., *Stellaria media* L., *Cephalaria gigantea* (Ledeb.) Bobrov) in the natural conditions of the Gadabay region.

Micromycetes may colonize plant surfaces or internal tissues, leading to tissue degradation, reduction of active metabolites, and production of toxic secondary metabolites such as mycotoxins (Namazov & Sultanova, 2015). These fungi not only pose phytopathological risks but may also threaten consumer health if contaminated plant materials are

used for medicinal purposes. Consequently, understanding the composition and ecological behavior of fungal communities associated with medicinal plants is essential for quality control and biosecurity (Mammadova, 2025).

Although mycological investigations of medicinal plants have been widely conducted in Europe and Asia, regional studies focusing on Azerbaijan's native flora remain limited. In particular, the mycobiota associated with medicinal plants growing under natural conditions in the Gadabay region has not been systematically studied. Therefore, the present research aims to fill this knowledge gap by characterizing the taxonomic diversity and ecolotrophic structure of fungi colonizing selected medicinal plant species.

2. Materials and Methods

2.1 Study Area

Gadabay district, known for its natural beauty, rich history and culture, is one of the charming corners of Azerbaijan. It is located in the high and middle mountainous belts of the Lesser Caucasus, in the area bordering the Gazakh and Tovuz districts in the west of Azerbaijan. Its area is 1232.98 square kilometers and is considered one of the ancient settlements of Azerbaijan. Goshabulag and Gojadagh are considered the highest peaks, as Goshabulag is 3549 m, and Gojadagh is 3317 m. Anthropogenic, Jurassic, Cretaceous and Paleogene sediments are mainly distributed here. White and black marble deposits are mainly found in the deposits.

The climate is typical of a mountainous region, as a mild subtropical climate prevails in the lower parts of the region, but colder climatic conditions prevail as

the altitude increases. High mountainous areas receive a lot of snowfall. A cold, dry, mountainous tundra climate prevails in winter. A mild, dry, warm climate prevails in summer. The air temperature in July is 10°C-20°C, and in January -2°C-10°C. Mountain-forest lands are observed in mountainous areas with a mild-warm climate between 700-200 m.

Yellow, black, brown and brown soils are found. Brown soils have developed under moist forests rich in rot. Brown soils are found in forests with a relatively dry climate. Yellow soils are widespread in the Talysh Mountains and Lankaran Lowland. The soil is red and yellow due to the iron it contains. A very dense river network is found. The upper reaches of the Akhinja and Zayam rivers are located in this region, and the upper reaches of the Shamkir river are located on the southwestern border of the region. One of the most important water sources is the Gamarli river. It is provided with various lakes and small reservoirs suitable for irrigation. The Tartar River is located in the southern part of Gadabay and is used for irrigation purposes. There are various mountains and mountainous valleys in the region. The most famous of these are the Gadabay Mountains, Murovdag and the Tartar River Valley (Climate atlas of the Republic of Azerbaijan, 1992).

2.2 Plant Material

Three medicinal plant species widely used in folk medicine were selected as study objects: *Urtica dioica* L., *Stellaria media* L., and *Cephalaria gigantea* (Ledeb.) Bobrov. Plant samples were collected from two natural sites within the Gadabay region during the vegetative period. Healthy and visibly affected plant organs were sampled to assess fungal diversity under different physiological conditions.

2.3 Fungal Isolation and Identification

Fungal isolation was carried out using standard mycological techniques. Plant fragments were aseptically placed on YGC (Yeast Extract Glucose Chloramphenicol) and DG18 (Dichloran Glycerol) agar media and incubated at 25–27 °C. Colony development was monitored daily, and pure cultures were obtained through subculturing.

Identification was based on macroscopic characteristics (colony morphology, pigmentation, growth rate) and microscopic features (conidia, hyphae, sporulation structures) using light microscopy. Taxonomic identification was performed according to international mycological keys and reference databases.

During the cultivation method of fungi, a number of equipment and supplies, nutrient media are used in the laboratory. When choosing a nutrient medium, it is necessary to know in which nutrient medium the fungi develop. When preparing the nutrient medium, an accurate weighing scale, a flask or a Pyrex bottle, a magnetic stirring oven, distilled water and powdered nutrient media were used. Ready-made YGC (Yeast Extract) and DG18 (Dichloran-Glycerol) agars were used as nutrient media. After weighing 22.5 g of YGC and DG18 agars with 1L of distilled water accurately on a scale and pouring them into the flask, distilled

water is measured using a beaker and added to the flask. After being placed on a magnetic stirrer for good mixing, it is autoclaved. The prepared sterile nutrient media are poured into petri dishes in a completely sterile manner in a UV box. The sample is separated using tweezers and sterilely taken and placed in a petri dish. A control (witness sample) is placed in the nutrient medium to ensure that no fungi fall from the outside and there is no external contamination. Then it is placed in an incubator at 25–27°C. The full development of the fungi is possible within 5 days. It is necessary to make sure that the incubators are working properly within 5 days. For this, the temperatures of the incubators should be measured and recorded every day. The development of the fungi is monitored from the 3rd day. The colonies that develop should be removed to a clean environment and the type determined.

After the colony is placed on a slide, it is examined under a microscope using the Gram stain method and the type is determined. For this, the database of the International Association of Mycology, as well as the CBC fund, is used.

It is possible to determine whether the growth and development of mushroom species differ by the Growth Factor. The Growth Factor is based on a special formula: D is the diameter of the colony (measured in millimeters), H is the vertical size (height) of the colony, S is the density indicator of the colony (evaluated on a scale from 1 to 5), and T is the cultivation period (in days).

The systematic location of plant species and their distribution frequency were calculated using the following formula:

$$N = n / s$$

Where, N is the frequency of use (units/ha), n is the number of specific medicinal plant species observed in a given area, and s is the total area of the studied area (in hectares).

The frequency of occurrence of fungal species in samples and the prevalence of diseases caused by pathogenic microorganisms were determined using the following formula:

$$P = (n / N) \times 100$$

Where, P is the frequency of detection of fungal species in samples or the prevalence of diseases caused by pathogens (in %), n is the number of specific fungal samples (plant individuals carrying the disease) observed, and N is the total number of plant samples studied in general."

The experiments were repeated at least three times, and the results obtained were analyzed using statistical methods. Only indicators that correspond to the formula

$$m/M = P \leq 0.05$$

Where M is the average value, m is the standard deviation, P is the probability level estimated by the Student test were considered reliable and included in the dissertation [14].

2.4 Statistical Analysis

The frequency of fungal occurrence and plant colonization was calculated using standard ecological indices. All experiments were conducted in triplicate,

and statistical reliability was assessed using Student's *t*-test, with significance accepted at $P \leq 0.05$.

3. Results

A total of more than 15 species of microscopic fungi were isolated from the investigated medicinal plants. These species were classified into two major phyla: **Ascomycota** and **Zygomycota**. Ascomycetous fungi represented the dominant group, accounting for approximately two-thirds of the identified taxa.

Within Ascomycota, members of the genera *Aspergillus* and *Penicillium* were particularly abundant. Zygomycetous fungi were represented mainly by *Mucor*, *Rhizopus*, and *Syncephalastrum*. The detected fungal taxa displayed distinct colony morphologies and growth patterns, reflecting their ecological adaptability.

4. Discussion

The dominance of Ascomycota in the mycobiota of medicinal plants is consistent with findings from other geographical regions. Many species of *Aspergillus* and *Fusarium* are known for their phytopathogenic potential and ability to produce mycotoxins, which may compromise the safety of medicinal plant materials.

In recent years, plant-microbiome studies have become of great importance in terms of studying biodiversity and establishing sustainable agricultural systems. Microorganisms living on and inside plants, especially microscopic fungi are directly related to the development, stress resistance and metabolic activity of plants. They play various ecotrophic roles and interact with plants as symbionts, pathogens or saprotrophs.

Saprotrophic fungi are fungi that live on and feed on dead and decaying matter. They play a very important role by breaking down dead organisms and ensuring the recycling of substances. Pathogenic fungi, unlike saprotrophic representatives, live on living organisms and cause crop losses in agriculture, as well as a number of serious diseases. Symbiont fungal species live together with other organisms and provide mutual benefit (mutualism). That is, the fungal organism transfers water and minerals from the soil to the plant. The plant transfers sugars produced as a result of photosynthesis to the fungus. Symbiont forms include mycorrhiza and symbionts.

During their long historical evolution, fungi have acquired adaptations and adaptations to environmental conditions, which have resulted in the formation of ecological groups. Therefore, they have formed various biologically active substances such as antibiotics, mycotoxins, vaccines, etc. They also play a significant role in the mineralization and biochemical transformation of dead organisms and living remains in the soil, in increasing soil fertility, and in soil formation.

The soil ecological group is a favorable habitat and food source. It is considered the main ecological environment for fungi. Gadabay region, one of the high mountainous zones of Azerbaijan, has a rich floristic diversity and various medicinal plants are distributed here in natural conditions. The mycobiota

formed on these plants is important both in terms of maintaining ecological balance and in terms of medical and agrobiotechnological potential.

The ecotrophic approach allows for the assessment of the ecological position and food acquisition strategies of fungi together. Within the framework of this study, fungi participating in the mycobiota of medicinal plants found in the flora of the Gadabay region were divided into parasitic, saprotrophic, symbiont, and endophytic groups.

The aim of this study is to determine and evaluate the composition of mycobiota formed on selected medicinal plants growing in the Gadabay region and the ecotrophic functions (parasitism, saprotrophism, symbiosis, and endophytism) of these fungi.

Fungi identified in this study exhibited diverse ecotrophic strategies. Saprotrophic species played a role in organic matter decomposition, while parasitic fungi were associated mainly with weakened plant tissues. Mutualistic or endophytic interactions were less common but may contribute to plant stress tolerance and metabolic activity (Namazov, 2019).

Environmental factors, plant age, and tissue integrity were found to influence fungal colonization patterns. These observations highlight the importance of ecological conditions in shaping plant-associated mycobiota.

5. Conclusions

The present study reveals that medicinal plants growing in the Gadabay region harbor a diverse and ecologically complex mycobiota dominated by Ascomycota. Pathogenic and saprotrophic fungi prevail, particularly in stressed or damaged plant tissues. These findings underline the importance of continuous mycological monitoring and strict hygienic measures during the harvesting, drying, and storage of medicinal plants to ensure their safety and therapeutic quality.

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