**REPUBLIC OF AZERBAIJAN**

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# ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

# BIOTECHNOLOGY OF OBTAINING FUNGICIDAL SUBSTANCES FROM SOME ESSENTIAL OIL PLANTS

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# INTRODUCTION

**Relevance and Extent of Research on the Topic.** One of the notable features of the modern era is the increasing anthropogenic impact on the environment, which has led to a rise in diseases, food poisoning, child mortality, and other undesirable outcomes, and has even contributed to the emergence of pandemic-causing illnesses. All this necessitates the implementation of measures aimed at preventing such problems and investigating their causes, making it an essential task for both theorists and practitioners.

Against the background of the aforementioned problems, plants play a crucial role in strengthening the human immune system and in the development of preparations with stronger therapeutic effects. It is therefore not accidental that the production volume and assortment of plant-derived preparations, particularly those effective against various diseases, are steadily expanding. Thus, nearly 80% of the world's population[1] currently uses plant-based preparations with varying degrees of pharmacological activity.

In this regard, essential oil plants constitute a group of particular interest. Currently, approximately 3,000 species in the world flora possess this characteristic, but only about 200 are utilized for industrial production purposes[2]. Essential oil plants are characterized by diversity in both life forms (trees, shrubs, and herbs) and life span (annual and perennial), and they are predominantly distributed in tropical and subtropical regions[3]. The flora of the Republic of Azerbaijan, despite its relatively small area, is also rich in essential oil plants; the total number of species in this group is approximately 800, some of which are officially registered in the

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[1] Balkrishna A., Sharma N., Srivastava D. et al. Exploring the Safety, Efficacy, and Bioactivity of Herbal Medicines: Bridging Traditional Wisdom and Modern Science in Healthcare//*Future Integr Med*., 2024, v.3(1), p.35-49. doi: 10.14218/FIM.2023.00086.

[2] Кузьменко И.Н., Колясникова Н.Л. Лекарственные и ядовитые растения: учебное пособие. – Пермь: ИПЦ «ПрокростЪ» – 2019, -104 с

[3] Farag, N.F., El-Ahmady Sh.H., Abdelrahman E.H. et al. Characterization of essential oils from Myrtaceae species using ATR-IR vibrational spectroscopy coupled to chemometrics//Industrial Crops and Products, 2018, v.124, p.870-877

world pharmacopoeia, as confirmed by the literature[4].

Medicinal preparations used in the treatment of various diseases should possess properties such as *antimicrobial and anti-cold activity, regulation of metabolic processes, improvement of blood circulation, enhancement of regeneration, and should not induce allergic reactions*[5]. The main characteristic of essential oil plants, both worldwide and in Azerbaijan, is defined by the presence of various phytocompounds with biological, including pharmacological activity, primarily represented by essential oils[6]. These components have long attracted attention for performing the aforementioned functions and as substitutes for certain synthetic medicinal compounds obtained through chemical synthesis.

Although the practical use of essential oil plants dates back to ancient times, research on their antimicrobial activity in various countries, including the Republic of Azerbaijan, primarily covers the recent past. Summarizing the results of these studies, it can be stated that medicinal plants, including essential oil plants, contain phytocompounds with diverse pharmacological effects. Their use is distinguished from other preparations by the long-lasting effect, minimal or rare side effects, and the lack of observed allergic reactions[7,8,9,10]. Nevertheless, the number of plants studied in this

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4 Mehdiyeva N.P. Azərbaycanın dərman florasının biomüxtəlifliyi. Bakı: "Letterpress", 2011, 186 s.

5 Rizvi SAA, Einstein GP, Tulp OL, Sainvil F, Branly R. Introduction to Traditional Medicine and Their Role in Prevention and Treatment of Emerging and Re-Emerging Diseases. Biomolecules. 2022 Oct 9;12(10):1442. doi: 10.3390/biom12101442.

6 De Sousa DP, Damasceno ROS, Amorati R, Elshabrawy HA, de Castro RD, Bezerra DP, Nunes VRV, Gomes RC, Lima TC. Essential Oils: Chemistry and Pharmacological Activities. Biomolecules. 2023 Jul 18;13(7):1144. doi: 10.3390/biom13071144.

7 Namazov N.R. Azərbaycan florasına aid olan efiryağlı bitkilərin mikobiotası, onların tərkib elementlərinin bakterisid və fungisid xüsusiyyətləri:/b.e.d. dissertasiyasının avtoreferatı/-Bakı, 2021, -64s.

8 Bunse M., Daniels R., Gründemann C. et al.Essential Oils as Multicomponent Mixtures and Their Potential for Human Health and Well-Being//Front Pharmacol., 2022, 24;13:956541. doi: 10.3389/fphar.2022.956541.

field remains limited. For some of the investigated plants, only the antimicrobial activity of their constituent compounds has been established, but the extent and nature of this activity have not been comprehensively clarified. Moreover, the effects of interactions between these plants and other organisms on their antimicrobial properties have not been sufficiently studied.

Furthermore, studies on the antimicrobial activity of essential oil plants primarily use classical cultures as test organisms. This conventional approach currently allows only limited application of the characteristics specific to essential oil plants. At the same time, there is growing interest in bioagents with broad-spectrum activity, particularly against toxigenic, allergenic, and opportunistic species of fungi, which are considered manifestations of their ecotrophic specialization. Therefore, taking these aspects into account in studies within this field will further enhance the relevance of research aimed at addressing the aforementioned issues.

**Aim and objectives.** The presented research aims to isolate fungicidal compounds from certain essential oil plants of the Azerbaijani flora and to clarify their potential applications.

For the realization of the stated aim, the implementation of the following objectives during the course of the research was considered appropriate:

1. Evaluation of the resource potential of certain essential oil plants of the Azerbaijani flora and selection of a method for extracting phytocomponents from them;
2. Isolation of test organisms from nature for the evaluation of fungicidal activity and selection of conditions for their cultivation;
3. Evaluation of the fungicidal activity of phytocomponents obtained from plants and selection of the active source;
4. Determination of the application areas of phytocomponents

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[9] de Sousa D.P., Damasceno R.O.S., Amorati R. et al. Essential Oils: Chemistry and Pharmacological Activities//Biomolecules, 2023, 18;13(7):1144. doi: 10.3390/biom13071144.

[10] Pandey V.K., Tripathi A., Srivastava S. et al. Exploiting the bioactive properties of essential oils and their potential applications in food industry. Food Sci. Biotechnol. 2023;32:885–902. doi: 10.1007/s10068-023-01287-0.

with high fungicidal activity obtained from plants.

**Methods of the research.** The methodology of the dissertation is based on the study of certain essential oil plants of the Azerbaijani flora and the generalization of literature data related to their investigation. Taking into account the set aim and the objectives considered essential for its realization, a plan for the implementation of the dissertation was developed, and the research object and methods were selected. The object of the dissertation was essential oil plants of the Azerbaijani flora, including *Apium graveolens, Artemisia absinthium, A. vulgaris, Cuminum cyminum, Foeniculum vulgare, Laurus nobilis, Mentha piperita, Olea europaea, Rosmarinus officinalis, and Salvia officinalis*, and their constituent compounds. These compounds consisted of the air-dried biomass of the plants, the fractions extracted with water and ethanol, and essential oils obtained by hydrodistillation. In the study of the fungicidal activity of the obtained components, fungi were used as test organisms. These fungi were isolated into pure cultures during the research and were provided by the Institute of Microbiology of the Ministry of Science and Education. They were distinguished according to manifestations of ecotrophic specialization. The assessment of fungicidal activity was carried out based on biomass extraction and the disk diffusion method. All experiments were performed in at least 4 replicates and the results were statistically analyzed.

**The main provisions of the defense**

- Although all constituent compounds of essential oils from the Azerbaijani flora generally inhibit the growth of test cultures, the magnitude of this effect may vary depending on the source of the component;
- The characterization of the effect of essential oil plants as fungicidal or fungistatic is determined by the phytochemical composition of the essential oil plant and the biological characteristics of the test organisms;
- For obtaining components with antifungal activity, it is more effective to collect plant materials during the flowering phase of their vegetation period;

- It is more appropriate to approach the effect and application of phytocomponents obtained from the studied plants based on the principle of "the effectiveness of a specific plant in a specific field of use".

**The scientific novelty of the research.** As a result of the conducted research, various bioagents obtained from 10 plant species belonging to the flora of Azerbaijan (*Apium graveolens, Artemisia absinthium, A. vulgaris, Cuminum cuminum, Foeniculum vulgare, Laurus nobilis, Mentha piperita, Olea europaea, Rosmarinus officinalis, and Salvia officinalis*) were evaluated for their effect on the growth of 12 fungal species belonging to toxigenic organisms. The obtained results revealed that aqueous extracts derived from plants exert a negative effect in all cases, and the level of this effect may vary depending on the plant species used, the characteristics of the test organisms, and the method of obtaining the bioagents. Thus, the growth of all fungi decreased by 40–61% under the effect of aqueous extracts (AE), while extracts prepared with 3% ethanol caused a reduction of 44–68%. When essential oil (EO) was added to the medium, fungal growth could be completely inhibited, or in some cases only slightly reduced (0,01%). In these reduction processes, the maximum effect was observed for the plant *Salvia officinalis* and for the fungus *Cladosporium herbarum*. Furthermore, it was found that adding 0.01% of EO obtained from sage to the medium affected all fungi, whereas the addition of EO from mint, common wormwood, and mugwort completely inhibited the growth of some fungal species.

The dried and crushed biomass of the aerial parts of the studied plants was used, and it was found that the antifungal activity of the dry biomass was the weakest compared to all other variants. Thus, growth occurred to some degree in all studied variants. On the other hand, weight loss was also observed in all variants, albeit with varying magnitudes. The relatively small difference between the maximum and minimum weight loss provides a solid reason to indicate that no significant effect was observed in this case.

For obtaining materials with antifungal activity, it is advisable to use ethanol extract and essential oils obtained from sage, mint, mugwort, and common wormwood collected during the flowering phase; in this case, adding 0.2% EtOH extract and 0.02% EO to the medium is recommended. Thus, the highest level of antifungal activity is observed at these concentrations, corresponding to the amount of plant-derived material used.

**Theoretical and practical significance of the research.** The results obtained in the conducted research provide factual material that contributes to expanding knowledge about the composition of certain essential oil plants of the Azerbaijani flora, their fungicidal activity, and potential areas of application.

The results obtained in the research can be effectively used in the development of new biopreparations with fungicidal activity and in determining their areas of application.

The information on the fungicidal activity of individual components of the studied essential oil plants is also significant in terms of their use in accordance with the principles of sustainable development of plant resources.

**Publication, approbation and application of the research.** 15 scientific works related to the topic of the dissertation have been published, 6 of which are scientific articles, and the rest are theses and conference materials. The materials of the dissertation were presented at the scientific-practical conference on "Current Issues in Modern Biology" (Baku, 2019), at the Republican scientific-practical conference on "New Trends and Innovations: Prospects for the Development of Microbiology in Azerbaijan" (Baku, 2022), at the Republican scientific conference on "Ways to Restore Biodiversity in Liberated Territories (Sumgayit, 2022), at the LXXXV International Scientific-Practical Conference on "Experimental and Theoretical Research in Modern Science" (Novosibirsk, Russia, 2023), at the international conference on "Science, Society, Innovation: Current Issues in Modern Research" (Penza, Russia, 2023), at the international conference on "Prospects for the Development of Science and Education" (Tambov, Russia, 2023), at the international conference on "Heydar Aliyev and the Nature of Azerbaijan" (Baku, 2023), at the international conference on "Current Issues in Microbiology, Biotechnology, and Biodiversity" (Astana, Kazakhstan, 2024), and at the Memorial Conference on Mycology

(Moscow, Russia, 2025).

**The organization where the dissertation work was performed.** The dissertation work was carried out in the Microbiological biotechnology laboratory of the Institute of Microbiology of the Ministry of Science and Education.

**The structure and volume of the dissertation.** The dissertation consists of the table of contents, introduction, literature review (Chapter I), research materials and methods (Chapter II), experimental section (Chapters III–VI), final analysis of the research, conclusions, and lists of references and abbreviations. Altogether, it comprises a total of 215,600 characters.

## CHAPTER I
## ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL PLANTS AND THE CURRENT STATUS OF THEIR USE

In section 1.1 of the dissertation, the general characteristics of essential oil plants are analyzed; in section 1.2, their chemical composition and antimicrobial activity are examined; and in section 1.3, the current state of application of essential oil plant components in biotechnology is reviewed, clarifying the level of study of the problem to be addressed.

## CHAPTER II
## METHODS AND MATERIALS USED IN THE RESEARCH

### 2.1. General characteristics of the collection sites of biological agents used in the research

The samples for obtaining phytocomponents from plants were primarily collected from the Absheron Peninsula (Fig. 1). Several studies have shown that the antifungal activity of bioagents obtained from the same plant species growing under different ecological conditions is relatively higher in those grown in the Absheron environment. On the other hand, Absheron is one of the driest regions of the Caucasus, with a total area of 5.42 thousand km², accounting for 6.3% of the country's territory[11]. The peninsula, more precisely the Absheron economic-geographical region, extends 124 km from north to south and 93 km from west to east.

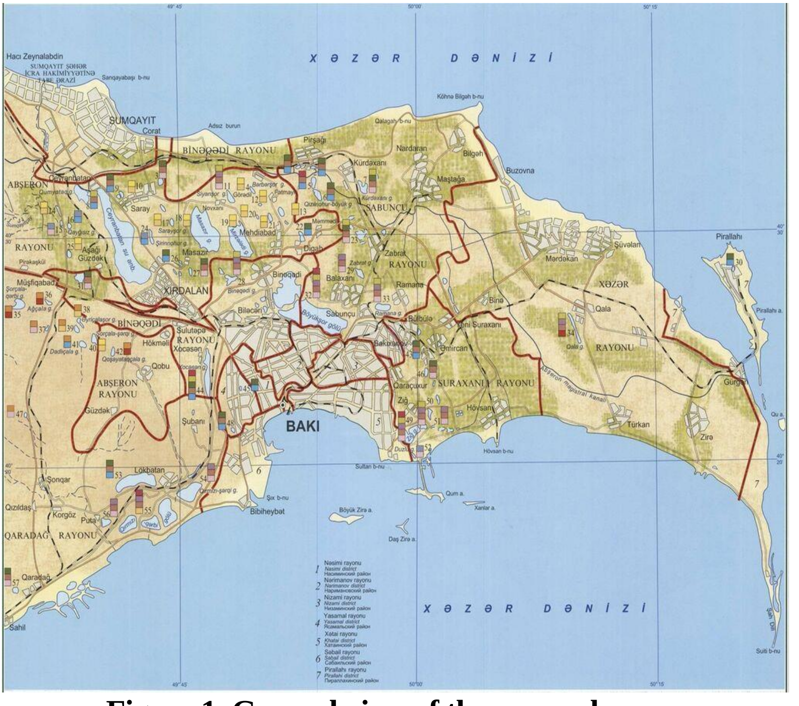


**Figure 1. General view of the research areas**

The flora and fauna of Absheron are relatively poor compared to other regions of the country. For example, only 22% of the plant species endemic to the country are found in Absheron[12]. Nevertheless, Absheron is one of the regions where a number of medicinal plants, including essential oil-bearing species, are widely distributed; plants such as alhagi, licorices, mint, common sage, cumin and olive serve as examples.

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[11] [11] https://gsaz.az/articles/view/110/

[12] Hacıyev V.C., Musayev S.H., Əkbərova Z.İ., İbadullayeva S.C. Azərbaycan florasının ali bitkilərinin biomüxtəlifliyinə dair.//AMEA-nın Botanika İnstitutunun elmi əsərləri, 2004, c.2, c.88-93

## 2.2. General characteristics of the methods used for analysis

Samples were mainly taken from the aerial parts of the plants, dried under normal conditions, and analyzed in accordance with the purpose of the study. Various phytocomponents were obtained from the plants, using different methods for their extraction.

1. Aqueous extract (AE). To obtain this, the dried biomass of a specific plant is mixed with distilled water at a ratio of 9 ml per 1 g and kept at 40°C for 2 hours. After this period, it is filtered, and the resulting solution is used.
2. Ethanol extract (EtOH extract). For this, 1 g of dried biomass is mixed with 9 ml of 1% ethanol and kept in a closed container at 40°C for 2 hours, with stirring every 30 minutes. Then it is filtered, and the obtained solution is used.
3. Essential oil (EO). This bioagent is obtained by hydrodistillation[13].
4. Dried biomass (DB), ground to a particle size of 2–5 mm

In the study, the mentioned bioagents were obtained from samples of 10 plant species, and 12 fungal species were used to determine their fungicidal activity. The isolation of some fungi and the determination of the mentioned characteristics were carried out during the course of the research(CR), while others were identified based on the results of studies conducted at the Laboratory of Microbiological Biotechnology of the Institute of Microbiology(MBİ MB).

Antifungal activity was evaluated using both the *disk diffusion method*[14], and *biomass yield*[15] (compared to control, in %). These

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[13] ГОСТ ISO 6571-2016 Пряности, приправы и травы. Определение содержания эфирных масел (метод гидродистилляции). – Москва: Стандартинформ, 2016, -9с.

[14] Определение чувствительности микроорганизмов к антибактериальным препаратам: Методические указания .— М.:ФЦ госсанэпиднадзора Минздрава России, 2004.— 91 с

[15] ГОСТР 56885- 2016. БИОМАССА. Определение общего количества твердых веществ стандартным методом. -Москва:Стандартинформ 2016, 5с.

methods and approaches are among the most widely used in current studies in this field.

For obtaining quantitative results, the experiments were carried out in at least four replicates, and the obtained data were *statistically* [16] processed.

# CHAPTER III
# ANTIFUNGAL ACTIVITY OF PHYTOCOMPONENTS OBTAINED FROM CERTAIN ESSENTIAL OIL PLANTS OF THE AZERBAIJANI FLORA

## 3.1. General characteristics of biological agents used in the research

Information on the plant samples collected during the research is summarized in Table 3.1. The selected plants are distributed not only in Absheron but also in other regions of Azerbaijan. Some are cultivated, some belong to the native flora, and others are introduced species.

The resource potential of the majority is considered sufficient for the country. Olive is the only exception to this characteristic; its resources are sufficient, but the majority are concentrated specifically in Absheron. Information about the fungi selected and used as test organisms is summarized in Table 3.2. As can be seen, among fungi, there are species corresponding to all three forms of ecotrophic specialization.

It should be noted that some fungi can simultaneously possess all or two of the mentioned characteristics.

Furthermore, as seen from the table, 50% of the fungi used as test organisms were isolated into pure cultures during the research and their properties determined, while the remaining 50% were provided by the Microbiological Biotechnology Laboratory, with their characteristics recorded.

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[16] Зверев А.А., Зефиров Т.Л. Статистические методы в биологии: учебно-методическое пособие / Казань, КФУ, 2013. - 42 с.

**Table 3.1.**

**General information on plant samples collected in the research**

| № | Plant species | Distribution in Azerbaijan | Fields of use |
|---|---|---|---|
| 1 | *Apium graveolens* | Throughout Azerbaijan | Food industry |
| 2 | *Artemisia absinthium* | Throughout Azerbaijan | - |
| 3 | *Artemisia vulgaris* | Throughout Azerbaijan | - |
| 4 | *Cuminum cuminum* | Throughout Azerbaijan | Food industry |
| 5 | *Foeniculum vulgare* | Throughout Azerbaijan | Food industry |
| 6 | *Laurus nobilis* | Throughout Azerbaijan | Food industry |
| 7 | *Mentha piperita* | Throughout Azerbaijan | Food industry |
| 8 | *Olea europaea* | Absheron | Food industry |
| 9 | *Rozmarinus officinalis* | Throughout Azerbaijan | Cosmetics |
| 10 | *Salvia officinialis* | Greater Caucasus | Medicine |

**Table 3.2**

**General information on fungi used as test organisms**

| № | Fungi species | The manifestation of ecotrophic specialization | Place of isolation | Provided by |
|---|---|---|---|---|
| 1 | Alternaria alternata | Allergen, Toxigen | soil | İMB MB |
| 2 | Aspergillus flavus | Toxigen | soil | CR |
| 3 | A.nidulans | Opportunist | plant | CR |
| 4 | A.niger | Universal | soil | İMB MB |
| 5 | Botrytys cinerea | Allergen, toxigen | plant | CR |
| 6 | Cladosporium herbarium | Allergen, toxigen | plant | İMB MB |
| 7 | Fusarium moniliforme | Toxigen | plant | İMB MB |
| 8 | F.oxysporium | Toxigen | plant | CR |
| 9 | Penicillium chrysogenum | Toxigen | soil | CR |
| 10 | P.cuclopium | Toxigen | soil | İMB MB |
| 11 | Trichoderma viride | Allergen | soil | CR |
| 12 | Verticillium album | Toxigen | plant | İMB MB |

## 3.2. Evaluation of the antifungal activity of agents obtained from the studied plants

Initially, experiments on the effect of aqueous and ethanol extracts obtained from plants on the growth of fungi used as test organisms showed that both agents generally inhibit the growth of the test organisms, and the level of this effect can vary depending on the plants used, the test organisms, and the source of the bioagent. As can be seen, the growth of all fungi varies between 40-61%, or more precisely, decreases, due to the effect of aqueous extracts (Table 3.3). In this reduction process, the maximum effect was observed for the plant *Salvia officinalis* and the fungus *Cladosporium herbarum*. This means that the aqueous extract of sage contains components with higher antifungal activity compared to others, and the mentioned fungus is more sensitive to such components than other fungi.

Disregarding some quantitative differences, a similar conclusion can be drawn regarding the components present in the ethanol extracts of the studied plants (Table 3.4).

Thus, in all cases, the quantitative indicator of antifungal activity of the ethanol extract is higher than that of the aqueous extract; in this case, the growth reduction ranges between 44–68%, which is 1.1–1.7 times higher compared to the aqueous extract. On the other hand, in this case, not only the sage but also the extract obtained from mint exhibited the highest antifungal activity, while fungi such as *Botrytis cinerea* and *Cladosporium herbarum* were the most sensitive. It would be more appropriate to attribute this to the stronger solvent properties of ethanol compared to water.

Unlike the aqueous and ethanol extracts, the antifungal activity of essential oil obtained from plants by hydrodistillation was higher, and in some cases it was characterized as fungicidal activity, that is, in some variants, the addition of EO to the medium caused a complete cessation of the growth ability of several fungi. (Table 3.5). So, the addition of 0.1 ml (i.e., 0.01%) of essential oil (EO) obtained from sage to the medium completely inhibited the growth of all fungi. The addition of EO derived from mint, common wormwood, and mugwort completely inhibited the growth of some fungi.

**Table 3.3**

**General information on the effect of aqueous extract obtained from plants on the growth of fungi used as test organisms (Biomass yield relative to control, %)**

| № | Fungi species | 1* | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Alternaria alternata* | 60 | 42 | 52 | 60 | 53 | 54 | 47 | 58 | 51 | 44 |
| 2 | *Aspergillus flavus* | 56 | 45 | 56 | 57 | 54 | 53 | 45 | 53 | 53 | 45 |
| 3 | *A.nidulans* | 53 | 41 | 53 | 59 | 50 | 56 | 42 | 57 | 56 | 41 |
| 4 | *A.niger* | 54 | 50 | 54 | 57 | 51 | 54 | 43 | 55 | 55 | 40 |
| 5 | *Botrytys cinerea* | 52 | 48 | 56 | 54 | 49 | 51 | 41 | 52 | 51 | 40 |
| 6 | *C.herbarium* | 50 | 43 | 51 | 53 | 48 | 50 | 40 | 50 | 49 | 39 |
| 7 | *Fusarium moniliforme* | 51 | 44 | 52 | 54 | 52 | 52 | 46 | 54 | 54 | 43 |
| 8 | *F.oxysporium* | 52 | 43 | 50 | 56 | 53 | 55 | 42 | 51 | 56 | 41 |
| 9 | *P.chrysogenum* | 57 | 49 | 55 | 58 | 55 | 58 | 49 | 58 | 59 | 45 |
| 10 | *P.cuclopium* | 55 | 46 | 54 | 53 | 54 | 56 | 46 | 53 | 57 | 43 |
| 11 | *Trichoderma viride* | 60 | 54 | 57 | 53 | 58 | 52 | 56 | 60 | 55 | 59 |
| 12 | *Verticillium album* | 53 | 50 | 55 | 56 | 55 | 51 | 49 | 51 | 50 | 48 |
| Control | | 100 | | | | | | | | | |

**Note***1-Apium graveolens, 2- Artemisia absinthium, 3 -Artemisia vulgaris, 4-Cuminum cuminum, 5-Foeniculum vulgare, 6 - Laurus nobilis, 7 - Mentha piperita, 8- Olea europaea, 9 - Rozmarinus officinalis, 10 -Salvia officinialis*



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**Table 3.4**

**General information on the effect of ethanol extracts obtained from plants on the growth of fungi used as test organisms (expressed as % relative to the control)**

| № | Fungi species | 1* | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Alternaria alternata* | 56 | 41 | 45 | 53 | 47 | 49 | 39 | 50 | 44 | 38 |
| 2 | *Aspergillus flavus* | 50 | 40 | 51 | 50 | 49 | 45 | 39 | 53 | 45 | 39 |
| 3 | *A.nidulans* | 47 | 41 | 50 | 51 | 44 | 49 | 37 | 43 | 48 | 34 |
| 4 | *A.niger* | 51 | 42 | 45 | 50 | 42 | 48 | 38 | 47 | 48 | 35 |
| 5 | *Botrytys cinerea* | 39 | 38 | 41 | 43 | 40 | 42 | 34 | 41 | 42 | 34 |
| 6 | *C.herbarium* | 41 | 35 | 40 | 43 | 40 | 41 | 32 | 41 | 40 | 32 |
| 7 | *Fusarium moniliforme* | 42 | 36 | 43 | 44 | 43 | 45 | 34 | 47 | 48 | 38 |
| 8 | *F.oxysporium* | 44 | 36 | 41 | 47 | 45 | 46 | 35 | 46 | 50 | 35 |
| 9 | *P.chrysogenum* | 50 | 41 | 45 | 48 | 46 | 49 | 39 | 49 | 50 | 37 |
| 10 | *P.cuclopium* | 47 | 40 | 47 | 45 | 46 | 47 | 39 | 45 | 48 | 35 |
| 11 | *Trichoderma viride* | 54 | 50 | 51 | 45 | 50 | 43 | 48 | 53 | 50 | 52 |
| 12 | *Verticillium album* | 45 | 44 | 45 | 50 | 49 | 43 | 41 | 45 | 44 | 41 |

**Note*** *1-Apium graveolens, 2- Artemisia absinthium, 3 -Artemisia vulgaris, 4-Cuminum cuminum, 5-Foeniculum vulgare, 6 - Laurus nobilis, 7 - Mentha piperita, 8- Olea europaea, 9 - Rozmarinus officinalis, 10 -Salvia officinialis*


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**Table 3.5**

**General information on the effect of EO obtained from plants on the growth of fungi used as test organisms (expressed as % relative to the control).**

| № | Fungi species | 1* | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Alternaria alternata* | 4.5 | 1.5 | 1.3 | 4.2 | 3.8 | 3.7 | 1.4 | 3.4 | 3.3 | 0 |
| 2 | *Aspergillus flavus* | 4.6 | 2.4 | 2.5 | 3.9 | 4.0 | 4.1 | 2.3 | 5.5 | 5.0 | 0 |
| 3 | *A.nidulans* | 3.7 | 3.0 | 3.3 | 4.2 | 3.6 | 3.9 | 2.3 | 3.3 | 3.7 | 0 |
| 4 | *A.niger* | 4.4 | 3.0 | 3.2 | 3.9 | 3.5 | 3.8 | 2.9 | 3.6 | 3.7 | 0 |
| 5 | *Botrytys cinerea* | 3.1 | 0 | 1.6 | 1.5 | 3.0 | 3.1 | 1.2 | 3.0 | 2.8 | 0 |
| 6 | *C.herbarium* | 2.7 | 0 | 0 | 4.7 | 3.0 | 3.4 | 0 | 3.4 | 2.8 | 0 |
| 7 | *F.moniliforme* | 3.2 | 2.0 | 1.8 | 4.1 | 3.3 | 4.2 | 3.7 | 3.5 | 3.5 | 0 |
| 8 | *F.oxysporium* | 3.4 | 2.3 | 2.7 | 3.1 | 4.0 | 3.6 | 1.7 | 3.2 | 3.2 | 0 |
| 9 | *P.chrysogenum* | 3.1 | 2.2 | 2.3 | 2.8 | 3.5 | 3.8 | 1.5 | 3.8 | 2.9 | 0 |
| 10 | *P.cuclopium* | 3.8 | 2.5 | 2.5 | 3.9 | 3.2 | 4.1 | 1.7 | 3.1 | 3.5 | 0 |
| 11 | *Trichoderma viride* | 4.5 | 4.6 | 3.1 | 3.3 | 5.5 | 4.1 | 3.4 | 5.3 | 5.5 | 0 |
| 12 | *Verticillium album* | 3.2 | 1.4 | 1.7 | 1.5 | 3.0 | 3.1 | 1.2 | 3.0 | 2.8 | 0 |

*Note*1-Apium graveolens. 2- Artemisia absinthium. 3 -Artemisia vulgaris. 4-Cuminum cuminum. 5-Foeniculum vulgare. 6 - Laurus nobilis. 7 - Mentha piperita. 8- Olea europaea. 9 - Rozmarinus officinalis. 10 -Salvia officinialis*



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Finally, in the studies, the dried and ground biomass of the aerial parts of the selected plants was used as a source of components with antifungal activity. In this case, antifungal activity was evaluated based on whether fungal growth occurred and on the loss of biomass weight. The results showed that the antifungal activity observed in the dry biomass was the weakest compared to all other variants. Thus, in all the examined variants, growth occurred to some extent. On the other hand, weight loss was also observed in all variants, with certain quantitative differences.

The relatively small difference between the maximum and minimum weight loss also indicates that no significant effect was observed in this case (Table 3.6). As a result, the minimum weight loss (9%) was observed in fungus A. alternata when dry biomass was used from sage, while the maximum weight loss (24%) occurred in fungus *T.viridii* when dry biomass was used from dill.

# CHAPTER IV
# INVESTIGATION OF THE FACTORS AFFECTING THE ANTIFUNGAL ACTIVITY OF PHYTOCOMPONENTS OF ESSENTIAL OIL PLANTS

Thus, although AE, etOH, EO, and DB obtained from plants selected as sources of phytocomponents with antifungal activity exhibited a certain effect against the fungi used as test organisms, the quantitative level of this effect varied depending on the indicated factors. The results obtained made it possible to conclude that the enhancement of antifungal activity occurred in the following directions:

**DB → AE → etOH → EO**

## 1.1. Factors affecting the activity of agents obtained from the studied plants

The growing interest in plants as a source of natural bioagents, including biologically and pharmacologically active substances, sometimes leads to their overexploitation. This highlights the need to develop approaches that ensure their efficient and, more precisely, sustainable use in accordance with the principles of sustainable development, making it a highly relevant issue.

Taking this into account, it has also been clarified in the study in what form the plant materials used align with the principles of sustainable development. Based on the conducted studies and analysis of literature data, the following general conclusion can be drawn:

For obtaining materials with antifungal activity, it is advisable to use ethanol extracts and essential oils from the studied plants, primarily sage, mint, mugwort, and common wormwood. Thus, although all extracts obtained using The most suitable stage for collecting plants indicated as sources of antifungal materials is during their flowering period, more precisely, in the middle of the vegetation stage.

Thus, at this stage, the extraction of EO from the aerial parts of the collected plants is higher (Table 4.3). As can be seen, the amount of EO is higher in the middle of the vegetation period, i.e., in summer, compared to the beginning and end of the vegetation period. different ethanol concentrations for 2 hours exhibited antifungal activity, the extract obtained with 5% ethanol showed higher antifungal activity against all test organisms, as clearly illustrated by the data from some plant examples (Table 4.1). As can be seen, reducing the ethanol concentration to 1% decreases the antifungal activity of the extract obtained from *Artemisia absinthium* by 1.19 times, whereas increasing it to 10% causes a decrease of 1.45 times. For other plants, the corresponding decrease ranges from 1.14–1.30 times and 1.37–1.64 times, respectively. It would be appropriate to clarify one point here. As can be seen, in the case of increasing ethanol concentration, the antifungal activity decreases more, which is related to the fact that ethanol itself also possesses antifungal effects. Regarding the optimal concentrations of ethanol extract and essential oil added to the medium, the results showed that 0.2% etOH

**Table 3.6**

**The weight loss of fungi used as test organisms during growth on plant-derived dried biomass (residual weight relative to control. %)**

| № | Fungi species | 1* | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Alternaria alternata* | 82 | 88 | 85 | 81 | 82 | 82 | 89 | 83 | 80 | 91 |
| 2 | *Aspergillus flavus* | 81 | 87 | 85 | 80 | 79 | 83 | 88 | 83 | 84 | 89 |
| 3 | *A.nidulans* | 78 | 81 | 82 | 76 | 80 | 79 | 83 | 76 | 78 | 84 |
| 4 | *A.niger* | 78 | 82 | 81 | 78 | 79 | 78 | 83 | 77 | 78 | 85 |
| 5 | *Botrytys cinerea* | 77 | 82 | 83 | 76 | 78 | 76 | 84 | 75 | 79 | 87 |
| 6 | *Cladosporium herbarium* | 79 | 85 | 84 | 78 | 80 | 79 | 86 | 78 | 80 | 88 |
| 7 | *Fusarium moniliforme* | 78 | 86 | 83 | 79 | 81 | 75 | 88 | 77 | 78 | 88 |
| 8 | *F.oxysporium* | 84 | 88 | 86 | 77 | 75 | 76 | 86 | 76 | 80 | 85 |
| 9 | *Penicillium chrysogenum* | 76 | 82 | 84 | 77 | 79 | 78 | 85 | 74 | 80 | 87 |
| 10 | *P.cuclopium* | 78 | 80 | 83 | 75 | 76 | 77 | 85 | 75 | 78 | 85 |
| 11 | *Trichoderma viride* | 76 | 79 | 80 | 78 | 76 | 77 | 81 | 77 | 77 | 82 |
| 12 | *Verticillium album* | 80 | 84 | 85 | 81 | 79 | 81 | 87 | 85 | 84 | 90 |

**Note***1-*Apium graveolens*. 2- *Artemisia absinthium*. 3 -*Artemisia vulgaris*. 4-*Cuminum cuminum*. 5-*Foeniculum vulgare*. 6 - *Laurus nobilis*. 7 - *Mentha piperita*. 8- *Olea europaea*. 9 - *Rozmarinus officinalis*. 10 -*Salvia officinialis*


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extract and 0.02% EO lead to the highest level of antifungal activity. At these concentrations, the ratio between the amount of plant used and the activity is considered optimal; that is, the activity per 1 g of plant was higher than that observed with both AE and DB.

This can also be clearly seen from the results obtained on the antifungal activity of essential oils extracted from plants such as *Artemisia absinthium* and *Salvia officinalis*. (Table 4.2). It is apparent that, increasing the amount of EO leads to an increase in antifungal activity, but the activity calculated per 1 g of plant is higher when it is 0.02% in the medium. For this reason, the mentioned concentration is considered the optimal value. A similar point can be made regarding ethanol extract. Making such a choice also reflects a more efficient use of the plants.

**Table 4.1.**

**Effect of the ethanol concentration used on the antifungal activity of plant extracts (in % relative to the optimum)**

| № | Fungi species | *A.absinthium* | | | *A.vulgaris* | | | *Mentha piperita* | | | *Salvia officinialis* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ethanol concentration used, % | | | | | | | | | | | |
| | | 1 | 5 | 10 | 1 | 5 | 10 | 1 | 5 | 10 | 1 | 5 | 10 |
| 1 | *A. alternata* | 90 | 100 | 75 | 87 | 100 | 71 | 85 | 100 | 73 | 81 | 100 | 71 |
| 2 | *A. flavus* | 88 | 100 | 74 | 87 | 100 | 70 | 84 | 100 | 73 | 85 | 100 | 69 |
| 3 | *A.nidulans* | 86 | 100 | 73 | 84 | 100 | 66 | 80 | 100 | 66 | 79 | 100 | 64 |
| 4 | *A.niger* | 91 | 100 | 72 | 83 | 100 | 68 | 80 | 100 | 67 | 79 | 100 | 65 |
| 5 | *B.cinerea* | 89 | 100 | 70 | 85 | 100 | 66 | 81 | 100 | 65 | 80 | 100 | 67 |
| 6 | *C.herbarium* | 84 | 100 | 70 | 80 | 100 | 67 | 78 | 100 | 63 | 82 | 100 | 66 |
| 7 | *F.moniliforme* | 86 | 100 | 73 | 87 | 100 | 65 | 82 | 100 | 67 | 81 | 100 | 68 |
| 8 | *F.oxysporium* | 87 | 100 | 72 | 86 | 100 | 63 | 81 | 100 | 70 | 77 | 100 | 62 |
| 9 | *P.chrysogenum* | 90 | 100 | 71 | 88 | 100 | 70 | 84 | 100 | 68 | 83 | 100 | 63 |
| 10 | *P.cuclopium* | 87 | 100 | 70 | 82 | 100 | 68 | 81 | 100 | 71 | 85 | 100 | 65 |
| 11 | *T.viride* | 85 | 100 | 72 | 84 | 100 | 71 | 80 | 100 | 68 | 82 | 100 | 61 |
| 12 | *V.album* | 87 | 100 | 69 | 83 | 100 | 70 | 83 | 100 | 69 | 84 | 100 | 62 |

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## FINAL ANALYSIS OF THE DATA OBTAINED IN THE RESEARCH

As is well known, one of the main features of the modern era is that increasing human interference with the environment has inevitably led to various problems. This is primarily manifested in the emergence of new (resistant) forms of existing pathogens and the occurrence of new diseases [17,18]

As a result, two tendencies are clearly observed in humans: an increase in the number of chronic infectious diseases caused by opportunistic microorganisms, and a weakening of human immunological resistance. Therefore, the above points underscore the necessity of searching for means that prevent these problems and strengthen the human immune system. Moreover, it is considered appropriate to address this issue using natural sources, primarily plants.

Thus, plants contain a wide spectrum of biologically active substances with varying effects. Among them, a considerable number possess pharmacological activity, and numerous studies have confirmed the effectiveness of using plants for
this purpose. Year by year, the number of plants involved in research in this direction is also increasing. Nevertheless, the number of plants selected for the production of medicinal products used for this purpose or possessing pharmacological activity is currently very small compared to the number of plants known to science. The Republic of Azerbaijan has rich nature, and the plants that are significant for the mentioned purposes, generally characterized as medicinal plants, exhibit a wide diversity. Currently, more than 1/3 of the plants included in the Azerbaijani flora correspond to this characteristic[19].

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**Table 4.2.**

**Effect of the concentration of the used essential oil in the medium on antifungal activity (based on biomass yield. g/L).**

| № | Fungi species | *Artemisia absinthium* | | | | *Salvia officinialis* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Concentration of the used essential oil, % | | | | | | | |
| | | 0.01 | 0.02 | 0.04 | 0.1 | 0.01 | 0.02 | 0.04 | 0.1 |
| 1 | *A. alternata* | 4.3 | 0.3 | 0.2 | 0 | 2.3 | 0 | 0 | 0 |
| 2 | *A. flavus* | 5.2 | 0.5 | 0.2 | 0 | 3.4 | 0 | 0 | 0 |
| 3 | *A.nidulans* | 6.0 | 0.1 | 0 | 0 | 4.4 | 0 | 0 | 0 |
| 4 | *A.niger* | 6.0 | 0.0 | 0 | 0 | 4.1 | 0 | 0 | 0 |
| 5 | *B.cinerea* | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | *C.herbarium* | 0.4 | 0 | 0 | 0 | 0.3 | 0 | 0 | 0 |
| 7 | *F.moniliforme* | 5.0 | 0.1 | 0 | 0 | 1.2 | 0 | 0 | 0 |
| 8 | *F.oxysporium* | 5.1 | 0.1 | 0 | 0 | 0.8 | 0 | 0 | 0 |
| 9 | *P.chrysogenum* | 5.4 | 0.2 | 0 | 0 | 0.9 | 0 | 0 | 0 |
| 10 | *P.cuclopium* | 5.6 | 0.2 | 0 | 0 | 0.5 | 0 | 0 | 0 |
| 11 | *T.viride* | 7.1 | 0.2 | 0 | 0 | 0.7 | 0 | 0 | 0 |
| 12 | *V.album* | 4.7 | 0.1 | 0 | 0 | 0.4 | 0 | 0 | 0 |

**Table 4.3**

**Variation of essential oil yield (%) in the aerial parts of plants depending on the vegetation period**

| *Artemisia absinthium* | | | *Artemisia vulgaris* | | | *Mentha piperita* | | | *Salvia officinialis* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration of the used ethanol, % | | | | | | | | | | | |
| spring | summer | autumn | spring | summer | autumn | spring | summer | autumn | spring | summer | autumn |
| 1.41 | 1.72 | 1.54 | 0.33 | 0.56 | 0.41 | 2.47 | 3.28 | 2.87 | 0.42 | 0.58 | 0.51 |

Nevertheless, except for some plants used in traditional medicine, no medicinal agents are currently produced from the plants growing in the country. Indeed, although some of these plants have been studied from botanical, pharmacological, mycological, and other perspectives, their comprehensive investigation from a biotechnological standpoint has not received adequate attention.

Taking into account all of the above, the present study aimed to evaluate the antifungal activity of the components of several medicinally important and sufficiently abundant plants of the Azerbaijani flora, as well as to assess their potential use from a biotechnological perspective.

The antifungal activity of four types of components (aqueous extract, ethanol extract, essential oil, and dry biomass) obtained from 10 essential oil–bearing plant species (*Apium graveolens, Artemisia absinthium, Artemisia vulgaris, Cuminum cyminum, Foeniculum vulgare, Laurus nobilis, Mentha piperita, Olea europaea, Rosmarinus officinalis, and Salvia officinalis*), which are mainly distributed in the Absheron Peninsula of the Republic of Azerbaijan and are sufficiently abundant throughout the country, was evaluated against 12 fungal species (*Alternaria alternata, Aspergillus flavus, A. nidulans, A. niger, Botrytis cinerea, Cladosporium herbarium, Fusarium moniliforme, F. oxysporum*, *Penicillium chrysogenum*, *P. cuclopium*, *Trichoderma viride, and Verticillium album*). Thus, the selected test organisms are characterized by a wide diversity of ecotrophic specialization manifestations, and among them, toxigenic, allergenic, opportunistic (conditionally pathogenic), and phytopathogenic fungi are encountered.

In the conducted studies, the antifungal activity of phytocomponents obtained from the investigated plants was evaluated, active producers were selected, and the factors influencing the activity of these phytocomponents were clarified. In addition, the optimal time and plant organ for obtaining the target component from the selected plants were determined. Baseline data of scientific and practical relevance were collected to enable their future practical application.

As a summary of the results obtained in the conducted research, it was deemed appropriate to present the following five key conclusions.

## CONCLUSION

1. Aqueous extracts (AE) obtained from 10 plant species included in the flora of Azerbaijan (*Apium graveolens, Artemisia absinthium*, *A. Vulgaris, Cuminum cuminum, Foeniculum vulgare*, *Laurus nobilis*, *Mentha piperita*, *Olea europaea*, *Rozmarinus officinalis and Salvia officinalis*) had a generally inhibitory (fungiostatic) effect on the growth of all 12 fungal species used as test organisms, and the level of this effect may vary depending on the plants used, the test organisms, and the source of bioagent. Thus, under the effect of AE obtained from the plants, the growth of all fungi decreased by 40–61%. The maximum reduction was observed for Salvia officinalis among the plants and for Cladosporium herbarium among the fungi [1-4, 6-8, 11, 13-15].
2. Extracts from the studied plants prepared with 3% ethanol also exhibited an inhibitory effect on the growth of the test fungi, causing a 44–68% reduction. On the other hand, in this case, not only sage but also the extract obtained from mint exhibited the highest activity. Fungi such as Botrytis cinerea and Cladosporium herbarium were the most sensitive [3-5, 6, 12].

3. The antifungal activity of the essential oil (EO) obtained from the plants was higher, and in some cases, the addition of EO to the medium completely inhibited the growth of certain fungi. Thus, the addition of 0.01% EO obtained from sage to the medium inhibited the growth of all fungi, while the addition of EO from mint, mugwort, and common wormwood completely inhibited the growth of certain fungi[6, 9].
4. Dried and ground biomass from the aerial parts of the studied plants was used, and it was determined that the antifungal activity in the dry biomass was the weakest compared to all other variants. Thus, growth occurred to varying degrees in all studied variants. On the other hand, weight loss was also observed in all variants, with certain quantitative differences. The relatively small difference between the maximum and minimum weight loss constitutes a strong basis for concluding the absence of a significant effect in this case [1, 2].
5. For obtaining materials with antifungal activity, it is advisable to use etOH extract and EO obtained from sage, mint, mugwort, and common wormwood collected during the flowering stage. In this case, 0.2% etOH extract and 0.02% EO should be added to the medium. Thus, at these concentrations, the antifungal activity corresponding to the amount of plant material used is characterized by the highest level [6, 9].

## PRACTICAL RECOMMENDATIONS

1. When obtaining a particular component from essential oil plants, it is advisable to collect them during the summer season and to use not the entire plant, but a specific organ.
2. Ethanol-extracted preparations and essential oils obtained from essential oil-bearing plants are more suitable for use. Their relatively high effect allows for the efficient use of resources.
3. The study of the fungicidal activities of plants such as sage, mint, mugwort, and common wormwood may serve as a

basis for the future development of preparations with similar activity.

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