

ENDOPHYTIC FUNGI ISOLATED FROM *ALTHAEA OFFICINALIS* L., THEIR SECONDARY METABOLITES AND BIOLOGICAL ACTIVITY

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Abstract

This article discusses endophytic fungi isolated from the medicinal plant *Althaea officinalis* L. (marshmallow), their ability to produce secondary metabolites, and the possible biological activity of these metabolites. *Althaea officinalis* L. is a pharmacologically valuable plant containing mucilaginous polysaccharides, flavonoids, phenolic acids, coumarins, and other bioactive compounds [1-5]. Endophytic fungi associated with medicinal plants have attracted scientific interest because they are capable of synthesizing secondary metabolites with antimicrobial, antifungal, antioxidant, cytotoxic, and anti-inflammatory activities [6-9]. The article presents a structured methodological approach for isolating endophytic fungi from roots, stems, leaves, and flowers of *Althaea officinalis* L.; obtaining pure cultures; characterizing isolates morphologically and microscopically; extracting secondary metabolites; and evaluating biological activity [9-12]. The topic is relevant because the fungal endophytes associated with *Althaea officinalis* L. remain insufficiently investigated, although they may represent promising natural sources of bioactive compounds for pharmaceutical and biotechnological applications [6-8].

Keywords: *Althaea officinalis* L.; marshmallow; endophytic fungi; secondary metabolites; antimicrobial activity; antifungal activity; antioxidant activity; biotechnology.

1. Introduction

Medicinal plants are important natural sources of biologically active compounds used in traditional medicine, phytotherapy, pharmacology, and biotechnology [1, 6]. They contain various classes of phytochemicals, including polysaccharides, flavonoids, phenolic acids, alkaloids, terpenoids, coumarins, and other secondary metabolites [1-5]. These

compounds are responsible for many therapeutic properties of medicinal plants and remain highly relevant in natural product research [1, 6].

Althaea officinalis L., commonly known as marshmallow, is a perennial medicinal plant belonging to the family Malvaceae [1]. The roots, leaves, and flowers of this plant are used in traditional and modern herbal medicine [1, 2]. Scientific literature reports that *Althaea officinalis* contains mucilaginous polysaccharides, flavonoids, phenolic acids, tannins, coumarins, steroids, triterpenoids, and other bioactive components [1-5]. These constituents are associated with antitussive, anti-inflammatory, antioxidant, antibacterial, gastroprotective, and mucosal-protective effects [2-5].

In recent decades, increasing attention has been paid not only to the phytochemical composition of medicinal plants, but also to the endophytic microorganisms inhabiting their internal tissues [6-9]. Endophytic fungi live within roots, stems, leaves, flowers, fruits, or seeds for at least part of their life cycle without causing visible disease symptoms [6, 9]. These fungi may contribute to plant growth, stress tolerance, resistance to pathogens, and ecological adaptation [6-8].

One of the most important characteristics of endophytic fungi is their ability to produce biologically active secondary metabolites [6-8]. Such metabolites include alkaloids, terpenoids, polyketides, phenolic compounds, peptides, lactones, quinones, steroids, and enzymes [7, 8]. Many of these compounds have demonstrated antimicrobial, antifungal, antioxidant, cytotoxic, anti-inflammatory, and enzyme-inhibitory activities [6-8, 13, 14]. Therefore, endophytic fungi are considered promising natural sources for the discovery of novel bioactive compounds [6-8].

Although the phytochemical composition and pharmacological properties of *Althaea officinalis* L. have been studied to some extent, the endophytic fungi living within its internal tissues, their secondary metabolites, and their biological activities have not been sufficiently investigated [1-5]. This knowledge gap determines the relevance of studying endophytic fungi associated with *Althaea officinalis* L. as potential producers of natural antimicrobial, antifungal, and antioxidant substances [6-8].

2. Aim and Objectives of the Study

The aim of this study is to isolate endophytic fungi from different tissues of *Althaea officinalis* L., characterize them based on morphological and microbiological features,

investigate their ability to produce secondary metabolites, and evaluate the biological activity of the obtained metabolites.

To achieve this aim, the following objectives were defined:

1. To collect root, stem, leaf, and flower samples of *Althaea officinalis* L. and prepare them for laboratory analysis;
2. To isolate endophytic fungi from plant tissues and obtain pure fungal cultures;
3. To characterize the isolated fungi based on morphological and microscopic features;
4. To investigate the capacity of endophytic fungi to produce secondary metabolites;
5. To evaluate the antimicrobial, antifungal, and antioxidant activities of fungal extracts;
6. To substantiate the scientific and practical significance of *Althaea officinalis* L. endophytic fungi as potential sources of bioactive compounds.

3. Materials and Methods

3.1. Research Material

The research material consists of healthy root, stem, leaf, and flower tissues of *Althaea officinalis* L. Samples should be collected from visually healthy plants because diseased or damaged tissues may contain pathogenic fungi rather than true endophytes [9, 10].

3.2. Isolation of Endophytic Fungi

Endophytic fungi are commonly isolated using the surface sterilization and tissue plating method [9, 10]. Plant samples are washed under running water to remove dust and soil particles, then surface-sterilized using 70% ethanol, sodium hypochlorite, and sterile distilled water [9-11]. After sterilization, the tissues are cut into small segments and placed on Potato Dextrose Agar (PDA) medium [9, 11]. Petri dishes are incubated at 25-28 °C until fungal colonies emerge from the internal plant tissues [9, 11].

A sterilization control is included to verify the effectiveness of surface sterilization [9, 10]. For this purpose, the final rinse water or tissue imprint is inoculated onto a separate PDA plate [9, 10]. The absence of microbial growth on the control plate confirms that external epiphytic microorganisms were successfully removed [9, 10].

3.3. Obtaining Pure Cultures and Morphological Characterization

Emerging fungal colonies are transferred to fresh PDA plates to obtain pure cultures [9, 11]. Repeated subculturing is performed until individual isolates show stable colony morphology [9]. The isolates are characterized according to colony color, shape, texture, margin, growth rate, reverse-side pigmentation, mycelial structure, and spore morphology [9, 12]. Microscopic examination is used to observe hyphae, conidia, spores, and reproductive structures [9, 12].

3.4. Secondary Metabolite Extraction

Pure fungal cultures are cultivated in Potato Dextrose Broth (PDB) or another suitable liquid medium for secondary metabolite production [11-14]. After fermentation, the culture broth is filtered to separate fungal biomass from the liquid phase [11, 13, 14]. Secondary metabolites are commonly extracted using ethyl acetate, and the crude extracts are concentrated and prepared for biological activity assays [11, 13, 14].

3.5. Evaluation of Biological Activity

The biological activity of fungal extracts is evaluated using antimicrobial, antifungal, and antioxidant assays [11, 13, 14]. Antimicrobial activity can be determined by agar well diffusion or disc diffusion methods against selected bacterial strains [11, 13]. Antifungal activity is assessed by measuring inhibition of pathogenic or test fungi [11, 13]. Antioxidant activity can be evaluated using DPPH radical scavenging or other free-radical neutralization assays [13, 14].

Table 1. Main methodological stages used for the isolation and study of endophytic fungi

Stage	Method	Purpose
1	Sample collection	Obtaining healthy plant tissues for analysis
2	Surface sterilization	Eliminating epiphytic microorganisms from the plant surface
3	Tissue plating on PDA	Allowing endophytic fungi to emerge from internal tissues
4	Incubation at 25-28 °C	Supporting fungal colony growth

5	Subculturing	Obtaining pure fungal cultures
6	Morphological and microscopic characterization	Preliminary identification and grouping of isolates
7	Fermentation in PDB	Stimulating secondary metabolite production
8	Ethyl acetate extraction	Obtaining crude fungal metabolites
9	Bioactivity assays	Evaluating antimicrobial, antifungal, and antioxidant properties

4. Methodological Problems and Challenges

- **Problem of surface sterilization:** Insufficient sterilization may allow epiphytic microorganisms to grow, whereas excessive sterilization may destroy true endophytic fungi living inside plant tissues [9, 10].
- **Risk of contamination:** Foreign bacteria or fungi may enter Petri dishes, culture media, or instruments during laboratory work, making it difficult to confirm the true endophytic origin of isolates [9].
- **Slow growth of some endophytes:** Some endophytic fungi grow slowly on artificial media, and fast-growing species may overgrow them, causing potentially useful isolates to remain undetected [9].
- **Difficulty in obtaining pure cultures:** Several colonies may emerge from one tissue segment, and repeated subculturing under strict aseptic conditions is required to obtain pure isolates [9, 11].
- **Limitations of morphological identification:** Colony morphology and microscopy are useful for preliminary characterization, but closely related species may require molecular identification, especially ITS-rDNA sequencing [9, 12].
- **Variation in metabolite production:** Secondary metabolite production depends on culture medium, temperature, incubation time, moisture, and other cultivation conditions [8, 11].

5. Results and Discussion

The expected result of this research is the isolation of morphologically diverse endophytic fungi from different tissues of *Althaea officinalis* L. Similar studies on medicinal plants indicate that fungal endophytes may differ in colony color, growth rate, texture,

sporulation, mycelial density, and microscopic structure [6-9]. Such diversity may indicate that marshmallow tissues host different groups of fungal endophytes [6-8].

The obtained isolates are expected to demonstrate varying capacities for secondary metabolite production. Ethyl acetate extracts of fermented cultures may contain compounds with antimicrobial, antifungal, and antioxidant potential, as reported in studies of endophytic fungi from medicinal plants [11, 13, 14]. If fungal extracts inhibit the growth of test microorganisms or demonstrate radical scavenging capacity, this would support the hypothesis that *Althaea officinalis* L. endophytes may serve as sources of biologically active substances [6-8, 13, 14].

The study is also important from a methodological perspective. Proper surface sterilization, contamination control, pure culture isolation, and reliable characterization are essential for distinguishing true endophytic fungi from external contaminants [9, 10]. Therefore, careful optimization of the isolation protocol is one of the central requirements of the research [9, 10].

6. Scientific Novelty

The scientific novelty of the study lies in the investigation of endophytic fungi associated with *Althaea officinalis* L., a medicinal plant whose phytochemical and pharmacological properties have been studied more extensively than its fungal endophytes [1-5]. The research focuses on isolating and characterizing fungal endophytes, assessing their secondary metabolite production, and evaluating their biological activity [6-9].

7. Practical Significance

The practical significance of the study is that endophytic fungi isolated from *Althaea officinalis* L. may be used as potential natural sources of antimicrobial, antifungal, and antioxidant compounds [6-8, 13, 14]. The results can be useful for future research in pharmacy, biotechnology, microbiology, and natural product chemistry [6-8]. They may also provide a scientific basis for the development of natural preparations, bioactive supplements, and environmentally safer biological agents [6-8].

8. Conclusion

Althaea officinalis L. is a medicinal plant rich in biologically active compounds and is therefore a promising object for endophyte-related research [1-5]. Endophytic fungi inhabiting internal plant tissues may produce secondary metabolites with antimicrobial, antifungal, antioxidant, and other useful biological activities [6-8, 13, 14].



The isolation and study of endophytic fungi from roots, stems, leaves, and flowers of *Althaea officinalis* L. may contribute to the discovery of new natural bioactive compounds [6-8]. However, methodological challenges such as surface sterilization, contamination control, slow growth of isolates, pure culture preparation, and limitations of morphological identification must be carefully addressed [9, 10].

Overall, endophytic fungi associated with *Althaea officinalis* L. represent a valuable and insufficiently explored source of natural bioactive metabolites. Further detailed studies, including molecular identification and chemical characterization of active compounds, are recommended [6-9].

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