# ANTI-INFLAMMATORY ACTIVITY OF ENDOPHYTIC FUNGI ASPERGILLUS SYDOWII STRAIN AV5L AND PENICILLIUM VERRUCOSUM STRAIN AV6L ISOLATED FROM ALOE VERA

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***Abstract.*** *The aim of this study was to evaluate the anti-inflammatory activity of ethyl acetate extracts of the endophytic fungi Aspergillus sydowii strain AV5L and Penicillium verrucosum AV6L. Anti-inflammatory properties were studied in vitro in a models of inhibition of egg and bovine serum albumin denaturation, as well as in a model of erythrocyte membrane stabilization. The studied extracts effectively demonstrated inhibition of protein denaturation. In the system with ovalbumin, the highest activity was demonstrated by the extract of P. verrucosum AV6L, providing 65% inhibition at a concentration of 500 µg/ml ($IC_{50}$ = 179.8 µg/ml), while for ascorbic acid this figure was 72% ($IC_{50}$ = 215.9 µg/ml). In the system with bovine serum albumin, the most pronounced activity among the studied extracts was demonstrated by A. sydowii strain AV5L — 71% inhibition at 500 µg/ml ($IC_{50}$ = 282.1 µg/ml), while ascorbic acid provided 78% inhibition ($IC_{50}$ = 267.9 µg/ml). In a model of red blood cells membrane stabilization, extracts at concentrations of 200–800 µg/ml were found to inhibit thermally induced hemolysis in a concentration-dependent manner. The maximum inhibition for P. verrucosum AV6L was 52.40 ± 0.40% at a concentration of 600 µg/ml ($IC_{50}$ = 360.80 µg/ml). Acetylsalicylic acid, used as a standard anti-inflammatory drug, provided 62.92 ± 0.75% inhibition at the same concentration ($IC_{50}$ = 388.48 µg/ml). The obtained results indicate a pronounced anti-inflammatory activity of the studied endophytic fungi and confirm the promise of further study of their bioactive metabolites as potential sources of anti-inflammatory compounds.*

***Keywords:*** *endophytic fungi, anti-inflammatory activity, inhibition of protein denaturation, stabilization of red blood cells membranes, biologically active metabolites.*

## Introduction

Inflammation is a complex protective response of the body to infectious agents, toxic substances, and tissue damage. Despite the physiological role of inflammation, its chronic course is a key factor in the development of many pathological conditions, including gastrointestinal

diseases, autoimmune disorders, and metabolic disorders [1]. In recent years, inflammation induced by microbial enzymes, among which urease plays a significant role, has attracted particular interest. Urease is a nickel-dependent enzyme that catalyzes the hydrolysis of urea to form ammonia and carbon dioxide. This enzyme is widespread among pathogenic microorganisms and plays a key role in their virulence. Specifically, *Helicobacter pylori* urease ensures the microorganism's survival in the acidic environment of the stomach by locally increasing pH, which promotes colonization of the mucosa and the development of inflammatory gastric diseases [2, 3].

Synthetic urease inhibitors, such as acetohydroxamic acid, are currently known, however, their use is limited due to significant side effects and toxicity. Similarly, the use of synthetic anti-inflammatory drugs is often accompanied by the development of side effects, sparking interest in natural sources of biologically active compounds [4,5].

Endophytic microorganisms that asymptomatically colonize the internal tissues of plants are considered as a promising source of biologically active secondary metabolites. According to a review by Strobel and Daisy (2003) [6], endophytes are capable of synthesizing a wide range of compounds with antimicrobial, antioxidant, and anti-inflammatory activity. Later, Kusari et al. (2012) [7] showed that many endophytic fungi produce metabolites identical or structurally similar to host plant compounds, emphasizing their importance for pharmacological and biotechnological research.

Among the metabolites of endophytic origin, phenolic compounds, flavonoids, terpenoids, alkaloids and peptides are of particular importance, characterized by the ability to inhibit enzymes, suppress inflammatory mediators and stabilize cell membranes by reducing the intensity of lipid peroxidation. We have previously established that ethyl acetate extracts of the endophytic fungi *Aspergillus sydowii strain* AV5L and *Penicillium verrucosum* AV6L possess pronounced urease-inhibiting activity [8]. Qualitative phytochemical analysis revealed the presence of flavonoids, terpenoids, tannins, cardiac glycosides, and peptides, which justifies the need for further study of their biological activity.

Taking into consideration the established phytochemical composition of the extracts and their pronounced ability to inhibit urease, the present study investigates the anti-inflammatory activity of secondary metabolites of the mentioned endophytes using *in vitro* models of protein denaturation inhibition and cell membrane stabilization. These methods are widely used for the initial assessment of the anti-inflammatory potential of natural compounds [9].

**Materials and Methods**

To evaluate the anti-inflammatory activity of the extracts, egg albumin (EA) and bovine serum albumin (BSA) were used, as well as a membrane stabilization test to confirm the universality and reproducibility of the inhibitory effects of the extracts on various proteins. The method was implemented according to Mizushima Y Kobayashi M (1968) with modifications proposed by C. Sree Kumari et al. (2015) [10,11]. All three experiments were performed in triplicate.

**Inhibition of denaturation of ovalbumin**. A 4 ml reaction mixture consisted of 0.2 ml of ovalbumin, 2.8 ml of phosphate buffer (pH 6.4), and 1 ml of extracts of varying concentrations. A similar volume of distilled water served as a control. The mixture was incubated at 37°C for 15 min and then heated at 65°C for 5 min. After the samples were cooled, the absorbance at 660 nm was measured spectrophotometrically. Acetylsalicylic acid served as a standard, from which a control was prepared at a concentration similar to the experimental extract. Protein denaturation inhibition (X) by the extract and standard was expressed as a percentage and calculated using the

formula: $X = \frac{A0 - At}{A0} \times 100$, where $A_0$ is the optical density of the control; $A_t$ is the optical density of the test sample.

**Inhibition of bovine albumin denaturation**. The reaction mixture (4 ml) consisted of 2 ml of bovine serum albumin (1% aqueous solution), 0.4 ml of fungi extract of varying concentrations and 1.6 ml of phosphate-buffered saline (pH 6.4). Samples were incubated at 37°C for 30 minutes and then heated at $65^0$C for 5min. After cooling absorbance was measured at 660 nm. 0.05 ml of distilled water was used instead of the extract as a control. The protein inhibition denaturation (X) by the extract and standard was expressed as a percentage and calculated using the formula: $X = \frac{A0 - At}{A0} \times 100$, where $A_0$ is the optical density of the control; $A_t$ is the optical density of the test sample.

**Membrane stabilizing activity**. Preparation of Red Blood cells suspension. Fresh whole human blood (10 ml) was collected and transferred to tubes to centrifuge. The tubes were centrifuged at 3000 rpm for 10 minutes and washed three times with an equal volume of a normal saline. The blood volume was measured, and a 10% (v/v) suspension in 0,9 % sodium chloride solution was prepared.

**Heat-induced hemolysis.** The reaction mixture (2 ml) consisted of 1 ml of the test sample and 1 ml of a 10% RBCs suspension. For the control, the drug was replaced with a saline. Acetylsalicylic acid was used as a standard. The tubes were incubated in a water bath at 56°C for 30 minutes and then cooled under running water. The mixture was then centrifuged at 2500 rpm for 5 minutes, and the optical density of the supernatant was measured at 560 nm. The protein inhibition denaturation (X) by the extract and standard was expressed as a percentage and calculated using the formula: $X = \frac{A0 - At}{A0} \times 100$, where $A_0$ is the optical density of the control; $A_t$ is the optical density of the test sample.

**Results and Discussion**

This study examined the anti-inflammatory activity of secondary metabolites of the endophytic fungi *A. sydowii strain* AV5L and *P. verrucosum* AV6L. Biological activity was assessed using in vitro models based on protein denaturation inhibition and membrane-stabilizing effects. These methods were chosen due to their ability to reflect key aspects of the inflammatory process, including damage to protein structures and disruption of cell membrane integrity.

***Table 1.***

***Anti-inflammatory activity of Aspergillus sydowii strain AV5L and Penicillium verrucosum AV6L determined by inhibition of ovalbumin denaturation (n = 3).***

| № | Concentration µg/ml | Acetylsalicylic acid % | *A. sydowii strain* AV5L, % | *P.verrucosum* AV 6L % |
|---|---|---|---|---|
| 1 | 100 | 37±0,33 | 43±0,45 | 33±0,23 |
| 2 | 200 | 40±0,41 | 45±0,65 | 45±0,45 |
| 3 | 300 | 58±0,68 | 50±0,33 | 49±0,23 |
| 4 | 400 | 50±1,33 | 30±1,44 | 51±0,68 |
| 5 | 500 | 72±0,45 | 42±0,35 | 65±0,40 |

Denaturation inhibition studies were conducted using egg albumin and bovine serum albumin models. Protein stabilization under these conditions is considered an indicator of anti-

inflammatory activity, as it prevents the formation of denatured forms that can induce an immune response and increase inflammation. The results showed that the extracts of the studied endophytic fungi effectively inhibit protein denaturation. The highest activity was demonstrated by *P. verrucosum* AV6L: at a concentration of 500 µg/ml, the inhibition rate of egg albumin denaturation was 65%, which is close to the activity of acetylsalicylic acid (72%) (Table 1).

Similar results were obtained in a study of the inhibition of bovine serum albumin denaturation, further confirming the anti-inflammatory properties of the secondary metabolites of the endophytic fungi studied. In this model, the standard anti-inflammatory drug acetylsalicylic acid demonstrated the highest activity among the control samples, with a denaturation inhibition rate of 78% at a concentration of 500 µg/ml. Among the studied fungi extracts, the most pronounced effect was observed with *A. sydowii strain* AV5L extract, which, at the same concentration, provided 71% inhibition of protein denaturation, close to the activity of the standard preparation. *P. verrucosum* AV6L extract also demonstrated significant anti-inflammatory activity, achieving 67% inhibition at a concentration of 500 µg/ml (Table 2). The obtained results indicate a pronounced ability of secondary metabolites of the studied endophytic fungi to suppress protein denaturation, which may indicate their potential as natural anti-inflammatory agents.

***Table 2.***

***Anti-inflammatory activity of A. sydowii strain AV5L and P. verrucosum AV6L determined by inhibition of bovine serum albumin denaturation (n = 3).***

| № | Concentration µg/ml | Acetylsalicylic acid % | *A. sydowii strain* AV5L, % | *P. verrucosum* AV6L, % |
|---|---|---|---|---|
| 1 | 100 | 25±0,60 | 23±0,45 | 31±1,05 |
| 2 | 200 | 20±0,55 | 29±0,32 | 34±0,45 |
| 3 | 300 | 45±0,33 | 44±0,60 | 40±0,60 |
| 4 | 400 | 50±0,45 | 49±0,55 | 54±0,33 |
| 5 | 500 | 78±0,60 | 71±0,32 | 67±0,32 |

The anti-inflammatory activity of the samples was assessed using $IC_{50}$ values, which reflect the concentration required to achieve 50% inhibition of protein denaturation. Higher $IC_{50}$ values indicate decreased anti-inflammatory activity.

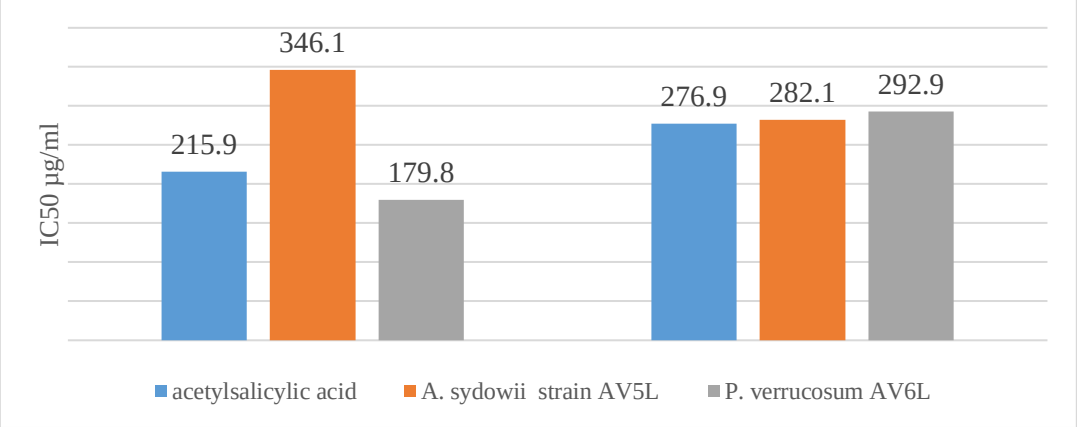



***Figure 1. $IC_{50}$ of ethyl acetate extracts of endophytic fungi A. sydowii strain AV5L and P. verrucosum AV6L for inhibition of denaturation of egg albumin (1) and bovine serum albumin (2).***

Figure 1 shows the $IC_{50}$ values of the ethyl acetate extracts of *A. sydowii strain* AV5L and *P. verrucosum* AV6L in the denaturation inhibition test of egg and bovine albumin. In the system with egg albumin, the lowest $IC_{50}$ value was demonstrated by the extract of *P. verrucosum* AV6L (179.8 μg/ml), indicating a more pronounced anti-inflammatory activity compared to *A. sydowii strain* AV5L (346.1 μg/ml) and acetylsalicylic acid (215.9 μg/ml).

In the bovine albumin model, the $IC_{50}$ values were 267.9 μg/mL for acetylsalicylic acid, 282.1 μg/mL for *A. sydowii* AV5L, and 292.9 μg/mL for *P. verrucosum* AV6L, demonstrating activity comparable to, though less pronounced than, that of the standard drug.

The obtained results are consistent with published data indicating the pronounced anti-inflammatory activity of secondary metabolites of endophytic fungi of the genus *Penicillium*. It has previously been shown that extracts of *Penicillium sp*. can effectively inhibit protein denaturation, stabilize biological membranes, and suppress the production of anti-inflammatory mediators, demonstrating activity comparable to standard nonsteroidal anti-inflammatory drugs (Govindappa et al., 2011; Kharwar et al., 2019; Zhang et al., 2020) [12-14].

Similar data on moderate anti-inflammatory activity were obtained for representatives of the genus Aspergillus, the metabolites of which are capable of inhibiting thermal denaturation of albumin and hemolysis of erythrocytes in a concentration-dependent manner (Mishra et al., 2017; El-Sayed et al., 2021) [15,16] (Table 1).

Overall, the obtained results indicate that both endophytic fungi, *A. sydowii strain* AV5L *and P. verrucosum* AV6L, have the ability to inhibit protein denaturation, confirming their anti-inflammatory properties. Moreover, *P. verrucosum* AV6L extract demonstrated higher efficacy in the ovalbumin denaturation inhibition model, while *A. sydowii strain* AV5L extract demonstrated slightly more pronounced activity in the bovine serum albumin system. The observed differences may be due to the peculiarities of the spatial structure of the substrate proteins used, as well as differences in the mechanisms of interaction of bioactive compounds of fungi extracts with protein molecules.

***Table 3.***

***Anti-inflammatory activity of Aspergillus sydowii strain AV5L and Penicillium verrucosum AV6L in stabilizing erythrocyte membranes (inhibition of thermally induced hemolysis, %).***

| № | Concentration μg/ml | Acetylsalicylic acid % | *A. sydowii strain* AV5 L% | *P.verrucosum* AV6L % |
|---|---|---|---|---|
| 1. | 100 | 44,21 ± 1,10 | 15,64 ± 0,40 | 20,29 ± 1,33 |
| 2 | 200 | 54,57 ± 0,91 | 20,30 ± 0,38 | 22,79 ± 0,67 |
| 3 | 400 | 63,45 ± 0,64 | 35,50 ± 0,60 | 48,33 ± 1,63 |
| 4 | 600 | 72,56 ± 0,58 | 47,90 ± 1,20 | 52,40 ± 0,82 |
| 5 | 800 | 62,92 ± 0,75 | 46,90 ± 0,45 | 50,44 ± 0,40 |
| | $IC_{50}$ (μg/ml) | 388.48 | 396.24 | 360.80 |

To further clarify the mechanism of anti-inflammatory action of the ethyl acetate extracts of *A.sydowii strain* AV5L and *P.verrucosum* AV6L, their effect on erythrocyte (RBC) membrane stabilization was examined. This method is widely used to evaluate the anti-inflammatory activity of natural compounds, as the ability to prevent thermally induced hemolysis correlates with membrane stabilization and potential anti-inflammatory activity.

The studied extracts at concentrations of 200–800 μg/ml were found to inhibit thermally induced erythrocyte hemolysis in a concentration-dependent manner (Table 3). The maximum inhibition level for the *P. verrucosum* AV6L strain was 52.40 ± 0.40% at a concentration of 600 μg/ml, while the $IC_{50}$ value was 360.80 μg/ml. Acetylsalicylic acid, used as a standard anti-inflammatory drug, demonstrated a higher inhibition level of 62.92 ± 0.75% at the same concentration (600 μg/ml), while the $IC_{50}$ value was 388.48 μg/ml.

The obtained results indicate a pronounced membrane-stabilizing activity of the studied extracts, which indicates their potential anti-inflammatory effect comparable to acetylsalicylic acid at maximum concentration. The observed activity may be due to the presence of flavonoids, tannins, and terpene compounds in the extracts, detected during phytochemical analysis (Vakhabova N., et al., 2025) [17]. Flavonoids are natural polyphenolic compounds with pronounced antioxidant and anti-inflammatory properties. They can reduce the formation of proinflammatory mediators, suppress the production of nitric oxide and cytokines, and inhibit the activity of enzymes involved in the development of the inflammatory process. Due to their pronounced antioxidant activity, flavonoids reduce the intensity of lipid peroxidation and protect cellular structures from oxidative and inflammatory damage.

Tannins, through their ability to form complexes with proteins and inhibit lipid peroxidation, help stabilize protein structures and cell membranes. Terpenoids, in turn, are known as inflammatory response modulators, capable of suppressing the synthesis of inflammatory mediators and reducing oxidative stress.

Thus, the established membrane-stabilizing activity of the studied extracts may be associated with the complex action of polyphenolic and terpene metabolites, which provide protection of proteins and biological membranes under conditions of inflammatory damage.

Thus, the established membrane-stabilizing activity of the studied extracts may be associated with the complex action of polyphenolic and terpene metabolites, which provide protection of proteins and biological membranes under conditions of inflammatory damage.

The obtained results confirm the potential of endophytic fungi as a source of multifunctional natural compounds with anti-inflammatory potential and substantiate the need for further isolation, structural identification, and study of the mechanisms of action of individual active components.

**Conclusion**

This study demonstrated for the first time that ethyl acetate extracts of the endophytic fungi *A. sydowii strain* AV5L and *P. verrucosum* AV6L, isolated from the medicinal plant Aloe vera, exhibit pronounced anti-inflammatory activity in in vitro models of protein denaturation inhibition and membrane stabilization. *P. verrucosum* AV6L extract was shown to exhibit higher activity in the egg albumin model, while *A. sydowii strain* AV5L demonstrated a comparable effect in the bovine serum albumin system.

The scientific novelty of this study lies in the identification and comparative evaluation of the anti-inflammatory potential of these endophytic strains, previously characterized as producers of biologically active metabolites with urease-inhibiting activity. For the first time, it has been demonstrated that these strains are capable of exhibiting a membrane-stabilizing effect comparable to a standard anti-inflammatory drug, expanding our understanding of the spectrum of their biological activity.

The established activity is probably due to the complex action of polyphenolic and terpene compounds identified during phytochemical analysis, which provide protection of proteins and

biological membranes under conditions of inflammatory damage. The obtained results confirm the potential of endophytic fungi as a source of multifunctional natural compounds with anti-inflammatory potential and substantiate the need for further isolation, structural identification, and study of the mechanisms of action of individual active components.

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