

# Effect of cherry leaves' extracts on *Aspergillus niger* in juice

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## Abstract

In this research, *Aspergillus niger* inhibition with ethanol and methanol extracts of cherry leaves in juice was investigated. *Aspergillus niger* concentration 3.2 log cfu/mL is selected. Ethanolic (air-dried, microwave-dried) and methanolic (air-dried, microwave-dried) extracts (1 mL) are added to juice (9 mL), separately. They were left for incubation at 25°C for 5 minutes. Then *Aspergillus niger* (1 mL) is put into test tubes on extracts and left for incubation at 25°C for 24 h. All extracts significantly reduced *Aspergillus niger* numbers in juice compared to control samples. The highest inhibitory activity was detected in the sample extracted with ethanol from air-dried cherry leaves (2.8 log cfu/mL). The effect of cherry leaves ethanol extract was found to be higher than methanol extract. The reason for this might be that the phenolic compounds in cherry leaves are more soluble in ethanol than in methanol. These results demonstrated that cherry leaves can be used for decrease *Aspergillus niger* in juice at 25°C.

**Keywords:** ethanol; mold; methanol; microwave

## 1. Introduction

*Aspergillus niger* emerges as a problem in food businesses. It develops minimum 6-8°C, optimum 35-37°C and maximum at 45-47°C. It is a xerophilic mold and it has been stated that it can grow at an aw value of 0.77. It also grows below pH 2 in environments with higher water activity than this value [1]. Its color varies between brownish black or black. It prefers warm environments where food is produced and stored. It protects black colored spores against ultraviolet rays of sunlight and this feature gives it an advantage against other molds. Generally, this mold is isolated from sun-dried products [2]. *Aspergillus* are hyphal structures that are widely found everywhere on earth. They are found in large amounts in soil and rotting foods. They participate in the carbon and nitrogen cycle. They decompose and use organic materials with the enzymes they have and live as saprophytes. Depending on environmental conditions, they can have pathogenic effects on plants, animals and humans. Their reproductive speed and capacity is high [3].

Cherries are among the earliest ripening fruits in temperate climates. Its homeland is the region

between the Caspian Sea and the Black Sea. Türkiye is one of the production centers of cherries [4]. Cherry trees can grow even in regions with cold winters and dry summers. However, they are deciduous trees that thrive especially in temperate climate regions. High temperature, precipitation and wind negatively affect the production of these species. Cherries are entomophilous species. Frequent watering is necessary throughout the cherry growing season, but rainfall during flowering or ripening can compromise production [5].

In this research, it is aimed to inhibit a strong pathogen *Aspergillus niger* with different extracts from cherry leaves at 25°C.

## 2. Materials and Method

### 2.1. Materials

Cherry (*Prunus avium*) leaves were collected from Karaman in June 2023. Leaves (50 g) were dried at 13 W/g (650 W) microwave power densities for 210-270 s in the microwave (M) drying process. Cherry leaves were dried on a clean surface at room temperature for one week in the air drying process (A). The dried leaves were ground with a

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commercial blender and stored at  $-18\text{ }^{\circ}\text{C}$  until extraction [6]. *Aspergillus niger* strain was isolated within the scope of the project previously carried out in our laboratory.

## 2.2. Methods

### 2.2.1. Production extracts

Methanol (Met) and ethanol (Et) extraction of leaves was carried out according to the method Özpınar et al. [7]. Methanol (100 mL) was added to 20 g of ground leaves in Met extraction. Ethanol (100 mL) was added to 20 g of ground leaves in Et extraction. They were kept in a shaking water bath set at  $25^{\circ}\text{C}$  and 150 RPM for 24 hours. The extracts were centrifuged at 4000 RPM for 10 minutes and the filtrate was obtained by passing the upper part through Whatman 1. The obtained filtrates were stored at  $-18\text{ }^{\circ}\text{C}$  until they were used.

### 2.2.2. *Aspergillus niger* inhibition

*Aspergillus niger* was used in the study. Mold strains from stock cultures were activated in Nutrient Broth (Merck, Darmstadt, Germany) at  $25^{\circ}\text{C}$  for 48 h. Concentration  $3.2\text{ log cfu/mL}$  is selected. Extracts (1 mL) are added to juice (9 mL), separately. They were left for incubation at  $25^{\circ}\text{C}$  for 5 minutes. Then *Aspergillus niger* (1 mL) is put into test tubes on extracts and left for incubation at  $25^{\circ}\text{C}$  for 24 h. Appropriate dilutions were inoculated on petri dishes with Potato Dextrose (PDA) Agar (with tartaric acid) using spread plate technique. It was incubated at  $25^{\circ}\text{C}$  for 5 days [8]. Results were calculated as log cfu/mL.

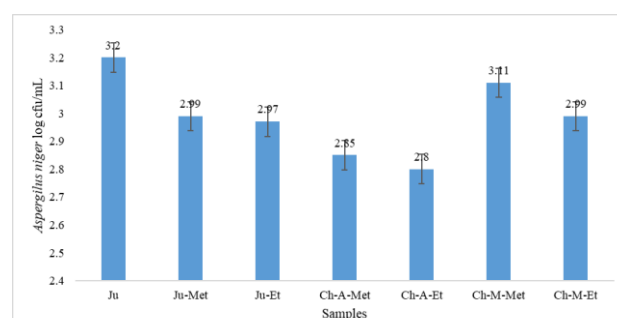
## 3. Results and Discussion

As can be seen in Figure 1, all extracts significantly reduced *Aspergillus niger* numbers in juice compared to control samples (Ju).

*Aspergillus niger* was cultured in juice as  $3.2\text{ log cfu/mL}$ . The highest inhibitory activity was detected in the sample extracted with ethanol from air-dried cherry leaves ( $2.8\text{ log cfu/mL}$ ). It was determined that the antifungal effect was the same in the sample extracted with methanol (Ju-Met) and in the sample extracted with ethanol (Ch-M-Et) from cherry leaves dried in the microwave. This is the lowest activity detected ( $2.99\text{ log cfu/mL}$ ). The effect of cherry leaves ethanol extract was found to be higher than methanol extract. The reason for this might be that the phenolic compounds in cherry leaves are

more soluble in ethanol than in methanol. Likewise, the inhibitory effect of air-dried extracts was found to be higher than microwave-dried extracts. Some of the phenolic compounds may have been damaged by the heat applied in the microwave. For this reason, the antifungal effect may have been detected lower.

Rasooli et al. [9] determined that thyme essential oils also have an inhibitory effect against *Aspergillus niger* [10]. In a study, researchers found nigella sativa extracts have antifungal effect on *Aspergillus* [11]. In another study, researchers determined methanolic extracts of *Spirulina platensis* have antifungal effect on *Aspergillus* [12]. Demir et al. [13] stated ethanolic extract of pomegranate have antimicrobial effect on a few microorganisms and *Aspergillus niger* is one of them. In the analyzes performed using 6 different solvents, it was reported that the highest antimicrobial activity was detected in ethanolic extracts.



(Ju: juice; Ju-Met: juice + methanolic extracts of cherry leaves; Ju-Et: juice + ethanolic extracts; Ch-A-Met: juice + air dried methanolic extract; Ch-A-Et: juice + air dried ethanolic extract; Ch-M-Met: juice + microwave dried methanolic extract; Ch-M-Et: juice + microwave dried ethanolic extract)

Figure 1. Inhibition of *Aspergillus niger* in juice

## 4. Conclusion

Juices are among the risky foods in terms of mold growth due to their high acidity. One of these molds is *Aspergillus niger*. There are various studies in the literature regarding the antifungal effects of various plant extracts on different molds. In this study, cherry leaf extracts were used for this purpose. Ethanolic extracts from air-dried cherry leaves showed the highest antifungal effect. The inhibitory activity obtained from methanolic and microwave dried leaves is also undeniable. Studies should be carried out to extend the shelf life of acidic foods

such as juice naturally, without using chemical preservatives, by trying different plant extracts against molds at different concentrations.

The results of this study show that as a natural food preservative cherry leaf can be used to effectively reduce the *Aspergillus niger* population in juice.

**Compliance with Ethics Requirements.** Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

**Disclosure statement.** No potential conflict of interest was reported by the authors.

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