

## Evaluation of Antimicrobial Effects of Fresh and Dried Fruit in Turkish Tombul Hazelnut Variety

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### Abstract

**Objective:** The aim of the study was to determine the antimicrobial properties of fresh and dried fruits of Turkish Tombul hazelnut (*Corylus avellana* L.) variety.

**Materials and Methods:** The Turkish Tombul hazelnuts used in the experiment were obtained from Helvacı Village/ Düzköy /Trabzon. Fresh hazelnuts were harvested in 2023, while dried hazelnuts were collected in 2022. The dried hazelnuts were dried at 105°C until they attained a consistent weight. This section was not created for fresh hazelnuts. The treated micelle was extracted for 4 hours with 100 ml of Ethyl alcohol (EtOH) for every 10 g of both the fresh and dried hazelnut samples crushed. The micelle (hazelnut+oil+solvent mixture) was filtered through coarse filter paper to remove particles before being blown away with a Rotary evaporator (Heidolph, Germany). The resulting extracts were used for antimicrobial testing. Antimicrobial activity of the strains was evaluated against *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC2468, *Enterococcus faecalis* ATCC51299, *Escherichia coli* ATCC2471, *Klebsiella pneumoniae* ATCC700603, *Salmonella typhimurium* ATCC13311, *Serratia marcescens* ATCC13880, *Staphylococcus epidermis* ATCC14990, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC 6633, *Streptococcus faecalis* ATCC 9790, *Yersinia pestis* ATCC 19428 and *Candida albicans* ATCC10351. One-way analysis of variance (ANOVA) was run to determine any significant differences in the study groups by Duncan multiple range test was performed through SPSS.

**Results:** Dried hazelnut extract was effective in all of them, while fresh hazelnut extract was only effective on *Candida albicans* ATCC10351 and *Klebsiella pneumoniae* ATCC700603 (F (3, 8) = 65.45, p<0.05). The most effective zone of inhibition obtained from dried nut extract was determined on *Streptococcus faecalis* ATCC 9790 (F (3, 8) = 15.07, p<0.05). It was determined to be above *Klebsiella pneumoniae* ATCC700603 in the least inhibition zone (F (3, 8) = 46.33, p<0.05).

**Conclusion:** After the necessary scientific research are carried out, hazelnut oil, which is produced under appropriate conditions, can turn into a market with high potential for related companies around the world and for Turkey in terms of cosmetic products and with the development of antibiotics.

**Keywords:** Antimicrobial, Hazelnut, Hazelnut extract, Microbiology, Turkish Tombul hazelnut

### Tombul Fındık Çeşidinde Yaş ve kuru Meyvenin Antimikrobiyal Etkilerinin Değerlendirilmesi

#### Öz

**Amaç:** Araştırmanın amacı Tombul fındık (*Corylus avellana* L.) çeşidinin yaş ve kuru meyvelerine ait antimikrobiyal özelliklerin belirlenmesidir.

**Materyal ve Yöntem:** Deneyde kullanılan tombul fındıklar Helvacı Köyü/ Düzköy /Trabzon'dan temin edilmiştir. Taze fındıklar 2023 yılında hasat edilirken, kuru fındıklar 2022 yılında toplanmıştır. Kuru fındıklar 105°C'de sabit bir ağırlığa ulaşana kadar kurutulmuştur. Bu bölüm taze fındıklar için oluşturulmamıştır. İşlenmiş misella, ezilmiş taze ve

kuru fındık örneklerinin her 10 g'ı için 100 ml Etil alkol (EtOH) ile 4 saat boyunca ekstrakte edilmiştir. Misella (fındık+yağ+çözücü karışımı) döner buharlaştırıcı (Heidolph, Almanya) ile uçurulmadan önce partikülleri uzaklaştırmak için kaba filtre kağıdından süzümüştür. Elde edilen ekstrakt antimikrobiyal testler için kullanılmıştır. Suşların antimikrobiyal aktivitesi *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC2468, *Enterococcus faecalis* ATCC51299, *Escherichia coli* ATCC2471, *Klebsiella pneumoniae* ATCC700603, *Salmonella typhimurium* ATCC13311, *Serratia marcescens* ATCC13880, *Staphylococcus epidermis* ATCC14990, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC 6633, *Streptococcus faecalis* ATCC 9790, *Yersinia pestis* ATCC 19428 ve *Candida albicans* ATCC10351'a karşı değerlendirilmiştir. Çalışma gruplarındaki anlamlı farklılıkları belirlemek için tek yönlü varyans analizi (ANOVA) yapılmış ve SPSS aracılığıyla Duncan çoklu aralık testi uygulanmıştır.

**Araştırma Bulguları:** Kuru fındık ekstrakt antimikrobiyal için kullanılan suşların hepsinde etkili olurken, taze fındık ekstraktı sadece *Candida albicans* ATCC10351 ve *Klebsiella pneumoniae* ATCC700603 üzerinde etkili olmuştur (F (3, 8) = 65.45, p<0.05). Kuru fındık ekstraktından elde edilen en etkili inhibisyon zonu *Streptococcus faecalis* ATCC 9790 üzerinde belirlenmiştir (F (3, 8) = 15.07, p<0.05). En az inhibisyon zonunun ise *Klebsiella pneumoniae* ATCC700603 üzerinde olduğu tespit edilmiştir (F (3, 8) = 46.33, p<0.05).

**Sonuç:** Gerekli bilimsel araştırmaların yapılmasının ardından uygun koşullarda üretilen fındık yağı, kozmetik ürünler ve antibiyotiklerin geliştirilmesi ile dünya çapında ilgili firmalar ve Türkiye için potansiyeli yüksek bir pazara dönüşebilir.

**Anahtar kelimeler:** Antimikrobiyal, Fındık, Fındık ekstraktı, Mikrobiyoloji, Tombul fındık

## Introduction

Plants have always been a significant source of nourishment for living creatures. Numerous research have revealed that the positive benefits of plants are attributable to active secondary metabolites in their structure, depending on technological advancements. Many plants' antioxidant, anticancer, antimicrobial, antiulcerative, antibacterial, antimutagenic, and antihistamine properties have been extensively studied in this area (Benetou et al., 2008; Güner et al.,

2019). At the same time, herbal natural products are employed as raw materials in the pharmaceutical industry (Lui, 2003). Hazelnut content, which is widely used across the world, is gaining popularity in the pharmaceutical industry due to its high levels of minerals, protein, dietary fiber, vitamins, and polyunsaturated fatty acids (Lui, 2004). At the same time, the presence of taxanes, which are active ingredients in chemotherapy treatments, in hazelnut products has increased the value of these herbal remedies (Falasca and Casari, 2012). While hazelnuts and hazelnut varieties are popular as food in Turkey, particularly in the Black Sea area, they have not gotten the expected attention in disciplines such as medicine and pharmacy (Güner et al., 2021).

Turkey is the world's biggest provider of hazelnuts, accounting for over 70% of total worldwide production, followed by Italy, the United States, and Spain (Ciarmiello et al., 2004; İslam, 2021). The Black Sea Region's climatic conditions make a great setting for hazelnuts and host several species of hazelnuts. In Turkey, grown hazelnuts may grow to be 5-6 meters tall and are hybrids of the "*Corylus avellana*" and "*Corylus maxima*" species (Köksal et al., 2006; Madesis et al., 2013). Hazelnuts cultivated in the Eastern Black Sea Region, in particular, contain more oil than hazelnuts grown in other places (Köksal, 2002). It is known to contain a high percentage of palmitic acid (3.8%), palmitoleic (3.7%), stearic (1.7%), oleic (84.5%), linoleic (10%), docosenoic (3.4%), linolenic (1.1%), and eicosaenoic acid (4.6%) (Turan, 2018). These numbers may differ depending on where the hazelnut is gathered, when it is collected, and the drying processes employed (Turan, 2019).

Today, interest in the discovery of natural antibiotic compounds that can be used as an alternative to both chemical preservatives and antibiotics is increasing. With new compounds to be found, it will be possible to reduce chemical preservatives and reduce the possibility of resistance to these chemicals in humans (Ramalhosa et al., 2011). Therefore, many studies have focused on the properties of antimicrobial agents and plant-derived active substances that have been used for some time to overcome infections in traditional medicine. Not many studies have been conducted to determine the antimicrobial potential of hazelnuts. Therefore, this study will prepare the ground for new studies. Nowadays, considering this rich content of hazelnut and its diversity in Turkey, its antimicrobial evaluation cannot be ignored.

Hazelnut oil contains flavour, fatty acid, phenolic and antioxidant profile, especially free radicals (Selli et al., 2022). It can be used as an alternative to preservative chemicals used especially in the food industry and can reduce the likelihood of antibiotic-related diseases in humans. For this reason, many studies have focused on antimicrobial agents (Ramalhosa et al., 2011). Particular attention has been drawn to the antimicrobial properties of plant-derived active ingredients. Although there are not many studies on this subject, two studies on hazelnut inside (Oliveira et al., 2008) and leaf (Oliveira et al., 2007) stand out. In both studies, minimal inhibitory concentration (MIC) values were determined for gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*). Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and fungi (*Candida albicans* and *Cryptococcus neoformans*) using radial diffusion based agar streak dilution method (Oliveira et al., 2007; Oliveira et al., 2008). In a study on hazelnut kernel, the effects obtained by looking at the effects of *Candida albicans*, *Escherichia coli* and *Serratia marcescens* in the antimicrobial activity section were found to be significant (Shataer et al., 2021). In addition, antimicrobial and antioxidant activities of hazelnut pollen structure were also evaluated. In these studies, the effects of different products of hazelnut (hazelnut inside, hazelnut leaf and hazelnut pollen) were also examined. Significant effects were observed by using 5 different bacteria in the study (Nikolaieva et al., 2019). In the investigation of the antimicrobial effect of both oil and extract of American hazelnut, effective results were obtained using 17 microorganisms (Gram positive, Gram negative bacteria and yeast strains) (Barta et al., 2020). All these studies emphasise the antimicrobial evaluation and importance of hazelnut products (hazelnut inside, hazelnut leaf, hazelnut pollen, hazelnut oil and hazelnut extract). The goal of this study is to assess the antimicrobial activity of extract extracted from Turkish Tombul dried (sun-dried) and fresh hazelnuts. Studies have shown that hazelnut extract and content have considerable health benefits that cannot be dismissed. Hazelnut is one of our important fruit species that is nutritious in terms of its high fat and protein content and has a lot of effects on human health (Turan, 2018; Turan, 2019). The goal of this study is to assess Turkish Tombul

hazelnut (*Corylus avellana* L.) extract's antimicrobial properties.

## Materials and Methods

### Obtaining hazelnut extracts

The Tombul hazelnuts used in the experiment were obtained from Helvacı Village/ Düzköy /Trabzon. Fresh hazelnuts were harvested in 2023, while dried hazelnuts (sun-dried) were collected in 2022. After the shells of the hazelnuts are peeled with the help of a hazelnut crusher, they were ground (pulverized) in a household blender (Tefal), and both the fresh and dried hazelnut powder obtained were used for oil extraction. The dried hazelnuts were dried at 105°C until they attained a consistent weight. It should be noted that this section is not applicable to fresh hazelnuts. The micelle to be treated was extracted for 4 hours with 100 mL of Ethyl alcohol (EtOH) with stringing for every 10 g of both the fresh and dried hazelnut samples ground at the room temperature. The micelle (hazelnut+oil+solvent mixture) was filtered through coarse filter paper (0.45µm) (Barta et al., 2020) to remove particles before being blown away with a Rotary evaporator (Heidolph, Germany). It was then treated at 80 rpm at 40 °C to remove ethyl alcohol. The commercial sample was transferred to an amber bottle and the extracts obtained from fresh and dried hazelnuts were transferred to a transparent bottle for visualisation. The extract samples were maintained in sealed bottles at 4°C until they could be utilized in antimicrobial tests after the amount of extract was determined to be ml extract /100 g dried sample (Figure 1). The same procedure was followed for the sample of fresh hazelnuts.

### Antimicrobial activity

The resulting extracts were used for antimicrobial testing. Antimicrobial activity of the extracts was evaluated against *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC2468, *Enterococcus faecalis* ATCC51299, *Escherichia coli* ATCC2471, *Klebsiella pneumoniae* ATCC700603, *Salmonella typhimurium* ATCC13311, *Serratia marcescens* ATCC13880, *Staphylococcus epidermis* ATCC14990, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC 6633, *Streptococcus faecalis* ATCC 9790, *Yersinia pestis* ATCC 19428 and *Candida albicans* ATCC10351. These selected microorganisms are also clinically important as they are human pathogens.



Figure 1. Hazelnut extracts, P: commercial product DHO: dried hazelnut extract FHO: fresh hazelnut extract

Firstly, Mueller-Hinton Agar (MHA) (1038720500, Merck) and Mueller-Hinton Broth (MHB) (1102930500, Merck) mediums were prepared in accordance with the manufacturer's instructions for bacterial strains and for the fungal strain were used Potato Dextrose Agar (PDA) (Merck 70139) and Potato Dextrose Broth (PDB) (Merck P6685).

The pH was adjusted to 7.2-7.4 for bacterial media and 5.3 for fungal media. After the media were autoclaved, MHA/PDA was dispensed in sterile circular Petri dishes with a diameter of 90 mm and a thickness of  $4 \pm 0.5$  mm, while the liquid one (MHB/PDB) was directly stored in a 4 °C fridge after cooling. The test microorganisms were grown in Mueller-Hinton Broth (MHB/PDB) and at 37 °C / 30 °C for 16-18 h. In the present study, disc diffusion test was used for the determination of antimicrobial activity according to Kirby-Bauer method (Barry et

al., 1970; Bauer et al., 1966). A hundred microliter of each test microorganism suspension adjusted at  $10^8$  cfu/ml was spread on MHA/PDA. The liquid cultures obtained at the end of 16-18 hours were measured at spectrophotometer  $OD_{600}$  and the results were adjusted with medium to  $10^8$  cfu/ml. The previously sterilized discs (5 mm diameter, Whatman no 1) were placed on the same plates. A 10  $\mu$ l of hazelnut extract was absorbed into discs. A product sold commercially in Turkey was used as a positive control. It is obtained from hazelnut kernel by cold pressing method. The plates were incubated at 37 °C for 16-18 h and *Candida albicans* was incubated at 30 °C for 16-18 h. In the study, 10mg/ml Kanamycin was utilized as a positive control for all bacterial groups, while 10mg/ml Penicillin solutions were employed in *Candida albicans* studies. The inhibition zone diameters were measured (Sharma et al., 2014) (Figure 2).

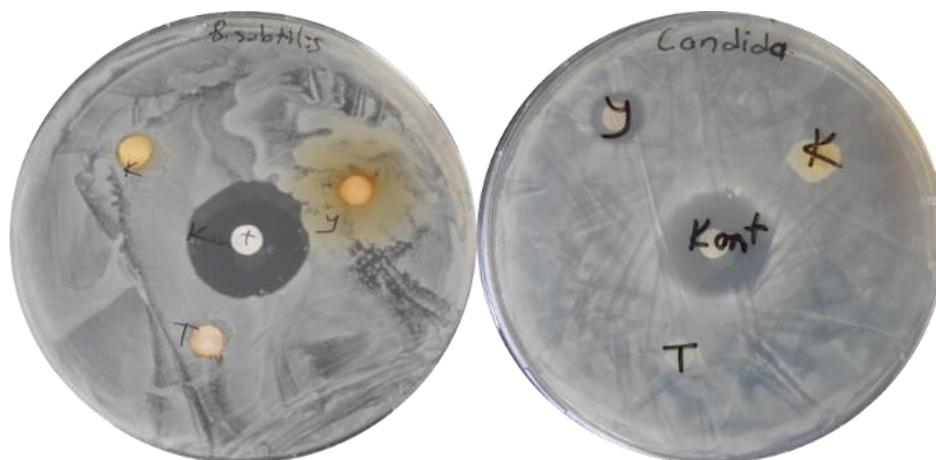


Figure 2. Determination of the inhibition effect of hazelnut extracts against some human patojen bacteria and fungal isolates by disc diffusion method (Y: fresh hazelnut extract, K: dried hazelnut extract, T: commercial product, Kont: control)

### Statistical analysis

One-way analysis of variance (ANOVA) was run to determine any significant differences in the study groups by Duncan multiple range test was performed through SPSS (Statistical Package for Social Sciences, version 28, Chicago, IL, USA) and the significance level was determined at  $p < 0.05$ .

### Results and Discussion

In the study, extract was obtained from both dried and fresh hazelnuts (Figure 1). In the antimicrobial trials of the obtained hazelnut extracts, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC2468, *Salmonella typhimurium* ATCC13311, *Serratia marcescens* ATCC13880, *Staphylococcus aureus* ATCC25923 and *Yersinia pestis* ATCC 19428 did not observe any antimicrobial effects ( $p > 0.05$ ).

Inhibition zones were observed *Enterococcus faecalis* ATCC51299, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* ATCC700603, *Staphylococcus epidermis* ATCC14990, *Escherichia coli* ATCC2471, *Streptococcus faecalis* ATCC 9790 and *Candida albicans* ( $p < 0.05$ ; Figure 2, Table 1). Oliveira et al. (2007) found that extractions from hazelnut leaves were particularly sensitive to gram-positive bacteria (MIC 0.1 mg/ml for *B. cereus* and *S. aureus* and 1 mg/ml for *B. subtilis*). In this study, it was determined that *B. subtilis* was sensitive especially in dried hazelnut extract. However, gram-negative bacteria and fungi showed much lower sensitivity because they were resistant to *P. aeruginosa* and *C. albicans*. In this study, both dried and fresh hazelnut extracts were found to be slightly effective on gram negative *Klebsiella pneumoniae* ATCC700603.

Table 1. Antimicrobial activity of hazelnuts- extracts against human pathogens bacteria (The indicated zones are given in mm. It shows measurements from outside the disc)

Bacteria	Dried Hazelnut extract	Fresh Hazelnut extract	Commercial Hazelnut extract	Control (Kanamycin)
<i>Escherichia coli</i> ATCC 2471	2.10±0.05 <sup>h</sup>	NZ	3.0±0.21 <sup>g</sup>	6.6±0.57 <sup>d</sup>
<i>Klebsiella pneumoniae</i> ATCC 700603	1.33±0.33 <sup>i</sup>	1.2±0.15 <sup>i</sup>	1.9±0.64 <sup>a</sup>	7.70±0.48 <sup>c</sup>
<i>Staphylococcus epidermis</i> ATCC 14990	2.46±0.88 <sup>gh</sup>	NZ	1.3±0.51 <sup>i</sup>	3.00±0.24 <sup>a</sup>
<i>Bacillus subtilis</i> ATCC 6633	2.00±0.57 <sup>h</sup>	NZ	2.2±0.61 <sup>gh</sup>	8.90±0.32 <sup>b</sup>
<i>Streptococcus faecalis</i> ATCC 9790	4.00±0.17 <sup>f</sup>	NZ	2.0±0.61 <sup>h</sup>	5.54±0.88 <sup>e</sup>
<i>Enterococcus faecalis</i> ATCC 51299	3.33±0.25 <sup>g</sup>	NZ	2.3±0.11 <sup>gh</sup>	7.20±0.53 <sup>c</sup>
<i>Candida albicans</i> ATCC10351	3.33±0.93 <sup>g</sup>	4.1±0.18 <sup>f</sup>	1.11±0.23 <sup>a</sup>	9.20±0.12 <sup>a</sup>

NZ: Non-Zone

Especially in these strains, dried hazelnut extract was effective in all of them, while fresh hazelnut extract was only effective on *Candida albicans* ATCC10351 and *Klebsiella pneumoniae* ATCC700603 (F (3, 8) = 65.45,  $p < 0.05$ ). The most effective zone of inhibition obtained from dried nut extract was determined on *Streptococcus faecalis* ATCC 9790 (Table 1; F (3, 8) = 15.07,  $p < 0.05$ ). It was determined to be above *Klebsiella pneumoniae* ATCC700603 in the least inhibition zone (F (3, 8) = 46.33,  $p < 0.05$ ).

According to the classification of Ramalhosa et al. (2011) hazelnut extracts have the following antimicrobial activity: inhibition zone <1mm – no antimicrobial activity, inhibition zone 2–3 mm – slight antimicrobial activity, inhibition zone 4–5mm – moderate antimicrobial activity, inhibition zone 6–9mm – high antimicrobial activity, and inhibition zone >9 mm – strong antimicrobial activity. With all this information, it was determined that dried and fresh hazelnut extract showed moderate antimicrobial activity especially in our study (Table 1).

Several plant oils have been shown to have antimicrobial properties in *in vitro* assays. Barta et al., stated in their study in 2020 that they could not see an effective antibacterial effect from American hazelnut oil and that the reason for this may be the microbial products in the extract they obtained. They found that only *Bacillus subtilis* and *Staphylococcus aureus* were effective among the 16 microorganisms they used. In this study, it was observed that dried and fresh hazelnut extracts showed effect against *Bacillus subtilis*. Different parts of the hazelnut show different antimicrobial activities. In the case of leaves, the samples showed antimicrobial activity against all microorganisms except *P. aeruginosa* and *C. albicans*. In the case of aqueous extracts of hazelnut kernels, high antimicrobial activity was found only against gram-positive bacteria, namely *B. cereus*, *B. subtilis* and *S. aureus*. On the contrary, gram-negative bacteria and fungi showed resistance to the tested extracts (Ramalhosa et al., 2011). In this study, it was observed that dried and fresh hazelnut extracts showed effect against *Bacillus subtilis* and *Candida albicans*.

In another study with hazelnut kernel, the effect of the compost obtained on *C. albicans*, *E. coli* and *S. aureus* was observed (Shataer et al., 2021). In this study, it was observed that dried and fresh hazelnut extracts showed effect against *Candida albicans*.

In addition to being used as a food source, hazelnut is also known to be used in the field of phytocosmetics (Athar and Nasir, 2005). It has astringent and antimicrobial effects due to the tannins it contains (Jiang et al., 2014; Michalak and Kieltyka-Dadasiewicz, 2019). It can be used in facial cleansing products due to its astringent and antimicrobial properties. Hazelnut oil is also found in skin care products as a moisturizer, regenerative and invigorating, due to its rich lipid content that ensures a long-lasting moisturizing effect (Athar and Nasir, 2005). In this study, it is shown that hazelnut extract has an antimicrobial effect.

When we look at the literature, an effect against *Candida albicans* is generally observed in antimicrobial studies made from different parts of hazelnut. This study also supports this information.

When the results of the study are analysed, it is seen that dried hazelnut extract is more effective than fresh hazelnut extract. The reason for this can be said to be the accumulation of more oil content in dried hazelnut (Özdemir et al., 1998). In the studies, the fat content in dried hazelnut was generally found to be higher (Özdemir et al., 1998). In studies conducted with fresh husk, more oil content was determined in fresh products (Barta et al., 2022). It is stated that the reason for this is the excess of phenolic compounds.

### Conclusion

The development of hazelnut and hazelnut oil production not only as a nutrient, but also in cosmetic products, and medicine, the development of new antibiotics for hospital infections, and as a supportive by-product in the healing of bed sores can create a new source of income for our country. After the necessary scientific research are carried out, hazelnut extract, which is produced under appropriate conditions, can turn into a market with high potential for related companies around the world and for Turkey in terms of cosmetic products and with the development of antibiotics.

### Conflict of Interest

There is no conflict of interest between authors of the article.

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### Author Contribution Statement

MU and SB: they contributed to get hazelnuts.

AS: he contributed to the extraction of hazelnut extract.

MU: She contributed to the design of the study, performing of laboratory studies and writing of the manuscript.

SB: She contributed to performing of laboratory studies, statistical analysis and writing of the manuscript.

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