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Antifungal Activity, Total Phenolic Content and Antioxidant Activity Properties of Some Spices Extracts as Alternative Natural Antimicrobial Agents

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ABSTRACT
In this study, extracts were obtained from rosemary, anise, cinnamon, ginger, peppermint, turmeric, fennel, clove, laurel leaves and thyme. The total phenolic content amount, antioxidant activity value and antifungal properties of these extracts were aimed to determine the extracts. Among the extracts, clove, cinnamon, turmeric and ginger were superior in terms of total phenolic content values, clove, cinnamon, turmeric, ginger, laurel leaves and rosemary extracts were superior in terms of antioxidant activity. The highest inhibition zone diameters among mold strains were determined by the use of extracts of cinnamon, turmeric, ginger, clove and laurel leaves against *Aspergillus oryzae*, *Penicillium digitatum* and *Aspergillus niger* strains. The results suggested the potential use of cinnamon and clove extracts as natural agents.

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Alternatif Doğal Antimikrobiyal Ajan Olarak Bazı Tıbbi Bitki Ekstraktlarının Antifungal Aktivitesi, Toplam Fenolik Madde İçeriği ve Antioksidan Aktivite Özellikleri

ÖZET
Bu çalışmada biberiye, anason, tarçın, zencefil, nane, zerdeçal, rezene, karanfil, defne yaprağı ve kekik' ten ekstrakt elde edilmiştir. Bu ekstraktların toplam fenolik madde miktarı, antioksidan aktivite ve antifungal özelliklerinin belirlenmesi amaçlanmıştır. Ekstraktlardan karanfil, tarçın, zerdeçal ve zencefil'in toplam fenolik madde miktarı daha yüksek iken antioksidan aktivite değerleri açısından karanfil, tarçın, zerdeçal, zencefil, defne yaprağı ve biberiye ekstraktları daha üstün olmuştur. Küf suşlarına karşı en yüksek inhibisyon zon çapları, tarçın, zerdeçal, zencefil, karanfil ve defne yaprağı ekstraktlarının *Aspergillus oryzae*, *Penicillium digitatum* ve *Aspergillus niger* suşlarına karşı belirlenmiştir. Tüm sonuçlar değerlendirildiğinde, tarçın ve karanfil ekstraktlarının doğal koruyucu ajan olarak potansiyel kullanımının yüksek olduğu tespit edilmiştir.

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INTRODUCTION

Antioxidant and antimicrobial substances have essential effects in preventing spoilage and increasing food's shelf life, quality, and safety in the food industry. Synthetic agents have toxicity, high costs, and less effect than natural agents (Ansari et al., 2013). Also, synthetic agents cause different negative health effects such as diabetes, allergenic reactions, asthma, hyperkinesis, cancer and cardiovascular diseases (Tewari et al., 2020).

Natural preservatives can be obtained from different sources as spices, animals and microbial (Ribes et al., 2018). Spice sources have important antifungal effects thanks to secondary metabolite components such as phenolic compounds, essential oils, flavonoids and alkaloids (Ciocan & Bara, 2007). Bioactive compounds having antimicrobial effects derived from spices are eugenol in cloves, thymol in thyme, carvacrol in oregano, vanillin in vanilla, allicin in garlic, cinnamic aldehyde in cinnamon and allyl isothiocyanate in mustard (Lopez-Malo et al., 2005).

The dominant active ingredient of anise is anethole;

eugenol and (E)-cinnamyl acetate of cinnamon; curcumin of curcuma; eugenol of ginger; transanethol of fennel; eugenol of clove and laurel leaves; bornyl acetate of rosemary; catechin of mint and carvacrol of thymeThese spices can be used as anticancer, antidepressant, antiviral, nematocidal, mutagenic, antispasmodic, antifungal, antibacterial and anti-inflammatory agents thanks to this bioactive component (Luigia & Giuseppe, 2005; Salehi Surmaghi, 2006; Fecka & Turek, 2007; Al-Bayati, 2008; Shojaii & Abdolahi Fard, 2011; Ribeiro-Santos et al., 2015; Koldaş et al., 2015; Kumar et al., 2011; Kumar et al., 2019; El-Saber Batiha et al., 2020). The spice extraction process ensures a great advantage that the removal of unwanted components as well as the preservation of bioactive components. For this reason, the evaluation of spice sources in extract form is safer, more effective and easier than in whole spice form (Karakaş, 2003).

Spice extracts are the new alternative to protect against the harmful effects of synthetic antioxidants and chemical preservatives and to prevent spoilage in the food industry. The total phenolic content of spice extracts is directly proportional to antioxidant activity and antifungal activity capacity. In addition to their antifungal activities, bioactive compounds including phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons increase the storage stability of food products thanks to their antimicrobial and antioxidant properties (Coşkun, 2021).

The antifungal activity of bioactive components occurs through two different mechanisms of action. One of these is that the components cause retraction of the mycelium cytoplasm with an attack on the cell wall, eventually causing cell membrane disruption, resulting in the death of the hyphae. Another mechanism is the death of the microorganism as a result of the intervention of bioactive components in the enzymatic reactions of cell wall synthesis, which affect the morphogenesis and growth of bacteria or molds (Carmo et al., 2008).

Considering the above-mentioned negative aspects of chemical preservatives, natural preservatives will have a great place in the food industry. For this reason, this study investigated the total phenolic content, antioxidant activity and antifungal activity (inhibition zone diameter, minimum inhibitory concentration and minimum fungicidal concentration) properties of spice extracts as natural preservatives and aimed to determine the spice extracts with the best properties that can be an alternative to chemical preservative food additives.

MATERIAL and METHOD

Materials

Rosemary, anise, cinnamon, ginger, peppermint, turmeric, fennel, clove, laurel leaves and thyme used in the study were obtained from a local market in Konya, Turkey. *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium digitatum* and *Penicillium camemberti* were obtained from Tokat Gaziosmanpaşa University Plant Protection Department and Yıldız Technical University Food Engineering Department.

Methods

Preparation of ethanolic extracts

Spice extract production was carried out by modifying the method of Grigoras et al. (2013). The raw materials were ground into powder in a laboratory grinder (Sinbo SCM2934, Turkey). Firstly, 100 g of sample with 600 mL solvent were mixed and subjected to agitation in a shaking water bath (Daihan Wisebath WSB-30, Gangwon, South Korea) for 3 h at 170 rpm at 25±1°C for ethanolic extraction and was filtered with whatman filter paper no: 1. Then, agitated with 400 mL of ethanol for 6 h and kept at room temperature for 12 h and the filtration process was repeated at the end of the period. Evaporation was carried out using a rotary evaporator (Büchi R20, Switzerland) at 40°C. Extracts were stored at 4°C until analysis.

Total phenolic content

The total phenolic content was performed colorimetrically as a method described by Maurya and Singh (2010) with slight modifications. For analysis, extracts of rosemary, anise, cinnamon, ginger, mint, turmeric, fennel, laurel and thyme were diluted 4000-fold and clove extract was diluted 7000-fold with pure ethanol. Briefly, 500 µL of extract samples are added 2.5 mL Folin -Ciolcalteu reagent (1/10, v/v in water) and 2 mL of sodium carbonate solution (7.5%, w/v, in water). The mixture was incubated for 60 min at room temperature (25±1°C) in darkness. After incubation, the absorbance was read against pure ethanol (≥ 99.5 %) at 760 nm with a UV-vis spectrophotometer (Hitachi-U1800, Japan). The total phenolic content value of each extract was expressed as milligram gallic acid equivalents per gram of extract (mg EAG g^{-1} extract).

Antioxidant activity

The antioxidant activity analyses of extract samples were estimated according to the DPPH (2-2-Diphenyl-2-picrylhydrazyl) methods described by Ahmad et al. (2013). The 4000-fold diluted extract samples (1 mL) were mixed with solution of DPPH (2 mL) and kept for 30 mins at room temperature (25±1°C). After incubation, The absorbance value was measured against pure ethanol (≥ 99.5 %) at 517 nm with a UV-vis spectrophotometer (Hitachi-U1800, Japan). The percentages inhibition of the DPPH radical were

calculated using the following Eq. (1).

% İnhibition=[(Abscontrol - Abssample)/Abscontrol] × 100(1)

Antifungal assay

Fungal cultures

Aspergillus flavus, *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium digitatum* and *Penicillium camemberti* cultures were used as test micro-organisms. Molds were subcultured on Potato Dextrose Agar (PDA) plates and incubated at 26.5°C for 7 days. Then, the spores were suspended by adding 10 mL of 0.01% Tween 80 (Sawai and Yoshikawa, 2004). The concentration of spores was adjusted to equal the 0.5 McFarland standard with absorbance 0.400-0.450 (1-5 × 106 CFU mL^{-1}) at 400 nm wavelength in a UV-spectrophotometer (Hitachi-U1800, Japan) (Kızılkeçili, 2007).

Disc diffusion methods

The antifungal activity was analyzed with agar disc diffusion method. Firstly, 0.1 mL of mold suspension adjusted according to 0.5 McFarland standard were inoculated over agar with a sterile pipette (Research Plus, Eppendorf, Germany) and spread uniformly using a glass spreader. Firstly, stock ethanol solutions of concentration 100 and 200 mg mL^{-1} of extract samples were prepared from each extract. Then, on the surface of plate were placed 4 discs, and 20 µL from 100 mg mL^{-1} extract samples, 200 mg mL-1 extract samples, negative controls (ethanol) and positive controls (2 mg mL^{-1} calcium propionate) were impregnated on the disk. The plates were incubated at 25°C for 72 h. After incubation, observations were recorded as the diameter of growth inhibition around the discs and were expressed in millimeters.

Minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC)

The MIC values of the extract samples were determined by the agar dilution method. Stock solutions of dissolved extract in ethanol were added to sterile melted PDA at 50°C to give a final concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 mg mL^{-1} with PDA at 50°C. The resultant dilutions were poured into petri plate in the amount of 12-15 mL and waited for 10 min. Then, 0.1 mL mold suspension prepared separately for *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium digitatum* and *Penicillium camemberti* was transferred in petri dishes and was spread homogeneously on the petri plate with a glass spreader. Finally, the petri dishes were incubated at 25°C for 48-72 h and the lowest concentration value at which no growth was determined as the MIC value (mg mL^{-1}).

Minimum fungicidal concentration was defined as the lowest concentration with no visible growth indicating 99.9% killing of the original inoculum. For MFC, the samples in the petri plate with no growth were transferred onto fresh PDA medium by streaking with a loop. Then, these plates were incubated at 25°C for 48-72 h, and the lowest concentrations without visible growth were recorded as MFCs.

Statistical analysis

For the statistical analysis, the JMP statistical program, version 5.01 (SAS Institute Inc., Cary, NC, USA) was used. The average values of the main variation sources were compared at $p < 0.05$ significance levels.

RESULTS and DISCUSSION

When the spice extraction in the literature is examined; water, ethanol and methanol are used as solvents in the extraction process and stated that the extract yield is higher with the ethanolic extraction process and antimicrobial properties than other solvents. Most importantly, ethanol is preferred among other chemical solvents as methanol due to its low toxicity (Ballesteros et al., 2014). Considering these data, ethanol was preferred as a solvent in the extraction process of spices used in the study.

Total phenolic content

The total phenolic content values of the extract samples obtained from spice sources are given in Table 1. The total phenolic content values of extract samples varied between 62.22 mg GAE g^{-1} and 461.38 mg GAE g^{-1}. When the results were examined, the highest total phenolic content value was determined with clove (461.38 mg GAE g^{-1}). Clove was followed by cinnamon (172.53 mg GAE g^{-1}), turmeric (173.53 mg GAE g^{-1}) and ginger (181.75 mg GAE g^{-1}), which gave statistically similar values. In a study investigating the amount and quality of the curcumin component in turmeric ethanolic extract, Himesh et al. (2011) reported that turmeric contains many phytochemical components, including curcumin, demetoxy curcumin, bisdemethoxycurcumin, zingiberene, curcum-enol, curcumol, eugenol, tetrahydrocurcumin, turmerine, turmerone and turmerin-onols, and besides, curcumin is found hydrophobic in nature and freely soluble in dimethylsulfoxide, acetone, ethanol and oils. Therefore, they applied ethanolic extraction to obtain a high percentage of curcumin component, which constitutes an important proportion of turmeric extract components. The lowest total phenolic content amount among the extract samples was obtained from anise with 62.22 mg GAE g^{-1}. The solubility of phenolic compounds is affected by solvent type, the polymerization degree of phenolics, phenolics-other ingredients interaction and insoluble complex formation (Falleh et al., 2008). Considering all these

different values, the total phenolic content values can effect significantly with different intrinsic and extrinsic factors, including the composition and amount of phenolics, spice genetics and varieties, soil and growing conditions, maturity and harvest conditions (Jeffery, 2003). The difference in total phenolic content amount may be due to the various efficiencies of extraction solvents to dissolve endogenous compounds (Anwar et al., 2009). Chirinos et al. (2007) and Chandra et al. (2014) reported that various extraction factors such as extraction time, sample, solvent ratio, temperature, pH, solvent type and extraction method, as well as crop growing factors such as soil, irrigation and climatic conditions, were effective on total phenolic content.

Table 1. Total phenolic content and antioxidant activity values of extract samples[1]
Çizelge 1 Ekstrakt örneklerine ait toplam fenolik madde ve antioksidan aktivite sonuçları[1]

Extract type *Ekstrakt Türü*	**Total Phenolic Content** *Toplam Fenolik Madde Miktarı* **(mg GAE g^{-1})**	**Antioxidant Activity** *Antioksidan Aktivite* **(%)**
Anise	62.22±1.10^{f}	48.70±1.62^{f}
Cinnamon	172.53±2.43^{b}	90.37±0.22^{ab}
Turmeric	173.53±4.64^{b}	89.18±0.15^{ab}
Ginger	181.75±10.61^{b}	87.10±0.29^{bc}
Fennel	82.45±1.68^{e}	39.44±0.15^{g}
Clove	461.38±3.04^{a}	91.83±0.07^{a}
Laurel leaves	124.78±0.59^{c}	89.13±0.52^{ab}
Rosemary	94.72±1.55^{d}	84.65±0.81^{c}
Peppermint	91.43±1.32^{de}	74.04±1.84^{d}
Thyme	77.20±2.46^{ef}	64.78±0.37^{e}

[1] Means followed by the different letters within a column are significantly different.

Antioxidant activity

The antioxidant activity values of the extract samples are given in Table 2. The antioxidant activity values of the extract samples were found to be between 39.44% and 91.83%. The highest antioxidant activity was found in clove extract with 91.83%, followed by cinnamon (90.37%), turmeric (89.18%), laurel leaves (89.13%), ginger (87.10%), rosemary (84.65%), peppermint (74.04%), thyme (64.78%), anise (48.70%) and fennel (39.44%) extracts. The relationship between antioxidant activity and total phenolic content may depend on many factors. Antioxidant activity potential has affected both synergy and structures of phenolic substances. Because the antioxidant activity level of the extract is not only dependent on the concentration of phenolic compounds but also on the structure and interaction between these compounds. This situation can explain the difference in antioxidant activities in similar total phenolic component concentrations in the sample (Piluzza & Bullitta, 2011). Contrary to all these, the total phenolic content value shows a generally compatible change with antioxidant activity of extracts. Considering the results obtained in our study, the high antioxidant activity values were determined in the extracts with high total phenolic content amount. As stated by Amer and Aly (2019), many factors lead to differences in antioxidant activity of extract despite using the same solvent. Because raw material chemical nature, particle size, storage time, the extraction conditions and interfering substances presence can significantly affect the activity of solvent (Naczk & Shahidi, 2004). A polar solvent such as ethanol can reveal more polar components together with non-polar components, and therefore antioxidant activity is obtained higher than other solvents (Liu et al., 2007). According to Silva et al. (2007), the antioxidant activity value of extracts can be increased with usage of suitable solvents by further recovery of phenolic compounds. Also, Ghasemzadeh et al. (2011) concluded that the extracts with high polarity solvents (methanol) have more effective radical scavengers compared to less polar solvents (acetone and chloroform), and this could be explained by the antioxidants found in ginger varieties or active compounds with different polarities. Chun et al. (2005) stated that the hydrophilic and hydrophobic ratio of phenolic has an important function in different antioxidant activity values of the extract samples.

Antifungal Activity

Inhibition zone diameter

The antifungal activity of anise, cinnamon, turmeric, ginger, fennel, clove, laurel leaves, rosemary, peppermint and thyme against *Aspergillus oryzae*, *Penicillium digitatum*, *Aspergillus flavus*, *Penicillum camemberti* and *Aspergillus niger* strains were determined with inhibition zone diameter using disc diffusion methods and values shown in Table 2. Phenolic compounds in extracts lead to damage to the cell walls, causing cell deformation, increasing cellular permeability and causing cellular contents to flow out and so, cell death occurs with the release of cellular contents out of the cell (Sharayei et al., 2020). Extract

samples were tested at 100 and 200 mg mL^{-1} concentrations. The negative and positive control group used ethanol and 2 mg mL^{-1} calcium propionate, respectively. The extract samples represented relatively high antifungal activity against the *Aspergillus oryzae* at 100 mg mL^{-1} and 200 mg mL^{-1} with percentage of mycelium growth inhibition varied between 14.7-39.2 mm and 15.90-55.20 mm. The highest inhibition zone diameter values against *Aspergillus oryzae* were obtained with cinnamon (39.2 mm) and clove (36.7 mm) extracts at 100 mg mL^{-1} concentration ($p<0.05$). This is followed by anise (21.4 mm), peppermint (18.8 mm), turmeric (18.0 mm), laurel leaves (16.7 mm), rosemary (16.6 mm), ginger (16.4 mm), fennel (15.4 mm) and thyme (14.7 mm). The high antifungal activity of cinnamon is associated with the presence of many bioactive components such as cinnamaldehyde, eugenol and cinnamic acid (Gill & Holly, 2004). When the antifungal activities of the extracts at 200 mg mL^{-1} concentration against *Aspergillus oryzae* were evaluated, anise, cinnamon, turmeric, ginger, fennel, clove, laurel leaves, rosemary, peppermint and thyme was determined an increase as 6.0, 16.0, 6.0, 3.6, 4.5, 14.1, 5.9, 5.6 and 1.2 units respectively, compared with the zone diameters at 100 mg mL^{-1} concentration.

All extract samples showed inhibition zone diameter against *Penicillium digitatum* as seen in Table 2 and the ethanolic extract of each of the ten spices formed a larger inhibition zone diameter in the discs compared to 2 mg mL^{-1} calcium propionate used as the positive control group (7.6 mm). The spice extract samples demonstrated more effective antifungal effect than calcium propionate used as an antimicrobial agent. The inhibition zone diameter values against *Penicillium digitatum* increased from 13.0-42.1 mm (100 mg mL^{-1}) to 16.8-57.4 mm (200 mg mL^{-1}) with increased concentration. The highest antifungal effect against *Penicillium digitatum* found with cinnamon extract with 42.1 mm in 100 mg mL^{-1} discs and 57.4 mm in 200 mg mL^{-1} discs, following clove extract with an inhibition zone of 41.0 mm in 100 mg mL^{-1} discs and 55.2 mm in 200 mg mL^{-1} discs. The high antifungal activity of cinnamon extract against *Penicillium digitatum* may be caused by cinnamaldehyde, an important bioactive component in cinnamon bark, and some studies have shown that cinnamaldehyde kills 80% of mold and bacteria (McCann, 2003). In addition, the presence of flavonoids, alkaloids, tannins, saponins, terpenes, steroids and essential oil in cinnamon extract may be responsible collectively or individually for the antifungal activity (Mahmoud, 2012). Fennel extract at 100 mg mL^{-1} showed the lowest antifungal activity against *Penicillium digitatum* with a 13.0 mm inhibition zone diameter value. The highest antifungal activity against *Aspergillus flavus* was recorded with cinnamon extract (44.2 mm) in disc containing 100 mg ml^{-1}. As seen in Table 2, increasing concentration has increased the inhibition zone diameters against *Aspergillus flavus*.

The inhibition zone diameter of 200 mg mL^{-1} extracts against *Aspergillus flavus* showed the highest effect with cinnamon and clove extracts at 50.2 mm and 44.5 mm. Inhibition zone diameters of the extract samples on *Penicillum camemberti* strain varied between 12.4-42.1 mm at 100 mg mL^{-1} concentration and between 16.5-54.7 mm at 200 mg mL^{-1} concentration. The highest inhibition zone diameter (54.7 and 51.7 mm) were observed by the concentration of 200 mg mL^{-1} of the cinnamon and clove extract samples and other extract samples were demonstrated similar values. The inhibition zone diameter values of anise, cinnamon, turmeric, ginger, fennel, clove, laurel leaves, rosemary, peppermint and thyme extracts increased at 4.1, 12.1, 2.9, 3.9, 3.7, 12.6, 2.7, 1.2, 2.2 and 2.4 units, respectively with the increase of the extract concentration from 100 mg mL^{-1} to 200 mg mL^{-1}. This high increase in cinnamon and clove extracts may be due to the high total phenolic content.

When the antifungal activity of the extract samples against *Aspergillus niger* was examined, the inhibition zone diameter of extracts prepared at 100 mg mL^{-1} concentration were determined 10.1-40.6 mm while 13.5-45.5 mm at 200 mg mL^{-1} concentration. The highest inhibition zone diameter at both 100 mg mL^{-1} and 200 mg mL^{-1} concentrations was found with the use of cinnamon, followed by clove, turmeric, laurel leaves and thyme were found to have statistically similar effects. The highest increase in the inhibition zone diameter with usage of high concentration was obtained with clove extract (9.7 units). Antifungal activity mechanisms of extracts are associated with low water-soluble properties and also with the easy incorporation of high hydrophobicity compounds into the plasma membranes and membranes of intracellular organelles (especially mitochondria) (Jing et al., 2014). These compounds can change the lipid membrane composition, such as lowering the levels of ergosterol, which is the main component of the cell membrane (Kedia et al., 2015). The decrease or absence of ergosterol can lead to maintaining cell function and integrity, membrane binding enzyme activity, cell viability and cellular transport systems, changes in cell permeability, disruption of cell organelles and cell death (Kiran et al., 2016). According to El Khoury et al. (2017), the antimicrobial activity mechanism of extracts occurs in three different ways. The first of these, enzymes responsible for intracellular functions change with the presence of -OH groups and form hydrogen bonds. The second of these explain by the loss of rigidity and integrity of the hyphae cell wall due to the interaction of these compounds with the membrane enzymes of the mold strains, while the third mechanism is expressed by

rupture of the cytoplasmic membrane, changes in the permeability of the cell membranes and granulation of the cytoplasm. Also, da Cruz Cabral et al. (2013) stated that some hydrophobic compounds in the extracts may cause to cross the cell membrane and change permeability of cations such as H+ - K+, thus changing the flow of protons and the pH of the cells, affecting chemical composition and activities. In addition to, the high phenolic compound levels can cause macromolecules loss from cell by changing of the mold cell permeability, and the deformation of structure and functionality by interacting with the membrane proteins (Fung et al., 1977).

In a study investigated compounds against *Aspergillus flavus* and *Aspergillus niger*, Kim et al. (2006) associated with targeting the mitochondrial oxidative stress defense system of compounds as action mechanism on growth inhibition of mold strain. Similar to our study results, Gupta et al. (2008) stated that the high antimicrobial activity of cinnamon may be due to cytoplasmic granulation, cytoplasmic membrane rupture and inactivation or inhibition of intracellular enzymes. Also, cinnamon have rich highly electro-negative cinnamaldehyde content (50.5%), and these electro-negative compounds affect biological processes containing electron transfer and also can be inhibit the growth of microorganisms as a result of the reaction with nitrogen-containing components (proteins and nucleic acids). In literature has been suggested that exposure of microorganisms to antifungal components may cause disruption of membrane integrity and function, which may slowly lead to loss of cell homeostasis, leakage of intracellular components, and ultimately cell death (Hammer & Carson, 2011).

Table 2. Inhibition zone diameter values against different molds of extract samples (mm)[1]
Çizelge 2 Ekstrakt örneklerinin farklı küf suşlarına karşı inhibisyon zon çapı değerleri (mm)[1]

Extract type *Ekstrakt Türü*	*Aspergillus oryzae*		*Penicillium digitatum*		*Aspergillus flavus*		*Penicillum camemberti*		*Aspergillus niger*	
	100 mg mL^{-1}	200 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}
Anise	21.4±0.57^{b}	27.4±1.27^{b}	15.9±0.71^{cd}	18.3±0.42^{de}	18.6±1.56^{c}	20.8±1.56^{b}	12.4±1.27^{c}	16.5±0.71^{b}	10.1±0.14^{ef}	16.2±0.71^{de}
Cinnamon	39.2±1.13^{a}	55.2±1.41^{a}	42.1±1.13^{a}	57.4±0.85^{a}	44.2±1.84^{a}	50.2±1.84^{a}	39.6±1.70^{a}	51.7±2.69^{a}	40.6±1.70^{a}	45.5±1.27^{a}
Turmeric	18.0±0.57^{bc}	24.0±0.66^{bc}	18.1±0.99^{bc}	20.6±0.57^{cd}	17.3±1.56^{c}	21.1±0.99^{b}	17.8±1.70^{b}	20.7±2.12^{b}	15.0±0.99^{cd}	21.3±0.57^{c}
Ginger	16.4±0.71^{c}	20.0±0.85^{cd}	18.4±0.57^{bc}	22.0±0.71^{bc}	15.3±0.42^{cd}	17.8±0.85^{bc}	16.9±1.56^{bc}	20.8±1.70^{b}	12.9±0.57^{de}	15.3±1.41^{de}
Fennel	15.4±0.57^{c}	19.9±0.85^{cd}	13.0±0.42^{d}	17.1±0.42^{e}	12.0±0.28^{d}	14.3±0.99^{c}	13.0±0.57^{bc}	16.7±1.84^{b}	10.6±0.57^{e}	13.5±0.99^{e}
Clove	36.7±2.26^{a}	50.80±2.40^{a}	41.0±0.57^{a}	55.2±0.85^{a}	37.7±2.26^{b}	44.5±1.98^{a}	42.1±1.13^{a}	54.7±2.12^{a}	27.1±0.42^{b}	36.8±1.56^{b}
Laurel leaves	16.7±0.28^{c}	22.6±1.70^{bc}	20.7±0.57^{b}	23.5±0.57^{b}	16.4±1.41^{cd}	22.9±1.56^{b}	16.4±0.71^{bc}	19.1±0.99^{b}	18.4±0.71^{c}	21.6±1.84^{c}
Rosemary	16.6±1.84^{c}	22.7±2.40^{bc}	14.3±0.71^{d}	16.8±0.14^{e}	16.0±0.42^{cd}	17.7±1.98^{bc}	15.6±1.84^{bc}	16.8±1.56^{b}	10.3±1.27^{e}	16.9±1.56^{cde}
Peppermint	18.8±1.41^{bc}	24.4±1.13^{bc}	19.0±0.85^{b}	21.8±0.71^{bc}	17.0±0.99^{cd}	18.6±1.13^{bc}	16.4±1.70^{bc}	18.6±1.84^{b}	10.4±1.56^{e}	16.4±0.57^{de}
Thyme	14.7±0.71^{c}	15.9±0.71^{d}	15.7±0.57^{cd}	18.3±0.57^{de}	14.8±0.42^{cd}	17.7±1.98^{bc}	16.0±0.28^{bc}	18.4±1.56^{b}	17.4±1.41^{c}	19.8±1.27^{cd}
Negative control[2]	-		-		-		-		-	
Positive control[3]	6.8±0.80		7.6±0.00		6.4±0.40		-		6.0±0.00	

[1]Means followed by the different letters within a column are significantly different. [2]Negative control: Ethanol, [3]Positive control: 2 mg mL^{-1} calcium propionate.

Minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC)

MIC and MFC were used to evaluate the fungicidal potential of cinnamon, turmeric, ginger, clove and laurel leaves extract against *Aspergillus oryzae*, *Penicillum camemberti*, *Penicillium digitatum*, *Aspergillus niger* and *Aspergillus flavus*. The results obtained were summarized in Table 3. The lowest MIC value on *Aspergillus oryzae* strain was obtained at 0.312 mg mL^{-1} with cinnamon and clove extracts, in contrast, turmeric extract was found to have the weakest antifungal activity with high MIC value. Similarly, the lowest MIC value against *Penicillum camemberti* strain was obtained with cinnamon and clove extracts, while the MIC value of turmeric and ginger extracts was found to be higher than 20 mg mL^{-1}. On the other hand, the MIC values for *Penicillium digitatum* strain was determined as 0.312 mg mL^{-1} for cinnamon, 0.625 mg mL^{-1} for clove, 5 mg mL^{-1} for laurel leaves, 10 mg mL^{-1} for turmeric and >20 mg mL^{-1} for ginger, respectively, and so *Penicillium digitatum* strain was observed to be more resistant to ginger extract. This result was consistent with the investigation conducted by Vieira et al. (2022), wherein evaluated the antifungal effects of green tea, rosemary, cinnamon, anise, clove, curcumin and lemon balm extracts against *Aspergillus* spp. and *Penicillium* spp.

As demonstrated by the study, extracts demonstrated antifungal activity against fungi species activity with MIC values in the range of 0.55-2.18 mg mL^{-1}. The obtained results agree with El-Fallal et al. (2019) who found that clove and cinnamon were recommended as the best anti-fungal spices that exhibited antifungal activity with a minimal concentration of 0.05 g L^{-1}. As seen in the literature, the use of spices in extract form can show an inhibition effect against fungus even at very low concentrations. According to Table 3, the MIC values of all extract samples except for turmeric extract against *Aspergillus niger* strain were obtained below 20 mg mL^{-1} concentration. Turmeric (>20 mg mL^{-1}) was found as the most resistant extract of *Aspergillus niger* strain, following ginger with 10 mg mL^{-1}, laurel leaves with 5 mg mL^{-1}, clove with 0.625 mg mL^{-1} and cinnamon with 0.078 mg mL^{-1}. The MIC values of turmeric and ginger extract samples against the *Aspergillus flavus* strain were obtained as >20 mg mL^{-1}, and hence low antifungal activity on mold strain. The lowest MIC value on *Aspergillus flavus* was determined with the use of cinnamon extract with 0.312 mg mL^{-1}. As a result, the highest inhibition properties were provided on *Aspergillus niger* for cinnamon (0.078 mg mL^{-1}), on *Penicillium digitatum* for turmeric (10 mg mL^{-1}), on *Aspergillus oryzae* for ginger (5 mg mL^{-1}), on *Aspergillus oryzae* and *Penicillum camemberti* for clove (0.312 mg mL^{-1}) and *Penicillium digitatum*, *Aspergillus niger* and *Aspergillus flavus* (5 mg mL^{-1}) for laurel leaves.

Table 3. Minimum inhibition concentration values of extract samples (mg mL^{-1})[1]
Çizelge 3 Ekstrakt örneklerine ait minimum inhibisyon konsantrasyon sonuçları (mg mL^{-1})[1]

Extract type *Ekstrakt Türü*	***Aspergillus oryzae***	***Penicillum camemberti***	***Penicillium digitatum***	***Aspergillus niger***	***Aspergillus flavus***
Cinnamon	0.312	0.312	0.312	0.078	0.312
Turmeric	>20	>20	10	>20	>20
Ginger	5	>20	>20	10	>20
Clove	0.312	0.312	0.625	0.625	0.625
Laurel leaves	10	20	5	5	5

[1]Means followed by the different letters within a column are significantly different.

The MFC values of extract samples were given in Table 4. Extract samples demonstrated fungicidal effect in response to *Aspergillus oryzae*, *Penicillum camemberti*, *Penicillium digitatum*, *Aspergillus niger* and *Aspergillus flavus* with concentration between 0.312 mg mL^{-1} - >20 mg mL^{-1}. The cinnamon extract exhibited stronger fungicidal activity against *Aspergillus oryzae* (0.625 mg mL^{-1}), *Penicillum camemberti* (0.312 mg mL^{-1}), *Penicillium digitatum* (0.625 mg mL^{-1}), *Aspergillus niger* (0.312 mg mL^{-1}) and *Aspergillus flavus* (0.625 mg mL^{-1}). Birhanu et al. (2014) reported that *Cinnamomum zeylanicum* extract has potential to inhibit microorganism growth at a very low concentration compared to other extract samples and stated that the MIC values against *Aspergillus* sp. of *Cinnamomum zeylanicum* extract specified as 30%, while as 20% againts *Penicillium* sp. The second greatest effect against mold strains was obtained with clove extracts. The effect can be attributed to the eugenol component in clove. Eugenol-induced inhibition may be due to caused by the permeability of cell membranes (Li et al., 2021), disruption of the cytoplasmic membrane, impaired proton motive force, increased permeability of the phospholipid bilayer (Zhang et al., 2019), electron flow, active transport, and coagulation of cell contents (Davidson et al., 2012). The antifungal activity presented by clove extract may be attributed to the demonstration of components in great amounts such as eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone (Chaieb et al., 2007), acetyl-eugenol, alpha-humulene, methyl salicylate, iso-eugenol, methyl eugenol (Yang et al., 2003). Turmeric extract showed a fungicidal effect with 20 mg mL^{-1} on only the *Penicillium digitatum* and *Aspergillus flavus* strains. On the other hand, the MFC value of ginger and laurel leaves extract was found to be greater than 20 mg mL^{-1} for all mold strains. According to the MIC and MFC values of cinnamon extract, *Aspergillus oryzae*, *Penicillum camemberti*, *Penicillium digitatum*, *Aspergillus niger* and *Aspergillus flavus* can be shown as the first extract having high sensitivity. For this reason, cinnamon is the most successful extract in preventing mold development. This high antifungal activity of *Cinnamomum* extract can be associated with cinnamaldehyde, eugenol, cinnamic acid and organic acids (Birhanu et al., 2014). *Aspergillus* and *Penicillium* species demonstrated the highest resistance against ginger and laurel leaves extract. The extract samples used in our study were not equally effective against all mold strains may be related to having different phenolic compositions and amounts of extracts. To Moreno et al. (2006), the antimicrobial effect of various phenolic complexes is associated with the inactivation of different cellular enzymes based on the penetration rate of substances into the cell and changes in membrane permeability, and this change in cell membrane permeability has been cited as the main factor in the antimicrobial effect of a particular compound. Also, phenolic compounds can completely disrupt cell membranes, affect cell integrity and cause eventual cell death.

Table 4. Minimum fungicidal concentration values of extract samples (mg mL^{-1})[1]
Çizelge 4 Ekstrakt örneklerine ait minimum fungisidal konsantrasyon sonuçları (mg mL^{-1})[1]

Extract type *Ekstrakt Türü*	***Aspergillus oryzae***	***Penicillum camemberti***	***Penicillium digitatum***	***Aspergillus niger***	***Aspergillus flavus***
Cinnamon	0.625	0.312	0.625	0.312	0.625
Turmeric	>20	>20	20	>20	20
Ginger	>20	>20	>20	>20	>20
Clove	5	10	10	2.5	5
Laurel leaves	>20	>20	>20	>20	>20

[1]Means followed by the different letters within a column are significantly different.

CONCLUSION

This study contributes to the current knowledge of the antifungal activity of different spice extracts. Rosemary, anise, cinnamon, ginger, peppermint, turmeric, fennel, clove, laurel leaves and thyme extracts were evaluated in terms of the total phenolic content amount, antioxidant activity value and antifungal properties. The five extract samples with high inhibition zone diameters against mold strains were selected and MIC and MFC values were determined. Clove extract had the highest total phenolic content amount compared to others. The current findings showed that ten selected spice extract samples had promising antifungal activity against tested all mold strains. According to results, the highest inhibition zone diameter was obtained with anise, turmeric, fennel, rosemary and peppermint extracts against *Aspergillus oryzae*; with cinnamon, ginger, clove and laurel leaves extracts against *Penicillium digitatum* and with thyme against *Aspergillus niger*. The MIC and MFC analysis carried out in this study revealed that lower concentration of cinnamon and clove were more effective against *Aspergillus* and *Pencillium* spp. according to turmeric, ginger and laurel leaves. As a result, cinnamon and clove extracts can be recommended as functional ingredients to improve cereal products without adverse effects on product quality.

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Author contribution

Mine Aslan: Investigation, Resources, Formal analysis, Writing – original draft. Nilgün Ertaş: Methodology, Project administration, Funding acquisition, Conceptualization, Supervision, Writing-review & editing. M. Kürşat Demir: Project administration, Supervision, Writing-review & editing.

Declaration of competing interest

The authors declare that they do not have any conflict of interest.

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