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ANTIBACTERIAL ACTIVITY OF CINNAMON (*CINNAMOMUM CASSIA*) EXTRACTS ON SOME *LACTOBACILLUS* SPECIES

Ayla ÜNVER ALÇAY[1,2*], Aysun SAĞLAM[3], Nagihan KALINTAŞ ÇAĞLAR[4], Sibel KAHRAMAN[5], Kamil BOSTAN[4]

[1]Istanbul Aydin University, Anadolu BIL Vocational School, Department of Food Technology, Istanbul, Türkiye
[2]Istanbul Aydin University, Food Application and Research Center, Istanbul, Türkiye
[3]Istanbul Aydin University, Anadolu BIL Vocational School, Department of Food Quality Control and Analysis, Istanbul, Türkiye
[4]Istanbul Aydin University, Faculty of Fine Arts, Department of Gastronomy and Culinary Arts, Istanbul, Türkiye
[5]Istanbul Aydin University, Faculty of Engineering, Department of Food Engineering, Istanbul, Türkiye

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ABSTRACT

This study is designed to investigate the antioxidant properties of *Cinnamomum cassia* extracts and their antimicrobial activities against selected probiotic *Lactobacillus* strains. For this purpose, ethanol, methanol, chloroform, and aqueous extracts of three different powdered cinnamon spices were produced, and the functional groups of the extracts were analyzed by ATR-FTIR. DPPH radical scavenging activities were determined with a UV-visible spectrophotometer, and the total phenolic content of the extracts was measured. It was determined that cinnamon extracts inhibited the growth of the selected probiotic lactobacilli (*Lacticaseibacillus rhamnosus, Lactiplantibacillus plantarum, Lactobacillus acidophilus, Lactobacillus gasserii, Lacticaseibacillus casei*) by using agar-well diffusion and microdilution methods. The cinnamon aqueous extract did not show antibacterial activity on any of the examined *Lactobacillus* strains. Chloroform extracts had less antimicrobial effect than the others. *Lactobacillus acidophilus* was found to be the most sensitive bacteria to the ethanol, methanol and chloroform extracts of cinnamon.

Keywords: Cinnamon extracts, *Cinnamomum cassia,* probiotics, antibacterial effects, minimum inhibition concentrations

TARÇIN (*CINNAMOMUM CASSIA*) EKSTRAKTLARININ BAZI LAKTOBASİLLUS TÜRLERİ ÜZERİNE ANTİBAKTERİYEL AKTİVİTESİ

ÖZ

Bu çalışma, *Cinnamomum cassia* ekstraktlarının antioksidan özelliklerini ve bazı probiyotik lactobasiller üzerine antimikrobiyel aktivitelerini araştırmak için tasarlanmıştır. Bu amaçla, üç farklı toz tarçının

[*]*Corresponding author* / Sorumlu yazar
✉: ayla.unver@hotmail.com ☎: (+90) 533 368 4060 🖷:(+90) 212 425 57 59

Ayla Ünver Alçay; ORCID no: 0000-0003-3254-155X
Aysun Sağlam; ORCID no: 0000-0002-4833-6107
Nagihan Kalıntaş Çağlar; ORCID no: 0000-0002-7700-5307
Sibel Kahraman; ORCID no: 0000-0002-8625-5471
Kamil Bostan; ORCID no: 0000-0001-7583-0066

etanol, metanol, kloroform ve sulu ekstraktları hazırlanmış; ekstraktların fonksiyonel grupları ATR-FTIR ile analiz edilmiştir. DPPH radikal temizleme aktiviteleri UV-görünür spektrofotometre ile belirlenmiş ve toplam fenolik bileşen içeriği ölçülmüştür. Tarçın ekstraktlarının bazı probiyotik bakteri suşları (*Lacticaseibacillus rhamnosus, Lactiplantibacillus plantarum, Lactobacillus acidophilus, Lactobacillus gasserii, Lacticaseibacillus casei*) üzerindeki in vitro antimikrobiyel aktiviteleri agar-kuyu difüzyon ve mikroseyreltme yöntemi kullanılarak değerlendirilmiş ve tarçın ekstraktlarının araştırılan probiyotik laktobasilleri inhibe ettiği belirlenmiştir. Tarçının sulu ekstraktları incelenen hiçbir laktobasil üzerinde antimikrobiyel aktivite göstermemiştir. Kloroform ekstraktları diğerlerine göre daha az antimikrobiyal etkiye sahipti. *Lactobacillus acidophilus*'un tarçının etanol, metanol ve kloroform ekstraktlarına en duyarlı bakteri olduğu saptanmıştır.
Anahtar kelimeler: Tarçın ekstraktları, *Cinnamomum cassia,* probiyotikler, antibakteriyel etkiler, minimum inhibisyon konsantrasyonları

INTRODUCTION

Cinnamon is a highly aromatic spice produced from the inner bark of an evergreen tropical tree of the *Lauraceae* family, *Cinnamomum*, which has wide use in cuisine and traditional medicine worldwide. *Cinnamomum zeylanicum, Cinnamomum loureirii, Cinnamomum burmanni*, and *Cinnamomum aromaticum* are the most well-known species with their sweet-spicy taste, and the enchanting scent is used in world cuisines to flavor drinks, desserts, appetizers, and side dishes (Hajimonfarednejad et al., 2019). Cinnamon, which also has a warming feature, is boiled with linden sticks and cloves and consumed as a hot winter drink. Recently, it has also been frequently used in formulations believed to support fat-burning and weight loss (Yazdanpanah et al., 2020).

Gulcin et al. (2019) used LC-MS/MS to assess the antioxidant activities and polyphenolic contents of cinnamon (*Cinnamomum verum*) in ethanolic and aqueous extracts. It was determined that the aqueous extract has a rich phenolic content. Cinnamon's familiar strong taste and odor are because of the predominant component, cinnamaldehyde. Cinnamon is known to have antibacterial, anti-allergic, anticancer, antilipemic, antidiabetic, antipyretic, antiulcerogenic, antihypertensive, anti-inflammatory, gastric-protecting, and immunomodulatory properties because of these components (Hajimonfarednejad et al., 2019).

The gastrointestinal microbiota plays a very important role in health. The microorganisms present here are involved in the metabolism of indigestible compounds and have functions such as supplying vitamins, preventing the colonization of pathogens, and helping to regulate immunity. Factors such as aging, diet, some drugs (especially antibiotics), and some diseases can cause changes in the intestinal microflora. It is known that imbalances in the composition and diversity of the microbiota in the gut cause various health problems. Probiotic bacteria are mainly from Lactobacillus and Bifidobacterium genera (Selle & Klaenhammer, 2013). According to the definition made by the World Health Organization (WHO) and the Food and Agriculture Organization (FDO), probiotics are "living microorganisms that provide health advantages when administered to the host in sufficient quantities" (Selle & Klaenhammer, 2013). *Lactobacillus* species such as *Lactobacillus acidophilus*, *Lactobacillus casei, Lactobacillus gasseri, Lactobacillus plantarum, Lactobacillus rhamnosus*, and *Bifidobacterium* species such as *B. adolescentis, B. animalis, B. bifidum*, and *B. breve* have been identified as probiotics. *Enterococci* such as *Enterococcus faecalis* and *Enterococcus faecium* are also considered probiotics (Maftei, 2019). Probiotics need to be antimicrobial-resistant to survive in the gastrointestinal tract. Probiotic bacteria must endure the preparation, storage, and subsequent passage through the stomach of food. These specific microorganisms should be alive, active, and abundant (cell numbers from 10^6 to 10^9 CFU/g) until the expiration date of the products (Casarotti & Penna, 2015).

Nowadays, adding plants and their extracts to foods is of great interest because of people's trends in healthy eating and consumption of natural foods. Currently, there are food supplement preparations containing cinnamon

extracts in the form of tablets, capsules, and powders, which are used for various health improvement purposes. It is a well-known fact that the use of food supplements with a protective effect has increased during the pandemic (Lordan, 2021). According to several research studies, cinnamon is highly preferred as a supplement in alternative medicine treatments (Fabian et al., 2011; Altun et al., 2021).

How the plant and its extracts affect desirable and beneficial gut bacteria, such as probiotic bacteria and beneficial microorganisms in probiotic foods, is an important question mark because they are consumed regularly as food supplements and used as natural food additives. Keeping probiotics alive in foods and the human gut is as important as taking them to maintain health. Probiotic activity is dependent on the survival of the microorganism in the gastrointestinal tract. Probiotic cultures must remain viable throughout the shelf life and during transit through the gastrointestinal tract and in the final product to effectively maintain probiotic activity (Selle & Klaenhammer, 2013). Many herbal extracts as food supplements are used by humans for their beneficial effects, but there are few studies on their effects on probiotics. Nutrition can cause significant changes in the microbiota, even for short periods (David et al., 2014).

The antimicrobial activity of cinnamon against many pathogenic microorganisms such as *Staphylococcus aureus* (Shan et al., 2005), *E. faecalis* (Gopalakrishnan et al., 2014), *Shigella* spp. (Akrami et al., 2021), *Acinetobacter baumannii* (Bayoub et al., 2010), *Pseudomonas aeruginosa* (Bayoub et al., 2010), *Escherichia coli* (Shan et al., 2005; Ismail et al., 2012), *Salmonella typhimurium* (Elgammal et al., 2020), *Listeria monocytogenes* (Shan et al., 2005; Bayoub et al., 2010), and *Candida* strains (Ismail et al., 2012) has been proven by many previous studies. This study aims to investigate the antimicrobial activity of various cinnamon extracts on some important probiotic strains and also determining the total phenolic content and antioxidant potential.

MATERIALS and METHODS

Collection and sampling of cinnamon powder

Three varied brands of cinnamon powder (*Cinnamomum cassia*) spices, two of Vietnamese origin and one of Indonesian origin were taken in closed packages from local markets in Istanbul/Türkiye. Cinnamon brands were selected by random sampling.

Preparation of cinnamon extracts

Four different solvents [ethanol (Merck 100986), methanol (Merck 106009), chloroform (Merck 102445)], and phosphate-buffered saline [(PBS, Merck 109439) pH 7] were used for obtaining each extract. All extracts were prepared by modifying the method of Gupta et al. (2008). For each type of extract, 15 grams of powdered cinnamon were weighed with the balance and transferred into conical flasks. Then, 60 mL of ethanol, 60 mL of methanol, 60 mL of chloroform, and 120 mL of phosphate-buffered saline were added per extract. The conical flasks were closed and put in a dark place for a maximum of 2 days. After two days, they were mixed with a homogenizer (WiseTis, HG-15A) for 2 minutes, and shaking with an orbital shaker (Scilogex, SK-O330-pro, 400 rpm) was continued for one more night at room temperature. The next day, these extracts were passed through Whatman No. 1 filter paper and then concentrated (at 40°C) using a rotary evaporator (Heidolph Hei-Vap Value HL/G6). Dimethyl sulfoxide (DMSO, Merck 802912) prepared with 1/10 distilled water was used to dissolve the underlying extracts. Dry extracts dissolved with dimethyl sulfoxide were stored in brown bottles in the refrigerator at -25°C until analysis.

Fourier transform infrared (FT-IR) spectroscopy

Functional groups in organic molecules selectively absorb infrared light, and FT-IR spectroscopy is a useful method for examining those groups. Commercially available cinnamon samples and cinnamon extracts were scanned using FTIR spectrometry in the wavelength range of 4000–400 cm^{-1} with a spectral resolution of 4 cm^{-1}, and characteristic peaks and their functional groups were detected. ATR-FTIR analysis was

done in the Istanbul Aydin University Health Sciences laboratory. The analysis was repeated three times at room temperature, and a comparison was made with commercially available literature using ATR-FTIR spectroscopy by the Bruker Invenio S ATR-FTIR instrument. The corrected spectrum and absorbance of the main peaks were plotted using Origin Pro software.

Total phenolic content assay

Cinnamon extract samples were analyzed for total phenolic content (TPC) using the procedure recommended by El-Baz et al. (2023). The test tubes were filled with 0.1 mL of the extract and then diluted in 4.5 mL of distilled water. Then, the tube was supplemented with 0.1 mL of Folin-Ciocalteu reagent and 0.3 mL of a 2% sodium carbonate (Na_2CO_3) solution. To achieve a full reaction, these solutions were properly mixed and placed in a dark place for two hours. A blank solution was used for comparison after measuring the absorbance of a T60 UV-visible spectrophotometer (Leicestershire LE17 5BH, UK) at 760 nm. A calibration curve was made using a gallic acid solution to quantify phenolic content. A unit of milligrams of gallic acid equivalent (GAE)/g of the dry substance was used for measurement expressions. The resultant value was calculated by taking the average of the three readings from each test repetition.

DPPH radical scavenging activity

An adapted version of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity process, originally suggested by El-Baz et al. (2023, was utilized to assess the antioxidant capacity of the extracts. A solution of the DPPH radical in methanol (20 mg/L) was prepared freshly just before the experiment. Then, 3.9 mL of the DPPH solution was combined with 0.1 mL of the test samples. The solution was blended and then kept at room temperature in darkness for half an hour after adding the samples to the DPPH solution. Then, the absorbances were recorded at a wavelength of 517 nm (T60 UV-visible spectrophotometer (Leicestershire LE17 5BH, UK). The method determines the antioxidant efficacy of the samples by calculating the percentage reduction in the DPPH absorbance using the given formula:

DPPH radical scavenging activity (%) = $(A_0 - A_1 / A_0) \times 100$

A_0 is the DPPH solution absorbance, and A_1 is the DPPH absorbance with antioxidants.

Determination of antimicrobial effects

In vitro antimicrobial activity was determined by considering the inhibition zones and minimum inhibition concentrations obtained using the agar-well diffusion and microdilution methods. For this purpose, probiotic strains were purchased from a commercial company.

Bacterial test isolates

In the study, five probiotic microorganisms (*Lacticaseibacillus rhamnosus* ATCC 53103, *Lactiplantibacillus plantarum* ATCC BAA-2838, *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus gasseri* ATCC 33323, *Lacticaseibacillus casei* ATCC BAA-2843) were used. Strains were purchased from BD Diagnostics (Germany). *Lactobacillus* species included in the study to determine the antibacterial activities of cinnamon extracts were selected for their probiotic and technological properties, which are described below:

Lactobacillus rhamnosus (renamed *Lacticaseibacillus rhamnosus, L. rhamnosus*) (ATCC 53103), also called *Lactobacillus rhamnosus* GG, reduces intestinal inflammation associated with food allergy in young children and infants with oral probiotic bacteriotherapy (Savino et al., 2020).

Lactobacillus plantarum (now known as *Lactiplantibacillus plantarum, L. plantarum*) is one of the most common bacterial species found in fermented foods, sourdoughs, fruits, vegetables, grains, meat, fish, and the gastrointestinal tract of mammals and probiotics (Yilmaz et al., 2022). Various beneficial health effects of *L. plantarum*, such as antimicrobial activity, antiobesity, immune-boosting, and anti-inflammatory effects, are known and have been used as a starter culture in various industrial fermented foods. As a probiotic, it has acid/bile tolerance and intestinal adhesion activity and alleviates acute and chronic inflammation (Yang et al., 2021).

L. acidophilus is an important member of the human intestinal natural microflora and is used in fermented foods for its potential probiotic effects. *L. acidophilus* ATCC 4356 strain, which is a human isolate, is reported to be an important resident of the gastrointestinal tract and therefore, is widely used in probiotic studies (Arslan et al., 2016).

Lactobacillus gasseri, a common homofermentative lactobacillus in the human gastrointestinal tract, is a microorganism whose use for health promotion has been proven by clinical trial data. It is a potential probiotic with known benefits in alleviating allergic diseases, anti-inflammatory effects, regulation of oxidative stress, protecting vaginal homeostasis, alleviation of *Helicobacter pylori* infection, and healing of diarrhea (Selle & Klaenhammer, 2013; Zhou et al., 2019; Sun et al., 2020). *Lactobacillus gasseri* ATCC 33323, whose whole genome has been sequenced, is known to play an important role in the defense and protection of the human gastrointestinal tract and therefore, is accepted as a probiotic candidate (Yarmohammadi et al., 2021).

Lactobacillus casei (renamed *Lacticaseibacillus casei, L. casei*) strain is a probiotic strain that inhibits pathogenic microorganisms with the metabolites it produces, provides the development of various sensory properties in the product, and creates positive effects on health. First identified isolates found in cheese are widely found in various environments such as raw and fermented dairy products, fresh vegetables, herbal fermented products, breast milk, the digestive system of humans and other warm-blooded animals, soil, and lakes. *L. casei* strains are used in probiotic products, in acid-producing starter cultures for milk and meat fermentation, in some cheese varieties, and in the production of yogurt, butter, ice cream, and beverages such as kefir, kumis, and yakult (Somer et al., 2012).

Determination of antibiotic susceptibility of the strains by disc diffusion method

To compare the effectiveness of the produced extracts, the antibiotic susceptibility of the examined strains was determined. For this purpose, antibiotics from different groups were selected, including a penicillin derivative (ampicillin), an aminoglycoside group (gentamicin), a fluoroquinolone (ciprofloxacin), a cephalosporin (cefotaxime), and quinine (optochin). All the test bacteria were subjected to a disc diffusion test using five different antibiotic disks. Probiotic bacteria were initially subcultured in MRS broth (Biolife, 4017294) for 48 h at 37°C in anaerobic conditions. 0.5 McFarland standard suspension of the isolate was made, and lawn culture was done on the MRS plate. Antimicrobial Susceptibility Discs containing 10 µg ampicillin (Oxoid, CT0003B), 10 µg gentamicin (Oxoid, CT0024B), 5 µg ciprofloxacin (Oxoid, CT0425B), 30 µg cefotaxime (Oxoid, CT0166B), or 5 µg optochin (Oxoid, DD0001) were placed on MHA plates. Plates were incubated at 37°C for 48 h, and zone diameters were measured.

Agar well diffusion method (AWD)

The antibacterial activities of cinnamon extracts were studied using the agar well diffusion method (Gupta et al., 2008). For quality control, 10 µg/disk-1 of gentamicin (Oxoid™ Gentamicin Antimicrobial Susceptibility Disc, Catalog number: CT0024B) was used as a positive control, and dimethyl sulfoxide as a negative control. For routine internal quality control, the *E. coli* ATCC 25922 strain recommended by EUCAST was used, and results were evaluated against EUCAST's acceptable limits in routine and extended internal quality control charts (Anonymous, 2022). Probiotic bacteria were initially subcultured in MRS broth for 48 h at 37°C in anaerobic conditions. To achieve confluent growth, 100 microliters of standardized inoculum (10^8 CFU/ml; 0.5 MacFarland) of each test bacterium were distributed over sterile De Man, Rogosa, and Sharpe (MRS) agar (Merck 110660). The plates were allowed to dry before drilling wells in the agar with a sterile cork borer (6 mm diameter). The extracts were then placed in wells of the agar plates in a 100 µL volume. The plates were left to stand for at least 1 h to allow for diffusion before being incubated. The strains were incubated at 37°C for 48 h in anaerobic conditions. The zone of inhibition was measured in millimeters to the closest size. Three

independent experiments were performed for each extract. The plaques were evaluated with the naked eye by holding them 30 cm away from the eye, and the zone diameters were evaluated by measuring the point of complete inhibition of growth with a digital caliper. Figure 1 (Fig. 1) shows the diameter of inhibition zones of cinnamon ethanol, methanol, aqueous (PBS) extracts, and chloroform obtained by the well diffusion test on MRS agar.

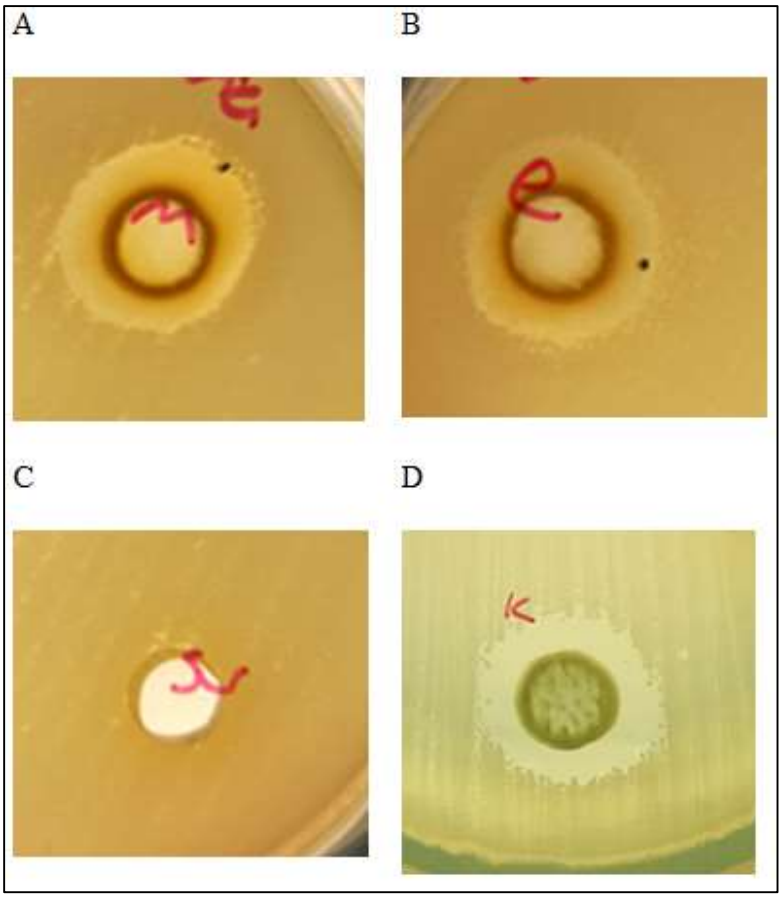


Figure 1. Photographs of the diameter of the inhibition zone of cinnamon ethanol (a), methanol (b), chloroform (c), and aqueous (PBS) extracts (d) obtained by the well diffusion test on MRS agar

Minimum inhibitory concentration (MIC)

The antibacterial effect of the extracts was confirmed by the microdilution method, and MIC values were determined (Boligon et al., 2013). The bacteria were cultivated anaerobically at 37°C for 48 hours in MRS broth. First, in sterile 96-well microtiter plates, two-fold dilutions of each extract were performed. To obtain 0.5 McFarland bacteria, each bacterium was suspended in Mueller–Hinton Broth (Merck 110293) (final density of 10^8 CFU/mL). Then, 50 µL of bacterial suspension was added to each well. Each microdilution plate contained a negative growth control (medium alone) and a growth control (100 µL of medium plus 100 µL of inoculum suspension). The 96-well plate was incubated for 48 h at 37°C in anaerobic conditions for probiotic bacteria. The lowest concentration of extracts at which no turbidity was observed in the culture, indicating the absence of bacterial growth, was determined as the MIC. From the 96-well plate, 10 microliters of liquid were taken from each well with a sterile pipette and transferred to MRS agar plates, and it was definitively confirmed which well was the last growth (Fig. 2). A confirmation test was carried out to be sure of the last pit where

turbidity ends in the wells. Three independent experiments were performed for each extract. Figure 2 shows photographs of the confirmation experiment of the microdilution test for MIC detection on MRS agar medium.

Figure 2. Photographs of the confirmation experiment of the microdilution test for MIC detection on MRS agar medium

Statistical analysis

The results of analyses are expressed as mean±standard deviation. The SPSS 25 (IBM) software was used to do a statistical analysis of the data collected in our study. The Games-Howell test and one-way analysis of variance (ANOVA) were used, and P <0.05 was considered statistically significant.

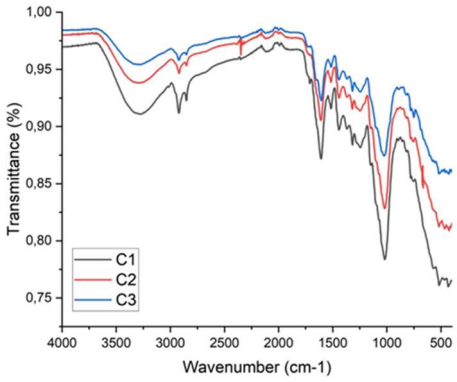
RESULTS and DISCUSSION

The antibacterial activity of cinnamon and some of its extracts against some pathogens has been determined by many studies. Cinnamon's antibacterial properties are attributable to bioactive components like cinnamaldehyde and eugenol (Cowan, 1995). However, these studies for probiotic microorganisms are negligible.

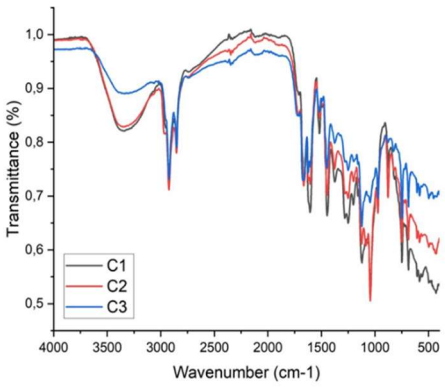
Mazimba et al. (2015) performed a phytochemical analysis of methanol extracts of *Cinnamomum verum* and found the presence of alkaloids, flavonoids, tannins, phenols, and saponins. The main component of cinnamon bark essential oil is cinnamaldehyde (3-phenyl-2-propenal phenol; C_9H_8O), and the main component of cinnamon leaf essential oil is eugenol (Jayawardena & Smith, 2010). Unlu et al. (2010) determined that the essential oil produced from *C. zeylanicum* bark has 68.95% (E)-cinnamaldehyde, 9.94% benzaldehyde, 7.44% (E)-cinnamyl acetate, 4.42% limonene, and 2.77% eugenol. Studies have revealed the high antimicrobial effect of aromatic substances such as cinnamaldehyde and eugenol (Sanla-Ead et al., 2012). Cinnamaldehyde, the primary component of cinnamon, has antibacterial properties since it affects cell wall biosynthesis, membrane functionality, and certain enzyme activity in microorganisms (Liu et al., 2017).

FT-IR spectrum analysis of cinnamon and extracts

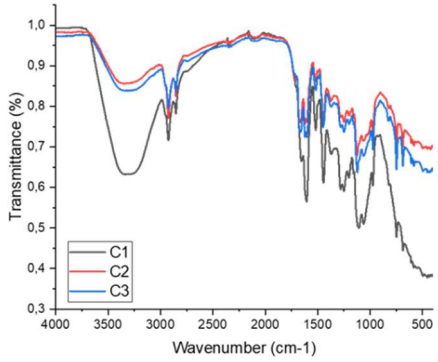
Before extraction, the IR properties peaks of *C. cassia* bark powder and the IR properties of the extracts were determined in the range of 4000–400 cm^{-1}, as shown in Figure 3. FT-IR spectroscopy in the mid-IR range of 450 to 4000 cm^{-1} provides detailed structural information about the molecular structure of the samples (Li et al., 2013).



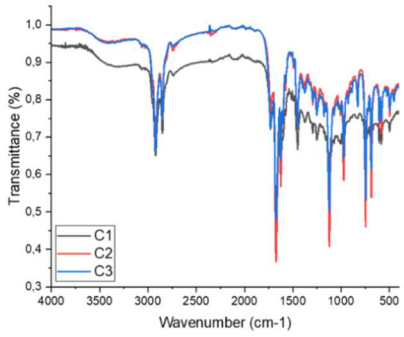
FTIR spectrum of the cinnamon powders



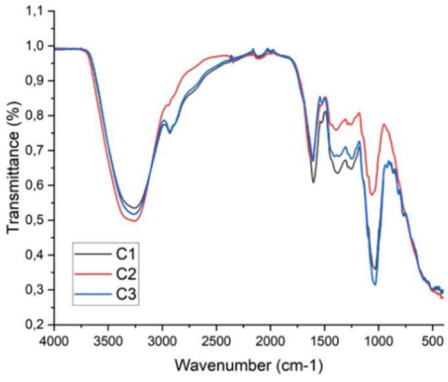
FTIR spectrum of the cinnamon ethanol extracts



FTIR spectrum of the cinnamon methanol extracts



FTIR spectrum of the cinnamon chloroform extracts



FTIR spectrum of the cinnamon water extracts

Figure 3. FTIR spectrum of the cinnamon powder and cinnamon extracts (C1: Cinnamon 1, C2: Cinnamon 2, C3: Cinnamon 3)

In all FT-IR spectra of cinnamon and extracts, a peak was seen in 1519-1515 cm^{-1}. The presence of the peaks in the 1600-1300 cm^{-1} region associated with nitro groups is consistent across all samples, with slight variations in the extracts. Jadhav et al. (2021) highlight that nitro group presence is reported at a peak at 1555 cm^{-1}, which is close to the range seen in Table 1, suggesting the presence of such compounds across.

Table 1. Functional groups of the *C. cassia* bark powder and extracts

Wavenumber (cm^{-1})	Assignment*	Cinnamon sample number	Cinnamon Powder	Cinnamon ethanol extract	Cinnamon methanol extract	Cinnamon chloroform extract	Cinnamon water extract
3400–3000	Phenols and carboxylic acid, O–H stretching, and C=O bending	C1	3246	3310	3310	3314	3273
		C2	3297	3361	3360	3385	3257
		C3	3368	3334	3320	3386	3270
3000–2500	Alkanes	C1	2926	2926	2924	2922	2927
		C2	2948	2925	2923	2920	2938
		C3	2946	2923	2926	2923	2933
2700–1600	Alkenes, amides, and ketones, C=O stretching	C1	1620	1712	1707	1730	1603
		C2	1610	1710	1710	1730	1616
		C3	1609	1716	1713	1731	1604
1600–1300	Nitro group	C1	1514	1519	1519	1513	1519
		C2	1517	1517	1517	1515	1521
		C3	1517	1517	1520	1516	1524
1300–1050	Ethers	C1	1245	1282	1282	1296	1246
		C2	1155	1279	1284	1293	1286
		C3	1246	1278	1283	1293	1246
1050–900	Esters	C1	1040	1044	1060	1069	1044
		C2	1021	1043	1060	1005	1046
		C3	1017	1045	1058	1006	1030

C1: Cinnamon 1, C2: Cinnamon 2, C3: Cinnamon 3

The C=O stretching signals, typically found between 1800 and 1600 cm^{-1}, were detected in the cinnamon powder within the range of 1620 to 1609 cm^{-1}; however, alterations in this measurement were detected in the extracts. Lin et al. (2017) reported a peak at 1727 cm^{-1}, like our findings, which can be related to aldehydes of the saturated fatty acids. It was also reported in the study that 1679 to 1626 cm^{-1} peaks are a sign of the aldehyde carbonyl, such as coumarin. The presence of the peaks at 1673 to 1603 cm^{-1} is a sign of the presence of coumarin in all spectrums. Goyal et al. (2019) mention that cinnamaldehyde exhibits characteristic vibrations around 1700–1600 cm^{-1} and that O-H in intermolecular hydrogen bonding is broad above 2600 cm^{-1}.

The 3400–3000 cm^{-1} range provided in Table 1 for phenols and carboxylic acids shows different absorption peaks for cinnamon powder and extracts, indicating variations in the concentration of these compounds after extraction. The peaks in this range shown in all cinnamon and extract spectrums are also related to –OH groups. These are caused by the presence of certain alcohols, such as eugenol, which have both carbonyl and hydroxy functional groups (Lin et al., 2017).

The peaks in 1300–1050 cm^{-1} and 1050–900 cm^{-1} show the presence of ethers and esters in our samples, respectively. Jadhav et al. (2021) reported peaks similar to our findings.

The total phenolic content of the cinnamon extracts

As shown in Table 2, the TPC values of the cinnamon extracts differ depending on the solvent type. While the highest TPC results were obtained using water solvent, the lowest results were obtained using chloroform. The solubility of phenolic substances is influenced by factors such as the nature of the solvent used, the degree of polymerization of these phenolic compounds, interactions between phenolics and other substances, and the formation of insoluble complexes as presented by Ghasemzadeh et al. (2011).

Table 2. The total phenolic content of the cinnamon extracts

	Total Phenolic Content mg GAE/g dry weight*			
	Ethanol Extract	Methanol Extract	Chloroform Extract	Water Extract
Cinnamon 1	44.53±1.12	45.48±2.10	2.97±0.33	85.94±2.02
Cinnamon 2	46.39±0.94	46.62±1.77	0.92±0.09	95.45±4.13
Cinnamon 3	44.22±4.15	45.84±1.54	1,82±0,57	86.03±3.46

* mean of three samples ±standard deviation

Mathew and Abraham (2006) reported a total phenolic content of 289±2.2 mg GAE/g for *Cinnamomum verum* barks. Abeysekera et al. (2013) found 33.43±0.51 mg GAE/g total phenolic content for ethanolic extract of *Cinnamomum zeylanicum,* which is lower than our results.

2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging activity of the cinnamon extracts

DPPH, being a diamagnetic molecule, possesses the capacity to undergo a color change. The DPPH free radical scavenging assay is widely employed for measuring antioxidant capability due to its easy application and accurate results (Liang et al., 2022).

% DPPH radical scavenging activity of the extracts can be seen in Table 3. When the results were compared, chloroform extracts showed the lowest results, while ethanol extracts showed the highest results. Ghasemzadeh et al. (2011) discovered that extracts made with highly polar solvents, like methanol, were more effective in scavenging free radicals than extracts made with less polar solvents, such as acetone and chloroform.

Table 3. % DPPH radical scavenging activity of the cinnamon extracts

	% DPPH Radical Scavenging Activity*			
	Ethanol extract	Methanol Extract	Chloroform Extract	Water Extract
Cinnamon 1	78.2±1.4	74.9±2.8	43.42±2.6	77.8±0.8
Cinnamon 2	79.10±1.4	77.86±1.1	43.64±1.3	76.6±2.7
Cinnamon 3	81.7±1.9	76.7±0.4	45.1±4.5	82.2±0.6

* mean of three samples ±standard deviation

Singh et al. (2018) reported that for the DPPH free radical scavenging assay, the ethanolic extract of *C. cassia* demonstrated the highest scavenging activity among the various extracts, exhibiting an activity of 88.26% ± 0.09%, while the methanolic and acetone extracts displayed scavenging activities of 63.08% ± 0.16% and 70.79% ± 0.20%, respectively.

Antibiotic susceptibility of the strains

Due to the diversity of lactic acid bacteria (LAB) genera and species, the wide range of resistance spectra, and the main focus of antibiotic resistance studies on clinical infections, there is still little known about the antibiotic resistance of LAB. The determination of LAB antibiotic resistance has certain issues. The Mueller-Hinton

medium used in conventional antibiotic susceptibility testing is not suitable for lactic acid bacteria. It is preferable to use the MRS medium for Lactobacilli rather than Mueller-Hinton. Observations made on this medium cannot be directly compared to standards determined with Mueller-Hinton agar, as zones of inhibition may vary depending on the diffusion medium (Hummel et al., 2007; Sharma et al., 2017).

According to the results obtained in our study, all test microorganisms were resistant to gentamicin and optochin (Table 4). Inhibition zone diameters of varying sizes (13.31±0.21-29.49±0.03 mm) were formed in all tested strains against ampicillin and cefotaxime. However, in the tests performed with ciprofloxacin, inhibition zone formation was observed only in *L. casei* (10.19±1.00 mm) and *L. rhamnosus* (11.15±0.16) strains. Similar results were obtained in a study investigating the antibiotic resistance of lactobacilli isolated from fermented fish products, and *L. casei* and *L. plantarum* strains were found to be ampicillin-sensitive and gentamicin-resistant (Liasi et al., 2009). Coppola et al. (2005) studied the antibiotic resistance of 63 different strains of *Lactobacillus rhamnosus* isolated from Parmigiano Reggiano cheese. Like our study, it was reported that all strains examined were susceptible to ampicillin, and the overwhelming majority were resistant to gentamicin.

Table 4. Diameter of inhibition zone (mm) of the cinnamon extract and antibiotic disks against test bacteria[a,b,c]

Bacteria number	Ethanol Extract	Methanol Extract	Chloroform Extract	AM10	CTX30	CIP5	CN10	OP
1	15.04±0.36^{cA}	15.04±0.21^{cA}	14.94±0.45^{bA}	12.83±0.01 ^{dB}	10.21±0.17 ^{cC}	11.15±0.16 ^{aC}	0.0	0.0
2	18.98±0.82^{bB}	17.69±0.79^{bB}	10.03±0.00^{dC}	29.49±0.03 ^{aA}	29.23±0.10 ^{aA}	0.00	0.0	0.0
3	30.68±0.58^{aAB}	30.17±0.26^{aA}	25.55±0.21^{aB}	18.00±0.15 ^{cD}	21.03±1.79 ^{bC}	0.00	0.0	0.0
4	14.93±0.17^{cC}	13.19±1.16^{dC}	13.75±0.17^{cC}	23.10±0.99 ^{bB}	26.24±0.45 ^{aA}	0.00	0.0	0.0
5	19.02±0.19^{bA}	17.78±0.07^{bA}	14.61±0.33^{bB}	13.31±0.21 ^{dB}	9.86±0.64 ^{cC}	10.19±1.00 ^{aC}	0.0	0.0

1: *L.rhamnosus*, 2: *L.plantarum*, 3: *L. acidophilus* , 4. *L. gasserii,* 5: *L. casei*
Differences between means shown with different letters in the same column are statistically significant at P <0.05
^{A,B} Differences between means shown with different letters in the same row are statistically significant at P <0.05.
AM10: ampicillin 10 μg disc, CTX30: cefotaxime 30 μg disc, CN10: gentamicin 10 μg disc, OP: optochin 5 μg disc

In a study investigating the resistance levels of 17 *L. casei* and 15 *L. plantarum* isolates to 10 antibiotics using gene-specific primers by the PCR method, the presence of 20 genes causing antibiotic resistance was determined in 11 of the examined isolates (Shao et al., 2015). At the study by Hummel et al. (2007), which investigated the antibiotic resistance of forty-five lactic acid bacterial strains belonging to *Lactobacillus*, *Streptococcus*, *Lactococcus*, *Pediococcus,* and *Leuconostoc* genera, according to MIC breakpoints, more than 70% of the isolates were resistant to gentamicin and ciprofloxacin. Charteris et al. (2007) have reported that more than 80% of LAB starter and probiotic strains are resistant to aminoglycosides. Hummel et al. (2007) reported *L. plantarum* BFE 7433 and BFE 7440 and *L. acidophilus* BFE 7444 strains to be resistant to ciprofloxacin and gentamicin, as in our study. While Hummel et al. (2007) determined that *L. rhamnosus* BFE 7442 and *L. casei* BFE 7445 were resistant to ciprofloxacin and gentamicin based on the EU Commission (EUC) breakpoint values, the diameter of inhibition zones was determined in our study using the disk diffusion method. However, due to the difference in strain and method, a complete comparison could not be made.

On the other hand, Zhao et al. (2023) reported that both *L. rhamnosus* X253 and *L. rhamnosus* GG strains are sensitive to both gentamicin and ampicillin. Tulini et al. (2013) found the *L. paraplantarum* FT259 strain isolated from Brazilian cheese to be resistant to ciprofloxacin and susceptible to gentamicin and ampicillin. It was thought that the different results obtained were due to the strain differences.

In vitro antibacterial activity results

In our study, the diameters of inhibition zones (DIZ) for the ethanol, methanol, and chloroform

extracts of cinnamon for the examined lactic acid bacteria were measured in the range of 14.93-30.68 mm, 13.19-30.17 mm, and 10.03-25.55 mm, respectively. In this study, the largest diameter of inhibition zones was determined for *L. acidophilus* (25.55±0.21 mm-30.68±0.58 mm), and the lowest for *L. gasserii* (13.75±0.17 mm-14.93±0.17 mm). The lowest diameter of inhibition zones was determined for *L. gasseri* (10.03±0.00 mm) with cinnamon chloroform extract, and the widest diameter of inhibition zones was for *L. plantarum* (30.68±0.58 mm) with cinnamon ethanol extract.

The number of studies on the antibacterial activity of *C. cassia* cinnamon extracts prepared using various solvents against Lactobacilli is limited. For this reason, it was not possible to compare the results of each of the diameters of inhibition zones found by investigating cinnamon extracts against the bacteria studied in this study with previous studies.

In our study, for probiotic bacteria, the solvent differences (ethanol, methanol, and chloroform) of the extracts with antimicrobial effects caused a significant change in the zone diameters, except for *L. rhamnosus* (P <0.05). In general, chloroform extracts had less antimicrobial effect on the tested bacteria than the others. *L. gasserii* and *L. casei* were found to be more resistant bacteria to methanol extract compared to ethanol extract. *L. acidophilus* is the most sensitive bacteria to all cinnamon extracts (P <0.05).

According to the results of this study, the inhibition zones of the ethanol extract against lactic acid bacteria ranged from 14.93 to 30.68 mm. The highest zone diameter of 30.68 mm was found for *L. acidophilus,* and the difference with the others was statistically significant (P <0.05). The methanol extract created inhibition zones between 13.19 and 30.17 mm. The highest mean zone diameter of 30.17 mm was obtained for *L. acidophilus,* and it was significantly different from the others (P <0.05). With chloroform extract, zones of 10.03 to 25.55 mm were obtained when the highest zone (25.55 mm) was determined for *L. acidophilus*. Also, the extracts obtained using PBS did not form any zones against the strains examined. Yields (mg/mL) of different extract types are shown in Table 5. Inhibition zones of cinnamon extracts and antibiotic discs against the investigated bacteria are shown in Table 4.

Table 5. Yields of different extract types (mg/mL)

Solvent Type	Cinnamon-1*	Cinnamon-2*	Cinnamon-3*
Ethanol	13.63±0.02	15±0.03	13.35±0.06
Methanol	18.325±0.05	26.33±0.03	17.92±0.01
Chloroform	13.38±0.01	14.75±0.01	14.4±0.01
PBS	6.61±0.02	8.64±0.01	6.62±0.02

* mean of three samples ±standard deviation

When the antimicrobial activity of cinnamon extracts was evaluated by both methods (AWD and MIC), the following ranking was obtained:
Ethanol extract: *L. acidophilus*>*L. casei*>*L. plantarum* >*L. rhamnosus*>L. *gasseri*
Methanol extract: *L. acidophilus*>*L. casei* >*L. plantarum* >*L. rhamnosus*>*L. gasseri*
Chloroform extract: *L. acidophilus*>*L. rhamnosus*>*L. casei*>*L. gasseri* >*L. plantarum*

There are some studies in which probiotic and cinnamon powders, extracts, or essential oils are added to the formulation to produce foods such as yogurt and kefir and provide functional properties (Behrad et al., 2009; Moritz et al., 2012; Gunes Bayir & Bilgin, 2019; Setiyoningrum et al., 2019; Sohrabpour et al., 2021). A study conducted by Gunes Bayir and Bilgin (2019) determined that different levels of cinnamon powder added to yogurts had antimicrobial activity on *Streptococcus thermophilus, Lactobacillus acidophilus,* and *Bifidobacterium animalis* ssp. *lactis* compared to the control. In that study, *B. animalis* ssp. *lactis* and *L. acidophilus* probiotic bacteria counts significantly decreased.

Similarly, another study conducted with a probiotic bacterium, *L. rhamnosus,* reported that the number of *L. rhamnosus* was found to be lower in yogurts containing cinnamon essential oil than in the control group (Moritz et al., 2012). In the study of Kuang et al. (2011), in which very finely ground cinnamon and clove powders were used to prevent the growth of microorganisms that cause spoilage in meat, the minimum inhibitory concentrations (MICs) of cinnamon powder were determined as 2.5% against *L. rhamnosus*.

In the present study, MIC values for ethanol, methanol, and chloroform extracts were found to be 0.26-10.41 mg/mL, 0.35-12.43 mg/mL, and 0.65-15.99 mg/mL, respectively. Although the literature is limited, MIC values of cinnamon essential oils against probiotic bacteria have been determined (Valdivieso-Ugarte et al., 2021). Valdivieso-Ugarte et al. (2021) evaluated the antimicrobial activity of different concentrations of cinnamon essential oil (0.1%, 0.01%, 0.001% v/v) on probiotic strains (*Lactobacillus paracasei* CNCM I-4034, *L. plantarum* 3547, *B. breve* CNCM I-4035, *L. rhamnosus* CNCM I-4036) using the disc diffusion method and MIC results. It was reported that the highest concentration applied in the study had an inhibitory effect on probiotics. Other studies have shown that, while using cinnamon as a spice and/or flavoring agent is harmless, using it in big doses or for an extended period can induce undesirable effects such as gastrointestinal issues **(**Hajimonfarednejad et al., 2019**)**. Ojagh et al. (2010) produced chitosan-based films (v/v) containing cinnamon essential oil at levels of 0.4%, 0.8%, 1.5%, and 2%. It has been reported that cinnamon essential oil enhances the antimicrobial activity of chitosan-based films against the bacteria *Listeria monocytogenes* PTCC1163, *E. coli* PTCC1399, *Lactobacillus plantarum* PTCC1058, *Lactobacillus sakei* PTCC1272, and *Pseudomonas fluorescens* ATCC 17482. MIC values for *L. plantarum* and *L. sakei* were determined as 500 µg/mL and 250 µg/mL, respectively.

In our study, MIC was determined for *L. plantarum* with cinnamon chloroform extract, and the prepared cinnamon aqueous extract did not show antibacterial activity on any Lactobacilli examined. The MIC values obtained in our study are shown in Table 6. The maximum and minimum MIC values obtained for ethanol, methanol, and chloroform extracts were determined as 0.26-10.41 mg/mL, 0.35-12.43 mg/mL, and 0.65-15.99 mg/mL, respectively. *L. acidophilus* has the lowest MIC value compared to the MIC values obtained by the ethanol extract. Based on the inhibition zone values obtained in our findings, *L. acidophilus* was shown to be the most sensitive bacteria to ethanol, methanol, and chloroform extract.

Table 6. Minimum inhibitory concentration (MIC) of cinnamon extracts (mg/mL)

Bacteria	Ethanol Extract (Mean)	Methanol Extract (Mean)	Chloroform Extract (Mean)	Water (PBS) Extract (Mean)
L. rhamnosus	7.00±0.01	9.09±0.02	2.14±0.05	ND
L. plantarum	4.47±0.05	5.64±0.05	15.99±0.13	ND
L. acidophilus	0.26±0.04	0.35±0.04	0.65±0.01	ND
L. gasseri	10.41±0.02	12.43±0.06	12.63±0.02	ND
L. casei	4.29±0.03	5.48±0.05	4.0±0.01	ND

ND Not Detected

* mean of three samples ±standard deviation

The antibacterial activity of cinnamon powder, extracts, and essential oils against both gram-negative and gram-positive pathogens of foodborne origin and gram-positive bacteria has been determined by many studies. Extracts and essential oils of cinnamon are thought to have antimicrobial activity strong enough to be recommended as an alternative agent for antibacterial supplementation (Vasconcelos et al., 2018). It is known that plants contain

components with antimicrobial properties, such as tannins, terpenoids, alkaloids, flavonoids, flavones, and flavanols. Since almost all of these components are aromatic or saturated organic compounds, they are generally extracted using ethanol or methanol (Cowan, 1999). Aqueous fractionation is avoided in most studies, except for those that are water-soluble, such as polysaccharides (e.g., starch) and polypeptides. Cowan et al. (1999) reported that cinnamon bioactive compounds are mostly soluble in alcohol, ether, and chloroform and less soluble in water. The water extract was also added to our study to make a detailed comparison because there is insufficient data in the literature on the probiotic bacteria we prefer. The water extracts did not have antimicrobial activity against all tested microorganisms in our research, which was in line with the literature, and we concluded that aqueous solvents were ineffective in extracting antimicrobial components. Behrad et al. (2009) reported that cinnamon aqueous extract did not affect the level of probiotic bacteria (*Lactobacillus acidophilus* LA-5 and NCFM, *Bifidobacterium* Bb-12, *L. casei* LC 10) found in yogurt during and after storage. However, there is also one study reporting that the aqueous extract of cinnamon causes a decrease in the survival of *L. acidophilus* (Sohrabpour et al., 2021).

Mazimba et al. (2015) determined the minimum inhibitory concentration against *S. aureus* as 2.5 μg/mL with *Cinnamomum verum* stem bark methanol extracts (100 μg/mL). In our study, the MIC values for cinnamon ethanol, methanol, and chloroform extract were 4.47 mg/mL, 5.64 mg/mL, and 15.99 mg/mL for *L. plantarum* and 4.29 mg/mL, 5.48 mg/mL, and 4 mg/mL for *L. casei* ethanol, methanol, and chloroform extract, respectively, and it is slightly higher than the values determined against *S. typhi* and *E. coli* ATCC 5087 by Ismail et al. (2012) and the MIC value of Mazimba et al. (2015) against *S. aureus*. In our study, MIC values of cinnamon ethanol, methanol, and chloroform extract were determined as 10.41 mg/mL, 12.13 mg/mL, and 12.63 mg/mL for *L. gasserii*, and 7.0, 9.09, and 2.14 for *L. rhamnosus*, and these results are higher than the MIC values (excluding chloroform extract against *L. rhamnosus*) determined by Ismail et al. (2012) for *S. typhi* and *E. coli* ATCC 5087.

In the study of Bayoub et al. (2012), the MIC of cinnamon (*Cinnamomum zeylanicum*) ethanol (90%) extracts (20 g/100 mL) against *L. monocytogenes* was determined as 0.4 mg/mL. This result is close to the MIC results (0.26, 0.35, 0.65) for *L. acidophilus* taken with cinnamon ethanol, methanol, and chloroform extracts in our study, but it is lower than the MIC values of *L. rhamnosus, L. plantarum, L. gasserii*, and *L. casei* that we have determined.

The amount of plant-specific antimicrobial compounds is affected by factors such as species, soil characteristics, climate, and irrigation. At the same time, factors such as the methods used in the extraction of these components and the nature of the solvent used in the extraction also determine the degree of antimicrobial effect. All these variables could explain why different researchers reach different conclusions about antimicrobial efficiency.

Previous studies have often examined pathogens, mainly because of the worrisome problem of antibiotic resistance, focusing on the potential use of cinnamon extracts as antibiotics and their use as antimicrobial food preservatives. The fact that these extracts can cause changes in the microbiota or cause problems when used in the production of probiotic and fermented foods has often been overlooked, and it has been the subject of truly little research. The results of these studies are also controversial. Based on the findings of our research, considering the MIC values found in this study may be beneficial when using probiotic bacteria together with cinnamon extract in food formulations.

CONCLUSION

Chemical components such as phenolics and essential oils, etc., contained in spices and edible plants have been known to have antibacterial properties. It was determined that cinnamon ethanol, methanol, and chloroform extracts have strong antimicrobial activity against lactic acid bacteria used as probiotics in this study. Very few scientific studies have been conducted to

investigate the inhibitory effects of the cinnamon herbal extract on probiotic bacteria in vitro and in vivo. Most of the studies focus on their pathogen-related effects. Among the examined bacteria, *L. acidophilus* was observed to be the most sensitive probiotic bacteria to the extracts obtained. There are no standards such as EUCAST or CLCI used to determine antibiotic activity for herbal antimicrobial extracts. There is a need for standardization in these matters. The results of this preliminary study with standard strains provide useful information for determining the specifications for the antimicrobial activities of herbal extracts. It is thought that the results obtained in this study will also be beneficial in the production of probiotic food supplements and in the development of functional foods that provide health benefits beyond basic nutrition.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ayla Ünver Alçay: Project management, preparation of extracts, FTIR analyses, determination of antibiotic susceptibility of the strains by disc diffusion method, agar well diffusion method, minimum inhibitory concentration (MIC) analyses, evaluation and interpretation of the results, writing the draft of the article and English translation, and final check of the article. Aysun Sağlam: Preparation of extracts, determination of antibiotic susceptibility of the strains by disc diffusion method, agar well diffusion method, minimum inhibitory concentration (MIC) analyses evaluation, and interpretation of the results. Nagihan Kalıntaş Çağlar: Determination of antibiotic susceptibility of the strains by disc diffusion method, agar well diffusion method, minimum inhibitory concentration (MIC) analyses, evaluation and interpretation of the results, preparation of the draft of the article, statistical analysis. Sibel Kahraman: (FT-IR) spectroscopy evaluation of the results, total phenolic content assay, DPPH radical scavenging activity analyses, and evaluation of the results. Kamil Bostan: Project management and consultancy.

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