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Original Article

In Vitro Antimicrobial Efficacy Assessment of Ethanolic and Aqueous Extracts of Cinnamon (Cinnamomum Verum) Bark against Selected Microbes

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ABSTRACT

To combat pathogens and reduce the major public health problem of antibiotic residues in animal products, scientists are looking for natural antibiotic substitutes that are effective against drug-resistant pathogenic microbes and spoilage fungi. The antimicrobial activity of three *Cinnamomum verum* extracts prepared with three different solvents (absolute ethanol, 50% ethanol, and aqueous extracts) was determined against two Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) and two Gram-negative bacteria (*Salmonella Typhimurium* and *Escherichia coli*) as well as two fungal strains. The antimicrobial activities of various *Cinnamomum verum* extracts against selected microbes were evaluated using the disc diffusion test, minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), minimum fungicidal concentrations (MFC), and the poisoned food technique.

Cinnamomum verum bark (CVB) extracts inhibited and killed microbial growth to varying degrees. Our findings also revealed that extracts prepared with alcoholic solvents, particularly absolute ethanol-CVB extract, were more active compared with aqueous solvents, suggesting that the cinnamon plant is a promising natural antimicrobial agent for food preservation.

INTRODUCTION

Antibiotic resistance is a major public health issue caused by antibiotic residue in animal products. Antibiotic-resistant microbes have emerged as a result of excessive and arbitrary antibiotic use (Jayalakshmi *et al.*, 2017; Samanta & Bandyopadhyay, 2019). Thus, researchers have renewed their efforts to reduce the risk of antimicrobial resistance by seeking natural antibacterial agents (phytocompounds) that are effective against drug-resistant pathogenic bacteria (Sharma *et al.*, 2018). Foodborne pathogens including *Salmonella, Escherichia coli*, and *Staphylococcus aureus* (*St. aureus*), as well as *Listeria monocytogenes* (*L. monocytogenes*) are among the hazards that cause significant concern in the food industry worldwide (Paswan & Park, 2020). Because of their potential pathogenicity, there is a significant impact on public health, the cost of treating these infections, and economic implications for exporters. *L. monocytogenes* is associated with a high mortality rate (approximately 20%) and is regarded as one of the most dangerous foodborne illnesses (Rothrock Jr *et al.*, 2017). Between 1999 and 2019, *Salmonella* was the most common foodborne pathogen in African food exports to the European Union (Somorin *et al.*, 2021). *Salmonella Typhimurium* has been isolated from eggs and egg-associated cases of human salmonellosis in Europe, and has been linked to outbreaks involving infected laying flocks and contamination of egg contents (Wales & Davies, 2011). Fungal contamination of food, feed, and

other agricultural products leads to massive spoilage or decay and a variety of food safety issues (Saladino *et al.*, 2016).

Medicinal plants and spices have received considerable attention in this respect. Active compounds may be extracted from herbal and traditional medicines used to treat disease and to improve health (Sam, 2019). Many plants, including cinnamon (*Cinnamomum verum*), have medicinal properties. Cinnamon is an aromatic evergreen tree with oval-shaped leaves, thick bark, and berry fruit (Vangalapati *et al.*, 2012). The leaves and bark of the cinnamon tree have aromatic oils. Significant attention has been given to cinnamaldehyde extracted from cinnamon, a major constituent, because of its numerous biological activities including fragrance and a spicy taste produced by the absorption of oxygen (Yeh *et al.*, 2014). The plant has also shown antioxidant activity (Sharma *et al.*, 2016), antibacterial effects (Friedman, 2017; Nematollahi *et al.*, 2020), antiviral activity (Fauvelle *et al.*, 2017), antifungal properties (Rahemi *et al.*, 2015), antidiabetic activity, cholesterol lowering effects (Faghani *et al.*, 2014), and anticancer activity (Hong *et al.*, 2016).

Although cinnamon has been studied for its antimicrobial activity, a comparison of its activity using extracts at high concentration has not been thoroughly investigated. In the present study, cinnamon extracts prepared with hot water, 50:50 ethanol and water, and 100% ethanol were compared for their antimicrobial activity against four bacteria (*St. aureus*, *L. monocytogenes*, *Escherichia coli*, and *Salmonella enterica* subspecies Typhimurium) and two fungal isolates (*Aspergillus fumigatus* and *Aspergillus niger*).

MATERIALS AND METHODS

This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

Proximate Analysis of *Cinnamomum verum* Bark

Cinnamon (*Cinnamomum verum*) dry bark was purchased in Riyadh, Saudi Arabia, from a local herb store. The bark was crushed and ground into a fine powder (particle size: 0.25–0.30 mm) when it arrived. The cinnamon bark powder was directly examined. The water content, crude protein, crude fat content, crude fiber content, and ash content of the dried ground cinnamon bark were determined using a drying oven, the Kjeldahl method (N X 6.25) with a 2020 digester and a Velp UDK 140 distillation unit, the Soxhlet apparatus, a Dosi-Fiber, and combustion of the dried samples at 600°C for 6 hours. Based on AOAC-International (2006), the acid detergent fiber content was determined, and the neutral detergent fiber content was analyzed according to Holst (1973). A bomb calorimeter was used to calculate gross energy (kcal/kg). The data were all expressed in terms of dry matter.

Phytochemical Analysis of *Cinnamomum verum* Bark

The dried CVB (20 g) was macerated with 200 mL of 50% methanol at 25°C for 24 hours, then filtered, and centrifuged. Methanol was evaporated as described by Humeera *et al.* (2013). The supernatant was then injected into a high-performance liquid chromatography (HPLC) column in the amount of 10µL. Agilent Technologies series HPLC equipped with Zorbax RP-C_{18} column (1.0 cm long × 4.6 mm i.d., Agilent Shimadzu, Japan) was used for the analysis. Gallic acid, catechin, caffeine, and chlorogenic acid were used as external standards to identify the presence of phenols, flavonoids, alkaloids, and polyphenols, respectively. The ultraviolet absorbance at 280 nm was measured for detection. The mobile phase was a mixture of water, acetonitrile, ethyl acetate, glacial acetic acid, and methanol (89:6:3:1:1 v/v/v/v/v) and flowed at a rate of 1 mL/min. All chromatographic analyses were carried out at a temperature of 25°C ± 2°C.

Cinnamomum verum bark extract was analyzed using GC-MS on an Agilent 6890N gas chromatograph outfitted with an Agilent HP-5MS column and a 5973N selective mass detector. The oven temperature was raised from 60-320°C at a rate of 6°C/min. The constituents were recognized by comparing their mass spectra, retention indices, and bioactive constitutes quality to those in the National Institute of Standards and Technology database library (NIST-based AMDIS software).

Preparation of CVB-derived extracts

The dried CVB powder extracts for antimicrobial testing were prepared using the method described by Muthuswamy *et al.* (2008). Briefly, CVB extracts were prepared using three solvents: absolute ethanol (C100EOH), 50% ethanol (C50EOH) and pure distilled water (DW) alone (CWA).

Then, 150 g each of CVB powder was weighed and distributed into flasks (50 g/flask). Each container was

mixed thoroughly in a tightly sealed flask and separately dissolved 1:10 (w/v) in 500 mL sterile distilled water for water extraction, 500 mL of ethanol/water (2:2, v/v) for ethanol/water extraction, and 500 mL of absolute ethanol for ethanol extraction. The flasks were then boiled for 30 min, 45 min, and 5 h with reflux at 37°C in a water bath. Next, the supernatants were collected and filtered through 0.22 µm Millipore membrane filters (Whatman filter paper No. 1) into new sterile flasks. The filtrate was completely dried or evaporated using a 45°C oven or using a rotary evaporator (Eyela, Irvine, CA). Prior to in vitro antimicrobial testing, the dried cinnamonic extracts were weighed and dissolved in dimethyl sulfoxide (DMSO) to obtain stock concentrations of 500 mg/ mL of each cinnamonic extract.

Determination of extract yield (% Yield)

The percent yield (%, w/w) of each dried extract was calculated by dividing the extract's weight by the cinnamon bark powder weight: Yield (%) = (W_1 × 100)/W_2, where W_1 is the extract weight after the solvent dried and W_2 is the weight of the powder of cinnamon bark before extraction (Julianti *et al.*, 2017).

Antibacterial activity of CVB extracts

Microorganisms and preparation of bacterial inoculum

The reference strain of two Gram-positive bacteria: *St. aureus* American Type Culture Collection (ATCC) 29737 and *L. monocytogenes* ATCC 13932, two Gram-negative bacteria: *Escherichia coli* ATCC 25922 (*E. coli*) and *Salmonella enterica* subsp. Typhimurium (*S.* Typhimurium) ATCC 14026 were obtained from standard stocks in our Laboratory at King Saud University.

The bacterial inoculate was created using the standard protocol of the Clinical and Laboratory Standards Institute (CLSI, 2006). Bacterial strains were activated for 24 h by suspending in nutrient broth before subculturing on sterile nutrient agar plates. To ensure the bacteria were fully activated, subculturing was repeated three times. The inoculum suspension from each experiment was created by taking four or five pure colonies from a new culture. The suspensions of the colonies were suspended in sterile normal saline and diluted sequentially until the turbidity was comparable to a 0.5 McFarland turbidity standard. This yielded an inoculum density of approximately 10^8 colony-forming units (CFU)/mL.

Disc-Diffusion Susceptibility Test

Cinnamon bark extracts (absolute ethanol, 50% ethanol, and aqueous extract) were tested for antibacterial activity against several bacteria (*St. aureus*, *L. monocytogenes*, *E. coli*, and *S.* Typhimurium) using an agar diffusion test based on the standard protocol of the with minor modification. Briefly, after adjusting for the turbidity of the inoculum suspension with McFarland turbidity standards, the adjusted suspensions of microbes were gently swabbed onto sterile nutrient agar plates with a sterile cotton swab. The inoculum was dried in a safety cabinet (Nuaire Class II biological safety cabinet, Plymouth, MN, USA) for 30 min. Whatman filter paper (No. 3) was used to prepare circular antibiotic assay discs with a 6 mm diameter. The sterile dried disks were aseptically applied to the inoculated agar plates after soaking in 40 µL of each cinnamon extract. The plates were left at 4°C for 1 h before incubating at 37°C for 24 h. The diameter of the inhibition zones were then measured (in mm). Inhibition zones with diameters of <12, 12 to 16, and >16 mm were classified as having low, moderately active, and highly active antibacterial activity, respectively (Indu *et al.*, 2006). Microbes were tested for sensitivity to the standard antibiotics, piperacillin (100 mg) and erythromycin (15 mg), as positive controls. Paper disks soaked with the corresponding extraction solvent (DMSO) were used as negative controls. The tests were done in triplicate.

Minimum Inhibitory Concentration Determination

The minimum inhibitory concentration (MIC) of the cinnamon extracts was determined using the CLSI protocol and the microdilution method (a microplate method) (CLSI, 2012). The MIC was calculated by selecting the lowest concentration of each CVB extract that did not result in visible bacterial growth (Zhang *et al.*, 2016). The adjusted inoculum suspensions of bacteria were further diluted (1:100) to 10^6 CFU/mL. The test was performed using sterile 96-well flat microtiter plates (Nunclon, Denmark). Each well contained 50 µL of sterile Mueller Hinton broth (MHB) as a diluent. Then, twofold serial dilutions were prepared by taking 50 µL from the stock solution (500 mg/mL) of each extract into the first well and mixing the broth with the extract, then transferring 50 µL from the first well to the next and so on and discarding 50 µL from the last well. We prepared 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2,048, and 1:4,096 dilutions in order to obtain 250, 125, 62.5, 31.25,

15.63, 7.81, 3.91, 1.95, 0.98, 0.49, and 0.24 mg/mL concentrations of the extract, respectively, using 50% DMSO. Then, 50 µL of the adjusted inoculum (10^6 CFU) was added to each well except for the negative control wells. Positive controls were adjusted inoculum suspensions without the extract, whereas negative controls consisted of the medium only. The plates were incubated at 37°C for 18 h, and the results were visually read and confirmed through measurements with a spectrophotometer plate reader (Bekkar *et al.*, 2021). Wells with absorbance values comparable to the positive control indicated the presence of turbidity or bacterial growth, whereas wells with the lowest extract concentration and absorbance comparable to the negative control indicated complete growth inhibition and were considered the MIC value.

Minimum bactericidal concentrations (MBC) determination

The MBC is defined as the smallest concentration of antimicrobial agent required to completely inhibit bacterial growth. To calculate the MBC, 10 µL from the wells with no bacterial growth (no bacterial turbidity) was inoculated into sterile nutrient agar plates. Plates inoculated with bacteria served as a positive control, whereas plates containing only nutrient agar served as negative controls. Bacterial growth was measured after 48 h of incubation at 37°C. The experiment was conducted in triplicate.

Antifungal activity

To test the activity of CVB extracts against pure fungal strains *Aspergillus niger* ATCC 6275 and *Aspergillus fumigatus* ATCC 28282, the agar disk diffusion method (ADDM) and the poisoned food technique were used. The MIC and MFC were also determined. *Aspergillus fumigatus* was obtained from our lab's standard incubator and *Aspergillus niger* was obtained from the King Saud University's College of Food and Agriculture's Laboratory of Food Microbiology.

Preparation fungal spores

The fungal suspension was prepared by the methodology of European Committee on Antimicrobial Susceptibility Testing-Antifungal Susceptibility Testing Subcommittee methodology against Aspergillus spp (EUCAST-AST- ASPERGILLUS) (Lass-Flörl *et al.*, 2006). Briefly, fresh, mature (2 to 5 days old) cultures are grown on sterile potato dextrose agar at 35°C to prepare inoculum suspensions. The colonies were covered with 1 mL of sterile water to which 0.1% Tween 20 was added. The conidia was collected rationally with a sterile cotton swab and transferred to a sterile tube. A rotating vortex blender at 2,000 rpm was used to uniformize the inoculum for 15 seconds. Then the conidia was diluted with sterile water counted in a hemocytometer and examined for hyphae and clumps. In case a significant number of hyphae or clumps were present (> 5% of the fungal structures), the inoculum was filtered through a sterile nylon grid filter with a pore size of 11 mm. Then the suspension was adjusted with sterile DW to 2-5 x 10^6 CFU/mL by counting the conidia in a hemocytometer. This suspension was then further diluted at 1:10 ratio with sterile DW to give a final working inoculum of 2-5 x 10^5 CFU/mL. All adjusted suspensions were quantified by plating on SDA plates.

Miconazole nitrate (20 mg/g) was dissolved in DMSO at a concentration of (20mg/mL) and stored at -20°C. The stored solutions were then diluted to the prescribed concentrations used in this study. The final concentration of DMSO in the reaction mixture was limited to less than 1% (v/v) (Oh *et al.*, 1993).

Antifungal susceptibility tested by the disk diffusion method

This technique was used to determine the suppressive activities of CVB extracts against *Aspergillus niger* and *Aspergillus fumigatus* according to Naeini *et al.* (2009). Briefly, 10 µL of an adjusted suspension of *Aspergillus niger* or *Aspergillus fumigatus* was spread evenly on a potato dextrose agar plates. The inoculated plates were allowed to dry at room temperature in a safety cabinet. Then, blank paper disks with a thickness of 5 mm were impregnated with 40 µL of selected herbal extracts on the surface of the previously inoculated plates. The plates were parafilm-sealed and incubated at 35°C for 48-72 h. Each plate was examined after 48-72 h of incubation. The Diameter of inhibition zone (DIZ) including the diameter of the disk were both measured. Miconazole nitrate and DMSO-soaked paper disks were used as positive and NC, respectively. The experiment was performed in triplicate.

Determination of minimum inhibitory concentrations (MICs)

The MICs of CVB extracts on *Aspergillus niger* and *Aspergillus fumigatus* were determined using a microplate method as described by Lass-Flörl *et al.* (2006). The adjusted inoculum suspensions (containing conidia or sporangiospores) were mixed with a vortexer (2-5 x10^5 CFU/mL). The extracts of CVB were dissolved in DMSO (10% of final volume) and diluted

to a concentration of 500 mg/mL. Sterile 96-well flat microtiter plates (Nunclon, Denmark) were used for this assay. Each well contained 100µL of sterile RPMI 1640 supplemented with 2% glucose as test medium. Then, serial twofold dilutions (the concentration range obtained was between 250 and 0.24 mg/mL) of each of the stock solutions of the extracts (500 mg/mL) were performed. Then, with the exception of the NC, 100 µL of the adjusted inocula was added to each well. The diluted inoculum suspensions without extracts served as active controls and the medium used without extracts was used as NC. The plates were incubated in the incubator at 35°C for 48 h. The MIC was defined as the lowest concentration of the extracts in the wells that demonstrated no visible growth.

Determination of minimum fungicidal concentrations (MFC)

From the wells that showed no growth 10µL were taken and inoculated onto potato dextrose agar plates to determine MFC. The plates inoculated with fungi were served as positive controls and the plates with only potato dextrose agar were served as NC. The plates were incubated at 35°C for seven days and fungal growth was assessed. The MFC was defined as the highest dilution (lowest concentration of extracts) at which no growth occurred on the plates. The test was carried out in three replicates.

Poisoned food technique

According to Shrestha and Tiwari (2009), the antifungal activity of the extracts was evaluated using the poisoned food procedure. A 0.5 mL of each extract (250 mg/mL concentration) was poured into the Petri plate, followed by the addition of 9.5 mL of sterile melted potato dextrose agar medium to a final volume of 10 mL and then gently swirled to achieve thorough mixing of the contents. After solidification, an *Aspergillus niger* or *Aspergillus fumigatus* inoculum disk of 5 mm in diameter was aseptically inoculated upside down in the center of the plates and incubated at 30°C for 7 days. The plates with potato dextrose agar without extracts were served as NC and the plates with miconazole nitrate were served as PC. Subsequently, the average diameters of fungal growth were measured on the 7th day of incubation and then used to compute an inhibition of mycelial growth (Rao & Srivastava, 1994). The percentage (%) of inhibition of mycelial growth was determined as $[(g_c - g_t) / g_c] \times 100$, where g_c is the average increase in mycelial colony growth in the NC and g_t is the average increase in mycelial colony growth on the plates treated with the selected extracts.

Statistical analysis

The Statistical Analysis System (SAS (2012) software was used to analyze the data. The antimicrobial efficacy was expressed as the mean ± standard deviation (SD), and a one-way variance analysis was used to determine whether there were differences in the parameters between groups. The means of data were separated using Duncan's new multiple range test for statistical differences. *P*-values less than 0.05% ($p < 0.05$) were considered statistically significant.

RESULTS

Proximate analysis and phytochemicals of CVB powder

Proximate analysis revealed the macronutrients in CVB with varying rations, as shown in Table 1. Caffeine concentration was 700 g/g, with a retention time of 5.164 min, an area of 132732, and a height of 2865 measured using HPLC test. In addition, another unknown component was larger than caffeine (Figure 1). Therefore, GC-MS was used to detect the major volatile compounds of CVB extracts. A total of 22 different volatile compounds (Cinnamaldehyde and other 21 volatile compounds) were identified, as shown in Figure 2 and Table 3. Hexadecanoic acid, methyl ester, pentadecanoic acid- 14-methyl- methyl ester, Cinnamaldehyde, (E)-2-propenal, 3-phenyl-, oxime- methoxy-phenyl-, 2-methyl-benzofuran, and Tridecanoic acid, methyl ester were the compounds with the highest quality detected by GC-MS in the CVB extract.

Table 1 – Nutrient composition (% DM) of *Cinnamomum verum* bark powder through approximate analysis.[1]

Item	%
Moisture (% as fed)	10.29
Dry matter (% as fed)	89.71
Crude protein	4.43
Ether extract (Lipid)	4.03
Ash	3.18
Total crude fiber	24.35
[2]Nitrogen-free extract	64.02
[3]Organic matter	96.82
Total fiber fractions:	
A. Acid detergent fiber	45.75
B. Neutral detergent fiber	65.34
Gross Energy (Kcal/Kg)	4974.52

[1]NB: Average of three replicates. [2]NFE (calculated by difference) = 100 – (protein+lipid+ash+fiber), [3]Organic matter=100- Ash.

Table 2 – Contents of individual phenols and flavonoids (µg/g) of Cinnamon determined by high performance liquid chromatography.

Items[1]	Retention time (min)	Area	Height	Concentration (µg /g)
Caffeine	5.164	132732	2865	0.003
Catechin	-	-	-	-
Chlorogenic acid	-	-	-	-
Gallic acid	-	-	-	-

[1] External standards have reputable antioxidant, anti-inflammatory, and antimicrobial activities were examined.

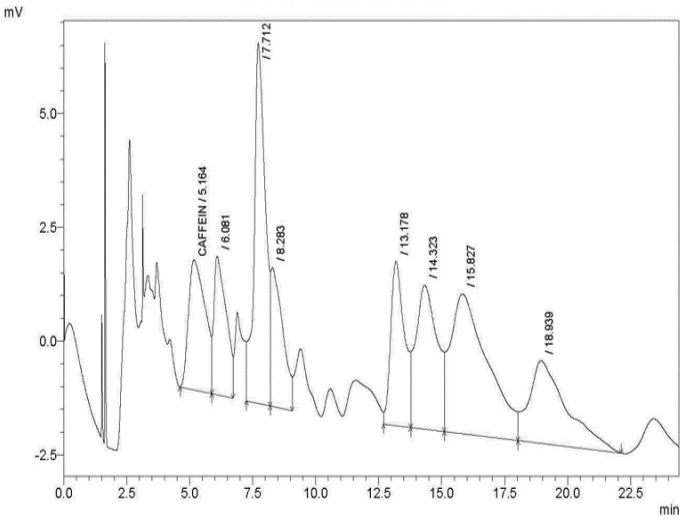

Figure 1 – *Cinnamomum verum* chromatogram of flavonoids and phenols mixture at 280 nm obtained by high performance liquid chromatography.

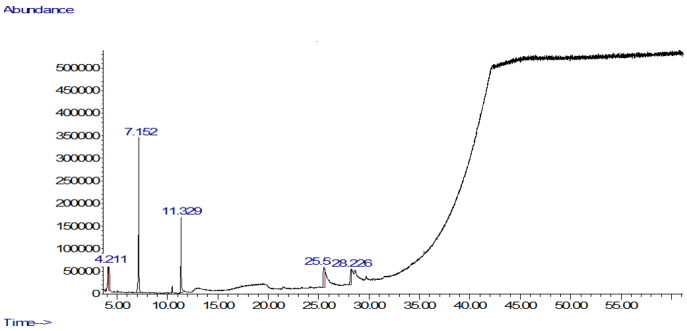

Figure 2 – *Cinnamomum verum* chromatogram of flavonoids and phenols mixture at 280 nm obtained by high performance liquid chromatography.

Cinnamon extracts yield

The yield from grinding 500 g of cinnamon bark was 475 g (95% w/w). The extraction solvent had a significant impact on yield. Fifty grams of cinnamon bark powder was extracted and concentrated using a 45°C oven. The final weight of the CVB extracts was 7.00, 6.75, and 6.24 g for 6.24, 6.75 and 7.00 g for C100EOH, C50EOH and CWA extract resulting in a yield percentage of 12.48, 13.50 and 14.00 (w/w), respectively.

Antibacterial activity

Screening for antimicrobial activity

The antibacterial effects of the three cinnamon bark extracts against four bacterial isolates (*St. aureus,*

Table 3 – Compounds identified in methanolic extract of *Cinnamomum verum* bark.

Retention Time (RT) (min)	Bioactive chemical constituents	Quality	Molecular weight (amu)	Molecular Formula
4.108	4-Ethylbenzoic acid, 2-butyl ester	78	206.131	$C_{13}H_{18}O_2$
4.108	2-Amino-6-methylbenzoic acid	59	151.063	$C_8H_9NO_2$
4.211	Oxime-, methoxy-phenyl-	87	151.063	$C_8H_9NO_2$
4.211	4-Ethylbenzoic acid, hexyl ester	50	234.162	$C_{15}H_{22}O_2$
7.152	Hexamethylcyclotrisiloxane	56	222.056	$C_6H_{18}O_3Si_3$
7.152	Silane, 1,4-phenylenebis[trimethyl-	50	222.126	$C_{14}H_{26}Si_2$
11.329	Cinnamaldehyde, (E)- 2-Propenal, 3-phenyl-	97	132.058	C_9H_8O
11.329	Benzofuran, 2-methyl-	87	132.058	C_9H_8O
11.329	Benzopyrimidine, 3,4-dihydro- 3,4-Dihydroquinazoline	59	132.16	$C_8H_8N_2$
11.329	2-Propenoyl chloride, 3-phenyl-, (E)-	53	166.6	C_9H_7ClO
11.329	4-Methylcoumarine-7-cinnamate	53	306.3	$C_{19}H_{14}O_4$
11.329	2-Nitrophenyl cinnamamide	50	268.085	$C_{16}H_{14}N_2O_4$
11.329	3-Butenoic acid, 2-oxo-4-phenyl-	50	176.17	$C_{10}H_8O_3$
25.536	Hexadecanoic acid, methyl ester	99	270.256	$C_{17}H_{34}O_2$
25.536	Pentadecanoic acid, 14-methyl-, methyl ester	98	270.5	$C_{17}H_{34}O_2$
25.536	Tridecanoic acid, methyl ester	83	228.37	$C_{14}H_{28}O_2$
25.536	Nonadecanoic acid, methyl ester	70	312.303	$C_{20}H_{40}O_2$
25.536	Octadecanoic acid, methyl ester	62	298.287	$C_{19}H_{38}O_2$
25.536	Methyl tetradecanoate	58	242.225	$C_{15}H_{30}O_2$
25.536	Pentadecanoic acid, methyl ester	58	256.24	$C_{16}H_{32}O_2$
25.536	Hexadecanoic acid, 15-methyl-, methyl ester	58	284.272	$C_{18}H_{36}O_2$
25.536	Heptadecanoic acid, methyl ester	58	284.272	$C_{18}H_{36}O_2$

L. monocytogenes, *E. coli*, and *S.* Typhimurium) were evaluated using the disc-diffusion antibiotic susceptibility test. The growth inhibition zones (mean ± SD) are presented in Table 4. Cinnamon extracts were found to have inhibitory effects against all of the tested isolates. For this test, the inhibition zones with a diameter greater than 6 mm were considered positive.

Compared to standard antibiotics (piperacillin), the extracts had the least significant antibacterial activity, but C100EOH similar effect to piperacillin. Piperacillin has been reported to have similar antimicrobial activity against these organisms when used as a standard. The Piperacillin antibiotic and absolute (100%) ethanol extract treatment resulted in the highest inhibition

Table 4 – Zone of inhibition (mm) of *Cinnamomum verum* bark extracts against selected bacteria.

Microorganism	C100EOH	C50EOH	CWA	Piperacillin	SEM	*p*-value
Salmonella Typhimurium ATCC 14026	14.33 ± 2.1^{a}	10.33 ± 2.1^{bA}	09.0 ± 1.0^{bA}	17.67 ± 1.7^{a}	1.08	0.0018
Escherichia coli ATCC 25922	12.8 ± 2.5^{b}	08.2 ± 0.3^{cB}	09.7 ± 0.6^{cdA}	16.17 ± 1.3^{a}	0.87	0.0008
Staphylococcus aureus ATCC 29737	13.0 ± 1.0^{b}	06.67 ± 0.3^{cB}	06.67 ± 0.3^{cB}	19.33 ± 0.9^{a}	0.456	<0.0001
Listeria monocytogenes ATCC 13932	11.33 ± 1.2^{b}	06.67 ± 0.3^{cB}	06.83 ± 0.3^{cB}	17.83 ± 0.2^{a}	0.363	<0.0001
Standard error mean (SEM)	1.031	0.618	0.354	0.833		
p-value	0.307	0.009	0.001	0.142		

Abbreviations: C100EOH, 100% ethanol; C50EOH, 50% ethanol; CWA, water alone cinnamon extraction; SEM. Values are mean±standard deviation of triplicate experiment; $^{a-c}$ Rows with different letters indicate statistically significant differences ($p<0.05$). ^{A,B} Columns with different letters indicate statistically significant differences ($p<0.05$).

zone (17.7 and 14.33 mm diameter, respectively) against *S.* Typhimurium ($p < 0.002$) compared with the 50% ethanol and aqueous extracts, which exhibited inhibition zones with diameters of 10.3 and 9.0 mm, respectively. When compared with cinnamon extracts, absolute ethanol extract treatment resulted in the largest diameter of inhibition zone (12.8, 14.3, 13.0, and 11.3 mm) against *E. coli* ($p=0.0008$), *S.* Typhimurium ($p<0.0008$), *St. aureus* ($p<0.0001$), and *L. monocytogenes* ($p<0.0001$). Therefore, the absolute ethanol extract (100% ethanol) was more active compared to the other Cinnamon extracts.

C100EOH has been reported to have the largest DIZ but showed insignificant effect against tested bacteria ($p=0.307$). However, C50EOH and CWA extracts of cinnamon bark were found to have different significantly antibacterial activity against the tested bacteria. C50EOH has been reported to have higher significantly ($p=0.009$) antibacterial activity against *S.* Typhimurium, and CWA has been reported to have higher significantly ($p=0.001$) antibacterial activity against *S.* Typhimurium and *E. coli* compared to other tested bacteria.

A microplate dilution test

The MIC and MBC values of the cinnamon extracts against *St. aureus*, *L. monocytogenes*, *E. coli*, and *S.* Typhimurium are summarized in Table 5. The CNB extracts have bacteriostatic and bactericidal effects on tested microbes at those MIC and MBC concentrations.

Table 5 – Minimum inhibitory concentrations (MIC) and Minimum bactericidal concentrations (MBC) values for *Cinnamomum verum* bark extracts on selected bacteria.

Microorganism	C100EOH	C50EOH	CWA	SEM	Probability
	Minimum inhibitory concentrations (MIC)				
Salmonella Typhimurium ATCC 14026	6.51 ± 2.3^{aB}	3.26 ± 1.1^{bB}	7.81 ± 0.0^{aC}	0.796	0.021
Escherichia coli ATCC 25922	7.81 ± 0.0^{B}	6.51 ± 2.3^{B}	7.81 ± 0.0^{C}	0.799	0.422
Staphylococcus aureus ATCC 29737	6.51 ± 2.3^{bB}	6.51 ± 2.3^{bB}	15.63 ± 0.0^{aB}	0.957	0.001
Listeria monocytogenes ATCC 13932	31.25 ± 0.0^{bA}	62.50 ± 0.0^{aA}	62.50 ± 0.0^{aA}	1.06	<0.0001
Standard error mean	1.031	0.618	0.354		
p-value	<0.0001	<0.0001	<0.0001		
	Minimum bactericidal concentrations (MBC)				
Salmonella Typhimurium ATCC 14026	13.02 ± 4.5^{aB}	6.51 ± 2.3^{bB}	15.62 ± 0.0^{aC}	1.67	0.021
Escherichia coli ATCC 25922	15.62 ± 0.0^{B}	13.02 ± 4.5^{B}	15.62 ± 0.0^{C}	1.501	0.422
Staphylococcus aureus ATCC 29737	13.02 ± 4.5^{bB}	13.02 ± 4.5^{bB}	31.26 ± 0.0^{aB}	2.123	0.001
Listeria monocytogenes ATCC 13932	62.50 ± 0.0^{bA}	125.0 ± 0.0^{aA}	125.0 ± 0.0^{aA}	0.001	<0.001
Standard error mean (SEM)	1.838	1.945	0.001		
p-value	<0.0001	<0.0001	<0.0001		

Abbreviations: Abbreviations: C100EOH: 100 percent ethanol; C50EOH: 50 percent ethanol; and CWA: water alone are used to extract *Cinnamomum verum* bark. Means of triplicates ±standard deviation. The lowest the value, the better; a,b Rows with different letters indicate statistically significant differences ($p < 0.05$). A–C Columns with different letters indicate statistically significant differences ($p < 0.05$). The concentrations of each extract were in the range of 500-0.12 mg.

The C100EOH showed antibacterial activity with MIC and MBC values of 6.51-31.25 mg/mL and 13.02-62.50 mg/mL, respectively. The MIC and MBC values for C100EOH were *S.* Typhimurium = *St. aureus* ≤ *E. coli* < *L. monocytogenes.* While, the C50EOH showed antibacterial activity with MIC and MBC values of 3.26-62.50 mg/mL and 6.51-125 mg/mL, respectively. The MIC and MBC values of C50EOH were in *S.* Typhimurium ≤ *St. aureus* = *E. coli* < *L. monocytogenes.* Furthermore, CWA extract exhibited the antibacterial activity with MIC and MBC values of 7.81-62.50 mg/mL and 15.62-125 mg/mL, respectively. The MIC and MBC values of CWA were in *S.* Typhimurium = *E. coli* ≤ *St. aureus* < *L. monocytogenes.*

Based on the MIC and MBC of the selected bacteria, the highest MIC (62.50 mg/mL) and MBC (125 mg/mL) were found in the presence of C50EOH and CWA against *L. monocytogenes.* On the contrary, the lowest MIC and MBC values were observed when C50EOH was tested with *S.* Typhimurium. Thus, the result revealed that the MIC values of all the extracts obtained from CVB tested here against selected bacteria were different and found to be the most active extracts. This was because these extracts had the lowest MBC values against tested bacteria except against *L. monocytogenes*. Very effective antimicrobial activity with bactericidal properties was observed at different concentration ranges against all clinical bacteria tested in this study. The results suggest that the phenols, tannins and flavonoids present in the herbal extracts might have increased toxicity against the pathogenic microorganisms.

Antifungal activity

The antifungal activity of CVB extracts against tested fungi (*Aspergillus fumigatus* and *Aspergillus niger*) using ADDM is presented in Table 6. The sensitivity test revealed antifungal inhibitory activity of C100EOH against *Aspergillus fumigatus,* with a DIZ of 11.33 mm, while the C50EOH and the CWA showed no inhibitory activity on *Aspergillus fumigatus*. The C100EOH and the CWA had inhibitory activity against *Aspergillus niger*, while the C50EOH had no inhibitory activity against *Aspergillus niger*. The CWA showed a stronger effect against *Aspergillus niger* than the other CVB extracts, with DIZ of 22.00 mm. Although C100EOH had an effect on fungal growth in both *Aspergillus fumigatus* and *Aspergillus niger* with a lethal effect with lowest MFC, the effect on these two isolates was less than the DIZ for miconazole. Therefore, C100EOH were the best fungi static and fungicidal compared to other cinnamon extracts.

Table 6 – *In vitro* antifungal activity (Zone of inhibition, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC)) of *Cinnamomum verum* bark extracts against *Aspergillus fumigatus* ATCC 28282 and *Aspergillus niger* ATCC 6275.

Fungal type/Extracts	C100EOH	C50EOH	CWA	Miconazole	SEM	Probability
	Inhibition zone (mm)					
Aspergillus fumigatus	11.3±1.7^{b}	6.0±0.0^{c} (R)	6.0±0.0^{c} (R)	15±0.82^{a}	0.667	<0.0001
Aspergillus niger	14.0±1.6^{b}	6.0±0.0^{c} (R)	22.0±1.0^{a}	20±1.87^{a}	1.051	<0.0001
	MIC (mg/mL)					
Aspergillus fumigatus	13.0±3.7^{b}	62.5±0.0^{a}	62.5±0.0^{a}	-	2.575	<0.0001
Aspergillus niger	1.3±0.5^{b}	6.5±1.8^{b}	13.1±0.0^{a}	-	1.692	0.008
	MFC (mg/mL)					
Aspergillus fumigatus	26.0±7.4^{b}	125.0±0.0^{a}	125.0±0.0^{a}	-	4.515	0.0079
Aspergillus niger	2.6±0.9^{b}	13.0±3.9^{b}	26.5±0.0^{a}	-	13.048	<0.0001

Abbreviations: C100EOH: 100 percent ethanol; C50EOH: 50 percent ethanol; and CWA: water alone are used to extract *Cinnamomum verum* bark; Means of triplicates ±standard deviation; The lowest the value, the better. $^{a–c}$ Row with different letters indicate statistically significant differences (p<0.05). R, resistance only disk zone "6 mm" without inhibition. (-) no tested. SEM: Standard error mean.

Table 7 showed the inhibitory effects of three CVB extracts on mycelial growth of selected fungi using food poisonous method. The cinnamon extracts showed high inhibitory activity (p<0.05) against *Aspergillus fumigatus* compared to the control; the degree of inhibition ranged from 59 to 100%, and the highest inhibitory activity was for C100EOH and C50EOH extracts (100% each), as the mycelial growth and speed were zero for both extracts. The CWA extract showed moderate inhibitory activity (59%) and had moderate mycelial growth (16.0±0.0) and mycelial velocity (3.64±0.03).

Similarly, the C100EOH extract exhibited the highest inhibitory activity (p<0.05) against *Aspergillus niger*. The inhibition threshold was 83.1±3.59 and 98.7±0.0 based on mycelial growth (19.33±0.0 mm and 1.0±0.0 mm) and mycelial velocity (4.13±0.66 and 1.69±0.03) for C50EOH and C100EOH, respectively. The water extract did not illustrate any inhibitory effect against *Aspergillus niger* as it could not prevent

Table 7 – *In vitro* antifungal activity of *Cinnamomum verum* bark extracts on mycelial growth of *Aspergillus fumigatus* (ATCC 28282) and *Aspergillus niger* (ATCC 6275) using food poisonous method.

Fungal type/ Extracts	C100EOH	C50EOH	CWA	Control (0 mg/mL)	SEM	Probability
	Mycelial growth (mm)					
Aspergillus fumigatus	0.0±0.0^{c}	0.0±0.0^{c}	16.0±0.1^{b}	39.00±0.0^{a}	0.001	<0.0001
Aspergillus niger	1.0±0.0^{c}	19.3±0.0^{b}	79.0±0.0^{a}	79.00±1.0^{a}	0.882	<0.0001
	Mycelial growth speed					
Aspergillus fumigatus	0.0±0.0^{b}	0.0±0.0^{b}	3.6±0.03^{a}	3.8±0.2^{a}	0.056	<0.0001
Aspergillus niger	1.7±0.1^{c}	4.1±0.6^{b}	21.0±0.0^{a}	21.0±0.0^{a}	0.190	<0.0001
	Growth inhibition (%)					
Aspergillus fumigatus	100.0±0.0^{a}	100.0±0.0^{a}	59.0±0.1^{b}	0.0±0.0^{c}	0.001	<0.0001
Aspergillus niger	98.7±0.0^{a}	83.1±3.6^{b}	0.0±0.0^{c}	0.0±0.0^{c}	1.116	<0.0001

The values are means of three replicates ± standard deviation; C100EOH: 100 percent ethanol; C50EOH: 50 percent ethanol; and CWA: water alone are used to extract *Cinnamomum verum* bark. a–d Row with different letters indicate statistically significant differences (p<0.05). SEM: Standard error mean.

mycelial growth (79.0±0.0 mm diameter) and mycelial velocity (21.0±0.0).

In general, the ethanol extracts of CVB showed the highest (p<0.05) inhibitory effect (≥83.1≤100%) on the growth of the selected fungi, while the water extract showed no or the least effect. In this *in vitro* experiment, miconazole was also used as a standard for antifungal activity, and it displayed high activity (15±0.82 mm for *Aspergillus fumigatus* and 20±1.87 mm for *Aspergillus niger*). The inhibition of mycelial growth of the tested fungi was 98.7-100%, 83.1-100%, and 0.0-59% for the extract of C100EOH, C50EOH, and CWA, respectively.

DISCUSSION

As far as we recognize, the search for herbal extracts with antimicrobial properties has increased due to their potential uses in a variety diseases. The current research focused on well-known medicinal plant, namely *Cinnamomum verum*. This is the first study to investigate the antimicrobial activity of CVB extracts using three solvents (absolute ethanol, 50% ethanol and water) against Gram-positive bacteria such as *St. aureus* and *L. monocytogenes*, Gram-negative bacteria (*S.* Typhimurium and *E. coli*) and fungal isolates (*Aspergillus fumigatus* and *Aspergillus niger*). Moreover, the extracts were tested for their antimicrobial activity using ADDM and plate dilution manner. The different extracts of CVB showed varying degree of inhibition against chosen bacteria. A positive result was detected when the zone of inhibition had a diameter >6 mm. Based on the obtained results, C100EOH extract had the highest antimicrobial activity against all bacteria. The ethanolic extracts of cinnamon was more effective than aqueous extract on both Gram-negative bacteria and Gram-positive bacteria (Mukhtar & Ghori, 2012). This was consistent with the results of this study based on solvent polarity, and it can be assumed that the lower the polarity of the extracts, the higher the antibacterial activity (Nawaz *et al.*, 2020). Although 100% ethanol is a good organic solvent for the extraction of most compounds, it is not as polar as water.

The extract yields indicated that the extraction solvents affected the recovery of crude powder extract prepared from *Cinnamon verum* bark.

The extraction yield, antioxidant properties, radical scavenging activity and phytochemical content were affected by the polarity of the extraction solvent because the antioxidant compounds in the plant parts have high affinity for more polar solvents compared to non-polar ones (Nawaz *et al.*, 2020).

They found that extraction in more polar solvents (water extract) resulted in a high yield but low flavonoid and phenolic content compared with that in non-polar solvents. These results suggested that absolute ethanol or a mixture of 50% (v/v) water with ethanol was the solvent of choice for yielding high levels of extractable solids compared with a water solvent alone.

Here in the ethanolic extract of cinnamon inhibited the mycelial growth of *Aspergillus fumigatus* and *Aspergillus niger* by 83.1-98.70 percent and 100 percent, respectively. Many studies have shown that cinnamon oil had inhibited numerous fungi including *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus versicolor*, *Aspergillus parasiticus*, and *Fusarium verticillioides* (Soliman & Badeaa, 2002; Nasir & Ali, 2020).

Several species of Cinnamon (family Asteraceae) have antimicrobial activity. However, the ADDM findings exhibited that the essential oils (EO) have a wide variety range of antimicrobial activity against pathogenic bacteria based on the presence of different minor and major constituents in the EO of each

plant due to the extraction method, plant species, plant part, microbial strains, geographical origin and environmental conditions, test conditions, test procedures and concentrations used … etc. (Oussalah *et al.*, 2007; Ozturk & Ozturk, 2007; Celikel & Kavas, 2008). Studying the antimicrobial activities of 22 EO, discovered discrepancies between ADDM and dilution tests. found that the ADDM is not suitable for samples that are difficult to dissolve in water or for nonpolar samples. In addition, there is no correlation between diffusion power and antimicrobial activity, nor between MIC values and growth inhibition diameter. The ADDM, on the other hand, was ideal for screening because it has a small sample size and can test multiple compounds against a single microorganism. A dilution method was recommended to determine MIC and MBC with the greatest precision and suitability for non-polar samples such as EO (Rios *et al.*, 1988; Khakhanang *et al.*, 2016). Therefore, the microplate dilution assay was used in this study to evaluate the potency of antimicrobial activity. The C50EOH extract had a lower minimal inhibitory effect on *S.* Typhimurium compared to the C100EOH. This extract was found to be inhibitory as well as bactericidal.

These data indicated that Gram-negative bacteria were more sensitive to CVB extracts than Gram-positive bacteria. Nevertheless, comparisons of bacterial susceptibility may differ. This was not only due to the antimicrobial agents in the EO, but also due to the diversity of microorganisms tested (Oussalah *et al.*, 2007; Kamazeri *et al.*, 2012). Another factor that may have contributed to the different results was the type of media used to test for antimicrobial activity. Bacterial growth conditions may differ when different types of media are used. For example, used nutrient broth medium in their study, used Muller Hinton agar medium in their study, while nutrient agar medium was used here. These aforementioned factors may be responsible for the contradictory results in each case. However, the promising antimicrobial agents and properties of the EO against bacteria and fungi, especially the extracts of CVB reported herein could recommend their use as antibiotics replacements in the cure of infectious diseases in animals.

For the same test strain, C100EOH revealed the highest antibacterial activity and DIZ (11.3 to 14.3 mm), and the C100EOH lowest MIC and MBC range, except for MIC and MBC against *S.* Typhimurium and *E. coli,* where C50EOH was lowest. Thus, C100EOH was much more sensitive to foodborne bacteria than the other extracts. These antibacterial activities may be attributed to the presence of the main bioactive compounds, especially (E)-cinnamaldehyde. These results suggest that the alcoholic ethanol extracts are superior to the water extracts and may be effective natural antibacterial agents. Thus, the CVB could be developed as a natural substitute for conventional food preservatives to extend the shelf life of food by preventing bacterial contamination during food production. *In vivo* experiments still need to be conducted to determine which bioactive compounds of cinnamon extracts are most effective against microbes. In addition, extra research is required to determine the synergistic antibacterial activity of cinnamon in combination with antibiotics or other herb extracts. reported DIZ for ethanol extracts against *L. monocytogenes*, *St. aureus*, *E. coli*, and *Salmonella anatum* ranging from 7.11 to 10.11 mm, while n-butane extracts yielded DIZ of 18.98 to 37.45 mm.

In this study, the DIZ ranged from 6.7 to 13.0 mm for Gram-positive bacteria and 8.2 to 14.3 mm for Gram-negative bacteria. These results are in agreement with those of Elisha *et al.* (2017), who found that Gram-negative bacteria were more responsive to extracts than Gram-positive bacteria. In contrast, Gram-positive bacteria were more sensitive to the cinnamon extracts than Gram-negative bacteria (Thirumurugan *et al.*, 2010; Raeisi *et al.*, 2015). These results of reported that both ethanol and n-butane extracts of cinnamon showed no significant differences between Gram-negative bacteria and Gram-positive bacteria. The difference in susceptibility between Gram-positive bacteria and Gram-negative bacteria may be the result of differences in cell wall structure (Branen & Davidson, 2004; Liang *et al.*, 2019). found that antimicrobial agents have difficulty penetrating the outer layer of Gram-negative bacteria. When antimicrobials such as monolaurin, nisin or EDTA were combined with EO of cinnamon, their effect on the selected bacteria, particularly *E. coli*, improved.

MICs of CVB ranged from 6.5 to 31.3 mg/mL for the absolute ethanol extract, 3.3 to 62.5 mg/mL for the 50% ethanol extract, and 7.8 to 62.5 mg/mL for the water extract. The MBC of CVB ranged from 13.2 to 62.5 mg/mL for the absolute ethanol extract, 6.5 to 125.0 mg/mL for the 50% ethanol extract, and 15.06 to 125.0 mg/mL for the water extract. This is somehow similar to the reports of who observed MBC values ranging from 20 to 160 mg/mL for ethanol extracts. Moreover, MIC and MBC of CVB extracts ranged from 3.3 to 7.8 for Gram-negative bacteria and from 6.5 to 62.5 mg/mL for Gram-positive bacteria. In contrast,

reported that MIC and MBC values ranged from 7.8 to 31.2 mg/mL for both types of bacteria. The C50EOH extract has significant bacteriostatic and bactericidal activity against *S.* Typhimurium.

The C100EOH extract showed bacteriostatic and bactericidal activity against the tested Gram-positive bacteria (*L. monocytogenes* and *St. aureus)*, which is confirmed by the sensitivity test results. Based on the obtained results, C100EOH can be used as an effective natural antimicrobial agent for safe preservation of food and drugs against bacterial contamination by *L. monocytogenes* and *St. aureus*. However, reported that absolute methanol extract of Chinese star anise had bacteriostatic and bactericidal activity against Gram-positive bacteria and Gram-negative bacteria that was less than or equal to that of 50% methanol and aqueous extracts of star anise. According to (El-Hack *et al.*, 2020), cinnamon oil may be an effective substitute for antibiotics in poultry by reducing fungi and coliform bacteria in the broiler chickens' intestinal contents (Krauze *et al.*, 2021).

The antimicrobial activity of cinnamon extracts has been associated with bioactive constituents of EOs, especially cinnamaldehyde, which is present in the dried bark (Firmino *et al.*, 2018). Carvacrol, eugenol, thymol, cinnamaldehyde, cinnamic acid, and perillaldehyde, all present in varying amounts in cinnamon, may also be considered for their potential synergistic and antagonistic activities (Burt, 2004). The antibacterial activity and mechanism of action of cinnamon and its derivatives on bacteria can be attributed to filamentous temperature-sensitive protein Z, biofilm inhibition, anti-quorum sensing activity, release of cellular components through loss of cell membrane integrity, permeability, morphological changes, effect on ATP levels and ATPase, and inhibition of bacterial acetyl-CoA carboxylase (López *et al.*, 2007; Doyle & Stephens, 2019). For example, the bacterial membrane integrity, permeability, and cell morphology are damaged when *E. coli* and *St. aureus* cells are exposed to MIC of cinnamaldehyde (0.31 mg/mL) (Shen *et al.*, 2015). In addition, the higher the concentration of cinnamaldehyde, the more severe the damage to the bacterial membrane. It is believed that the antibacterial effect of (E)-cinnamaldehyde results from its ability to hinder the cell membrane of bacteria and its structures, leading to ion leakage (Unlu *et al.*, 2010). (*E*)-cinnamaldehyde showed stronger antibacterial activity compared to eugenol and linalool (Herman *et al.*, 2016; Marchese *et al.*, 2017). Moreover, reported that 80% methanol extract of *Cinnamomum burmannii* exhibited antibacterial activity with significantly higher MBC against *E. coli* and *St. aureus* compared to water extraction of EO. discovered that the antimicrobial activity of EO of *Cinnamomum zeylanicum* was more effective compared to 50% ethanol extract. reported that a high concentration of phenolics extracted from the stem bark of *Cinnamomum verum* with a methanol solvent showed higher inhibitory activity against some organisms compared to an ethyl acetate extract. Thus, Cinnamon and its derivatives have various modes of action that will contribute to the development of new antibacterial agents in the future.

In addition, further research is needed on studying more details of *in vitro* cinnamaldehyde alone or in combination with other antibacterial products found in cinnamon on other pathogenic bacteria infected broiler chickens. The vast majority of plant extracts have tested positive for antibacterial activity. This confirms previous research that plant pathogenic fungi are more resistant to plant extracts than plant pathogenic bacteria (Heisey and Gorham, 1992). They found that 13 extracts inhibited bacterial growth, but only 5 inhibited fungal growth.

When agar gel inoculated with selected fungi is impregnated with disks of CWA alone or in combination with ethanol, it shows resistance without any inhibitory effect, except the effect of CWA on *Aspergillus niger*. This may be attributed to the fact that C100EOH extract produces more secondary bioactive components that cannot be extracted by other extracts, and that have an inhibitory effect on fungi. The mean diameter of mycelial growth of the tested fungi in the poisoned food plates was significantly smaller than that of the control plates, indicating that the extracts have antifungal potential. The inhibition was dependent on the alcohol concentration. Among the tested fungi, extract efficacy revealed that ethanolic CVB extracts inhibited the mycelial growth of the isolated fungi more than CWA alone. However, the average diameter of mycelial growth of *Aspergillus niger* in poisoned food plates with CWA was similar to that of control plates. Thus, the results of the susceptibility test of CWA against *Aspergillus niger* contradicted the food poisoning method, indicating that CWA had good efficacy to *Aspergillus niger,* but poor mycelial growth inhibition. The obtained results showed that the C100EOH extract with a much lower concentration compared to C50EOH and CWA extract exerted inhibitory (MIC) and lethal (MFC) effects on *Aspergillus fumigatus* and *Aspergillus niger*. Compared to control plates, the antifungal effect of extracts

in poisoned plates is demonstrated as a decrease in mycelial growth of selected fungi. Several researchers have used this method to evaluate the antifungal activity of herbs (Debonne *et al.*, 2018; Hu *et al.*, 2019). According to Gwa & Ekefan (2018), some plant extracts (neem, tobacco, ginger, papaya, black pepper, and mancozeb) have fungicidal activity against yam disease caused by *Aspergillus niger*. Cinnamaldehyde and its derivatives possess activity against a variety of pathogenic fungi such as *Aspergillus fumigatus* (Abd Rashed *et al.*, 2021), *Aspergillus niger* (Sun *et al.*, 2020), *Aspergillus flavus* (Bisceglie *et al.*, 2020), and *Candida spp* such as *Candida albicans* and *Candida glabrata* (Bakhtiari *et al.*, 2019). Interestingly, both geometric isomers of cis-cinnamaldehyde and trans-cinnamaldehyde have potential antifungal properties (Shreaz *et al.*, 2016). They confirmed the potential of cinnamaldehyde derivatives as antifungal alternatives to current remedies. Cinnamon oil was highly effective against *Aspergillus fumigatus* at a dose of 1 mL/kg ration and can be used in poultry industry to replace other antifungal drugs (Sadiek *et al.*, 2019).

The obtained food poisoning method results partially support the ADDM results suggesting that the C100EOH extract could be used as an effective natural fungicide in the food industry to prevent fungal contamination with *Aspergillus fumigatus* and *Aspergillus niger*. As well as the CWA extract against *Aspergillus niger*.

In the current study, the antifungal activity of cinnamon may be associated with the high content of cinnamaldehyde (97%) and other important bioactive constituents. Which interfere with several biological processes including electron transfer, and may react with nitrogen containing molecules (Gupta *et al.*, 2008). However, the majority of aldehydes and phenols in cinnamon EO can cause severe lesions and secondary damage to the membrane, resulting in increased antifungal activity (Tu *et al.*, 2018; Hu *et al.*, 2019). Consequently, the differences in bioactive substances produced by different CVB solvents may contribute to different antifungal extracts diversity. Moreover, natural antifungal agents are readily biodegradable, highly volatile, low toxic, produce little residue, and are harmless to non-target organisms. The inhibitory effect of plant EOs on many fungi is attributed to the components with high lipophilicity and low molecular weight, which can easily disrupt cell membranes and cause cytoplasm leakage (Chao *et al.*, 2005). Clove and cinnamon EOs as edible spices had the strongest antifungal activity and inhibited mycelial growth and spore germination of *Aspergillus niger, Aspergillus ochraceus,* and *Aspergillus oryzae* fungi isolated from moldy wheat bread (Hu *et al.*, 2019). discussed the mechanisms of action of EOs and most of their constituents as antifungal agents, as well as their role in blocking cell communication channels, mycotoxin production, and fungal biofilm formation. The CWA had the least activity on the fungi tested. The presence of more lipid soluble components in CVB could be the cause behind superior antifungal activity of the ethanol extracts. The high inhibitory activity of EOs and cinnamaldehyde against the selected fungi could be due to the presence of cinnamaldehyde in the oil (Hu *et al.*, 2019). Therefore, this can be used as a natural fungicide to control plant diseases and to preserve fruits and vegetables.

CONCLUSION

To summarize, four bacteria were isolated from pure colonies cultured and identified as St. aureus, L. monocytogenes, E. coli, and S. Typhimurium. The edible cinnamon bark spice extracts obtained with different solvents displayed varying degrees of inhibitory activity against these four bacteria. For the same test strain, the absolute ethanol extract of cinnamon exhibited the highest antibacterial activity, the largest diameter inhibition zone, the lowest MIC and MBC range, and the best inhibition of mycelial growth of fungi. Thus, the absolute ethanol extract of cinnamon bark was much more effective against foodborne bacteria than other extracts. CWA exhibited good effectiveness against Aspergillus niger but poor mycelial growth inhibition. The best MIC and MBC values were found with C50EOH against S. Typhimurium.

These antibacterial activities can be attributed to the presence of the principal bioactive constituents, particularly (*E*)-cinnamaldehyde. The outcome of this study indicates that ethanol alcoholic extracts are superior to water extracts and may represent effective natural antibacterial agents. Thus, the bark of *Cinnamomum verum* could be developed as a natural replacement for traditional food preservatives to extend the shelf life of food products by preventing microbial contamination during food manufacturing. *In vivo* trials need to be performed to determine which bioactive compounds of cinnamon extracts are most effective against microbes. Furthermore, more research is needed to determine the synergistic antibacterial activity of cinnamon when combined with antibiotics or other plant extracts. Field studies recommend testing the bioactive extract against the selected bacteria or

fungi. In addition, the results obtained in this study would help to develop new antimicrobial agents in the future.

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