Journal of Advances in Microbiology Research

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2020; 1(2): 42-50

www.microbiojournal.com
Received: 27-01-2020
Accepted: 29-02-2020

Ahuokpeme Ifunanyachukwu C
Department of Pharmaceutical Microbiology and Biotechnology, Madonna University Elele Rivers, Nigeria

Osuala Oluchi J
Department of Pharmaceutical Microbiology and Biotechnology, Madonna University Elele Rivers, Nigeria

Ezemba Chinyere C
Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University ULI, Anambra, Nigeria

Ezemba Arinze S
Chychy Gilgal Laboratory and consulting services Ichida, Anambra, Nigeria

Etikudike Victor O
Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University ULI, Anambra, Nigeria

Otolorin Isaac G
Department of Pharmaceutical Microbiology and Biotechnology, Madonna University Elele Rivers, Nigeria

Correspondence
Ahuokpeme Ifunanyachukwu C
Department of Pharmaceutical Microbiology and Biotechnology, Madonna University Elele Rivers, Nigeria

Antimicrobial effect of cinnamon bark extracts on microbial isolates

Ahuokpeme Ifunanyachukwu C, Osuala Oluchi J, Ezemba Chinyere C, Ezemba Arinze S, Etikudike Victor O and Otolorin Isaac G

Abstract
The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. The aim of this study is to evaluate the antimicrobial activity of *Cinnamomum zeylanicum* (cinnamon) stick/bark extract on microbial isolates. The cinnamon barks were grounded and divided into two, one was macerated in ethanol and the other was macerated in water for 72hrs. The extract was tested for its antimicrobial effect on *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus sp* and *Candida albicans* using agar well diffusion methods. GC-MS was carried out to determine its bioactive compounds. For the ethanol extract, 200mg/ml had the highest zone of inhibition for *S. aureus, P. aeruginosa. Bacillus sp* and *Candida albicans* with 9.50mm, 14mm, 19.50mm, 18mm respectively and the highest for *E. coli* was 25 mg/ml at 8.80mm. The lowest zones of inhibition was found at 6.26mg/ml for all isolates. For the aqueous extract, 200mg/ml had the highest zone of inhibition and the lowest was found in 6.25mg/ml for all isolates. The minimum inhibitory concentration for all the organism for both the aqueous and the ethanol extracts is 25mg/ml. There is no bactericidal action for the aqueous extract but for the ethanol extract, 200mg/ml was the minimum bactericidal concentration *for E. coli, P. aeruginosa* and *S. aureus* while 100mg/ml was the minimum bactericidal concentration for *Bacillus sp., and Candida sp.* In the aqueous extract, 2H-1-Benzopyran-2-one-4-diethylphosphonate was found to be the most abundant compound with a percentage area of 28.09%. In the ethanol extract, Octamethylcyclotetrasiloxane and, Dodecamethylpentasiloxane were found to be the most abundant compounds with percentage areas of 10.98% and 6.64% respectively. From the results presented, it showed that cinnamon barks have antimicrobial properties and also certain compounds that have been reported to contribute to its properties.

Keywords: Antimicrobial, phytochemicals, bactericidal concentration, pharmaceutical

Introduction
Natural products, especially those derived from plants, have been used to help humankind sustain its health since the dawn of medicine. Over the past century, the phytochemicals in plants have been a pivotal pipeline for pharmaceutical discovery. The fundamental of traditional medicine is their biological activity which makes use of the pharmacological effectiveness of original compounds found in the herbal preparations for the treatment of different human ailments [1]. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove the efficiency of these plants [2]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant [3]. Herbs and spices have been used since ancient times, not only as antioxidants and flavoring agents, but also for their antimicrobial activity against degradation induced by foodborne pathogens and food spoilage bacteria. There are many edible and medicinal plants with high antimicrobial effects, such as thyme (*Thymus vulgaris* L.), tea (*Camellia sinensis* L.), garlic (*Allium sativum* L.), turmeric (*Curcuma longa* L.), berries belonging to Rosaceae family, and cinnamon (species belonging to *Cinnamomum* genus). Cinnamon is of the genus *Cinnamomum* from the family Lauraceae. The term cinnamon commonly refers to the dried bark of *C. zeylanicum* and *C. aromaticum* [4] used for the preparation of different types of chocolate, beverages, spicy candies and liquors [5]. Cinnamon bark, leaves, flowers and fruits are used to prepare essential oils which are used in cosmetics or food products.

Antimicrobial resistance (AMR) happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. That means the germs are not killed and continue to grow. Factors that have contributed to the growing resistance problem include: increased consumption of antimicrobial drugs, both by humans and animals; and improper prescribing of antimicrobial therapy. Overuse of many common antimicrobials agents by physicians may occur because the choice of drug is based on a combination of low cost and low toxicity [6]. There may also be improper prescribing of antimicrobials drugs, such as the initial prescription of a broad-spectrum drug that is unnecessary or ultimately found to be ineffective for the organism(s) causing the infection [7]. The danger is that excessive use of antibiotics in humans leads to emergence of resistant organisms [8-9]. Continued increases in antimicrobial resistance have led to fewer treatment options for patients and an associated increase in morbidity and mortality. This has led to more severe infections needing more extensive treatment and longer courses of illness often requiring extended hospitalization the aim of this study is to evaluate the antimicrobial activity of *Cinnamomum zeylanicum* (cinnamon) stick extract on microbial isolates.

Method

Plant collection and Identification

The cinnamon bark strips were purchased from Wuse Market, Abuja. The samples were stored appropriately before the identification. It was identified by the botanist (Mr. Adeleke K.O.) of the Pharmacognosy department of Madonna University, Elele, Rivers State.

Extraction of active ingredients

The dried bark sticks of *Cinnamomum zeylanicum* were grounded to fine powder using a mechanical grinder. A 180g of quantity was macerated in 750ml of ethanol and 275ml of water for 72 hours in conical flasks. The mouth of the conical flask was then covered with an aluminum cork to prevent evaporation of solvent. After 72 hours, the mixtures were filtered properly using a filter paper to separate the marc from the extract which was collected in a beaker. The beakers were then transferred to a water bath where the extracts were concentrated until all the solvent was evaporated leaving the extracts behind.

Preparation of the Serial dilution

The work bench was first disinfected with ethanol before preparing the serial dilution. A serial dilution of the two extracts was prepared using 2-fold dilution method.

2g of the aqueous and ethanol extracts were weighed out into beakers and dissolved in 10mls of Dimethylsulfoxide (DMSO) to get a stock solution of 200mg/ml. 6 test tubes labeled A-F with their corresponding concentrations that would be prepared in them (i.e. A = 100mg/ml, B = 50mg/ml, C = 25mg/ml, D = 12.5mg/ml, E = 6.25mg/ml and F=200 mg/ml) and were arranged in a test tube rack and 5mls of DMSO was introduced into each test tube. 5mls was then transferred from the stock solution (test tube F) to test tube A giving the resulting concentration of 100mg/ml. The process repeated for the rest of test tubes until the final dilution in test tube E.

Confirmation of test isolates

The isolates were sub-cultured from preserved agar slants onto different selective media (media for bacteria) and Saboraud Dextrose agar (all-purpose media for fungi) 24 hours prior to use for the bacteria and 72 hours prior to use for the fungi. Biochemical tests were also used to confirm the isolates gotten from the lab. The biochemical tests used were catalase test, coagulase test, indole test, oxidase test and gram staining and lactophenol cotton blue staining for fungi.

Standardization of inoculums

The required number of cells to be used as inoculum is 1.5 x 10^8 cfu/ml which is the 0.5 McFarland standard. This was achieved by using a spectrophotometer to dilute the cells to 0.08-0.1 OD at a wavelength of 600nm.

Antimicrobial Susceptibility Testing

To test for the susceptibility of the test isolates to the extracts, the agar well diffusion technique was used. Agar diffusion test using punch-hole method was carried out to determine the susceptibility of the microbial isolates to the plant extract. Mueller-Hinton agar plates were prepared and the isolates were swabbed on the surface of the sterile plates and a sterile cork borer of 8mm was used to bore holes on the solidified agar plates and 50 µl of the extract was introduced into the bored holes. Muller-Hinton agar is a loose agar that allows for better diffusion of the antibiotics than most other media. A better diffusion leads to a truer zone of inhibition [10]. This criterion is missing in Nutrient agar.

For the susceptibility testing of *Candida albicans*, the Mueller- Hinton supplementary agar plate was prepared with the Mueller-Hinton agar supplemented with 2% glucose and 0.5µg/ml of methylene blue. The fungi isolate was swabbed across the plates. Using a sterile standard cork borer of 8mm, wells were bored at equal distance around the plates. A 50µl volume of each concentration of each extract was aseptically introduced into each well on the agar plates. The plates were left for 30 minutes for the extracts to diffuse across the agar plates. The inoculated plates were then inverted and incubated for 24 hours. The inhibition zone diameter (IZD) was measured for all wells using a well calibrated ruler [11].

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the aqueous and ethanol extracts on the microbial isolates was obtained using the agar dilution technique which was plated out and observing for visibility after 24 hours [12]. For this process, 6 bijou bottles containing 19mls of Mueller hinton agar were autoclaved. Before pouring them into the petri dishes, 1ml of each concentration for the ethanol extract was added to the corresponding bijou bottles to make up 20mls volume. The mixture was shaken slightly for proper mixing before being poured into the petri dishes and allowed to solidify. The petri dishes were divided into four equal quadrants, where each quadrant was swabbed with the corresponding bacterial organism. This same process was followed when using the aqueous extract.

For the fungi *Candida albicans*, a total of 12 agar plates were prepared to check the MIC using both the ethanol and aqueous extracts. Following the same method used to check the MIC of the bacteria, 6 bijou bottles containing 19mls of Mueller-Hinton supplementary agar were autoclaved. After which 1ml of each concentration of the ethanol extract was

added to a corresponding bottle to make up 20mls volume and they were slightly shaken to ensure proper mixing before being poured into the petri dishes and left to stand. The fungi organism was then swabbed properly unto the petri dishes and observed for 24 hours to check for growth.

Minimum Bactericidal Concentration (MBC)
The minimum bactericidal concentration of the aqueous and ethanol extracts on the microbial isolates was obtained from the MIC after incubating of the petri plates of the 24hrs MIC plates for another 24hrs.

GC-MS Methodology
The GC-MS analysis was carried out using GC-MS-QP 2010 Plus Shimadzu system and gas chromatograph interfaced with a mass spectrometer system using the following conditions. Elite - 1 column fused silica capillary column (30 m x 0.25 mm 1 D x µL composed of 100% dimethyl polysiloxane). An electron ionization system with ionization energy of 70 eV was used. 99.99% helium gas was used as the carrier gas with flow rate of 1 mL/min with an injection volume of 2 µL. injection temperature of 280°C and ion source temperature of 280°C was used. The oven temperature was programmed at 110°C. The relative percentage amount of each component was matched with the data available in the National Institute of Standard and Technology (NIST) library.

Data Analysis
The experiments were carried out in duplicates and the results were expressed as mean and standard error of mean (x±SEM). The results were analyzed using the one-way ANOVA method with a significance value of p=0.05. The statistical package used was the graphpad prisim 5 software.

Results

Standardization of Extract
The aqueous and ethanol extract of *Cinnamomum zeylanicum* were prepared and tested for its antimicrobial properties against *Escherichia coli, Bacillus sp, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans.*

Ethanol extract
Weight of powdered sample = 180g
Weight of solvent = 750ml
Weight of extract =12.88g
Concentration of extract = 200mg/ml

Aqueous extract
Weight of powdered sample= 180g
Weight of solvent = 275ml
Weight of extract = 12.58g
Concentration of extract= 200mg/ml

Morphological and biochemical results of microbial isolates
The biochemical tests on the organisms confirmed the presence of *E.coli, S.aureus, P.aeruginosa, Bacillus sp* and *C.albicans.*From the results seen in Table 1, the gram staining test confirmed gram negative rod for *E.coli* and *P. aeruginosa,* gram positive cocci for *S. aureus,* gram positive rod for *Bacillus sp.* Catalase test showed a positive result for all the bacteria isolates. The coagulase test showed negative for *E. coli, P. aeruginosa* and *Bacillus sp* while *S. aureus* had positive results. For the indole test, *E.coli* was the only organism that tested positive while the other organisms were negative. The oxidase test had positive results for *P. aeruginosa* while *E.coli, Bacillus sp* and *S. aureus* had negative results.

Table 1: Microbial and biochemical results of microbial isolates

Microbial Isolates	Cultural Characteristics	Gram Staining	Catalase	Coagulase	Indole	Oxidase	Lactophenol cotton blue staining	Organism
Escherichia coli	On Eosin methylene blue agar, colonies are 2-3mm in diameter and have a greenish metallic sheen in reflected light.	-ve rod	+ve	-ve	+ve	-ve	ND	Affirmed
Pseudomonas aeruginosa	On Cetrimide Agar, colonies are medium sized and irregular growth is observed due to the swarming of the bacterium. Pigment production is enhanced and diffused in the medium imparting characteristic greenish-blue coloration to the medium.	-ve rod	+ve	-ve	-ve	+ve	ND	Affirmed
Staphylococcus aureus	On Mannitol Salt Agar, colonies are 2-3mm in diameter, with a smooth, shiny surface, opaque appearance and often pigmented golden yellow	+ve cocci	+ve	+ve	-ve	-ve	ND	Affirmed
Bacillus sp	On Nutrient agar, colonies appear rough, opaque, fuzzy white or slightly yellow with jagged edges.	+ve rod	+ve	-ve	-ve	+ve	ND	Affirmed
Candida albicans	On Sabouraud Dextrose agar, *Candida albicans* shows white colored, smooth and yeast-like appearance.	ND	ND	ND	ND	ND	Circular bluish colonies appearing in clusters	Affirmed

Key: +ve = Positive; -ve = Negative; ND-Not determined

Antimicrobial studies of *Cinnamomum zeylanicum* on microbial isolates
Agar well diffusion method was carried out with the aqueous and ethanol extracts of cinnamon bark on *Escherichia coli, Bacillus sp, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*. Table 2 and Table 3 show the different zones of inhibition from different concentrations of the aqueous and ethanol extracts respectively of cinnamon on *Escherichia coli, Bacillus sp, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans.* The result shows the mean zones of inhibition calculated from the duplicates of the experiment.

Table 2: Antisusceptibility Activity of Ethanol Extract of *Cinnamomum Zeylanicum* on Microbial Isolates

Concentrations	MZI (X±SEM)				
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Bacillus sp***	***Candida sp***
200mg/ml (mm)	19.50±0.5	7.50±5.50	14.00±3.0	19.50±1.5	18.00±0.0
100mg/ml (mm)	16.00±2.0	5.50±5.50	11.50±0.50	18.50±1.5	14.00±2.0
50mg/ml (mm)	13.00±1.0	5.00±5.00	8.50±1.50	12.00±2.0	8.50±1.5
25mg/ml (mm)	9.50±2.5	8.50±1.50	6.00±2.00	8.50±1.5	7.50±0.5
12.5mg/ml (mm)	7.00±0.0	8.00±1.50	3.50±2.50	5.50±0.5	6.00±1.0
6.25mg/ml (mm)	2.00±0.0	3.50±3.50	1.00±1.00	4.50±0.5	6.00±1.0
Ciprofloxacin (mm)	26.50±0.5	22.00±2.00	15.00±1.00	18.00±4.0	ND
Fluconazole (mm)	ND	ND	ND	ND	13.00±1.0
DMSO (mm)	0	0	0	0	0

The values are expressed as mean ± SEM. There is no significant difference in the analysis between the columns with P Value = 0.6570; $p>0.05$ significance when compared between columns using One-way ANOVA.
Key: x = mean; SEM = Standard Error of Mean; ND = Not determined.

Table 3: Ant susceptibility Activity of Aqueous Extract of *Cinnamomum Zeylanicum* on Microbial Isolate

Concentrations	MZI (X±SEM)				
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Bacillus sp***	***Candida sp***
200mg/ml (mm)	7.00±1.0	10.00±2.0	6.00±0.0	7.00±2.0	8.00±2.0
100mg/ml (mm)	5.00±1.0	7.50±3.5	5.00±0.0	6.00±1.0	6.00±1.0
50mg/ml (mm)	4.00±0.0	6.00±3.0	5.00±0.0	3.00±0.00	5.00±1.0
25mg/ml (mm)	3.00±0.0	5.00±2.0	3.50±0.5	0.00±0.0	3.00±1.0
12.5mg/ml (mm)	2.50±0.5	3.00±3.0	3.00±0.0	0.00±0.0	1.5±1.5
6.25mg/ml (mm)	0.00±0.0	0.00±0.0	0.00±0.00	0.00±0.0	1.00±1.0
Ciprofloxacin (mm)	26.50±0.5	22.00±2.00	15.00±1.00	18.00±4.0	ND
Fluconazole (mm)	ND	ND	ND	ND	13.00±1.0
DMSO (mm)	0	0	0	0	0

The values are expressed as mean ± SEM. There is no significant difference in the analysis between the columns with P Value = 0.9321; $p>0.05$ significance when compared between columns using One-way ANOVA.
Key: x = mean; SEM = Standard Error of Mean; ND = Not determined.

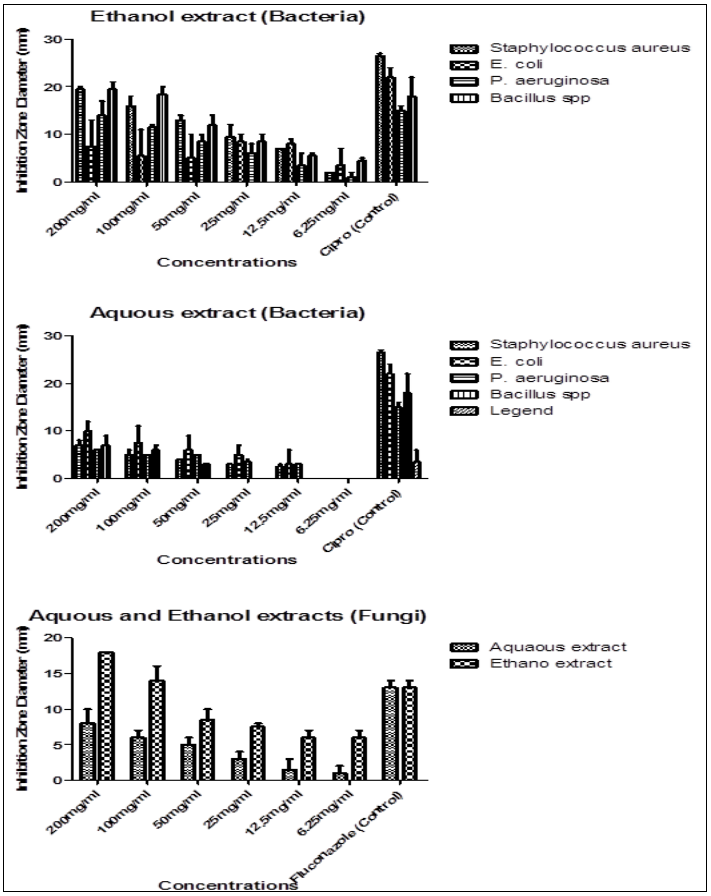

Fig 1: Graph showing the Mean zone of inhibition of the extracts against the Microbial Isolates

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the aqueous and ethanol extracts on the microbial isolates was obtained using the agar dilution technique. From table 4, the MIC of the aqueous and ethanol extract of *C. zeylanicum* on all the organisms evaluated is 12.5mg/ml.

Table 4: Minimum Inhibitory Concentration Aqueous extract

Isolates	200mg/ml (Stock solution)	100mg/ml (A)	50mg/ml (B)	25mg/ml (C)	12.5mg/ml (D)	6.25mg/ml (E)
E. coli	-	-	-	-	+	+
P. aeruginosa	-	-	-	-	+	+
Bacillus sp	-	-	-	-	+	+
S. aureus	-	-	-	-	+	+
C. albicans	-	-	-	-	+	+

Key: + = growth= no growth

Table 5: Minimum Inhibitory Concentration Ethanol extract

Isolates	200mg/ml (Stock solution)	100mg/ml (A)	50mg/ml (B)	25mg/ml (C)	12.5mg/ml (D)	6.25mg/ml (E)
E. coli	_	_	_	_	+	+
P. aeruginosa	_	_	_	_	+	+
Bacillus sp	_	_	_	_	+	+
S. aureus	_	_	_	_	+	+
C. albicans	_	_	_	_	+	+

Key: + = Positive= Negative

Minimum Bactericidal Concentration (MBC)

There is no bactericidal activity for the aqueous extract of *C. zeylanicum* on the isolates evaluated.

For the MBC of the ethanol extract of *C. zeylanicum*, the MBC of *E. coli*, *P. aeruginosa* and *S.aureus* was 200mg/ml. The MBC of *Bacillus sp* and *C. albicans* were 100mg/ml.

Table 6: Minimum Bactericidal Concentration Aqueous extract

Isolates	200mg/ml (Stock solution)	100mg/ml (A)	50mg/ml (B)	25mg/ml (C)	12.5mg/ml (D)	6.25mg/ml (E)
E. coli	+	+	+	+	+	+
P. aeruginosa	+	+	+	+	+	+
Bacillus sp	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+
C. albicans	+	+	+	+	+	+

Key: + = Positive

Table 7: Minimum Bactericidal Concentration Ethanol extract

Isolates	200mg/ml (Stock solution)	100mg/ml (A)	50mg/ml (B)	25mg/ml (C)	12.5mg/ml (D)	6.25mg/ml (E)
E. coli	_	+	+	+	+	+
P. aeruginosa	_	+	+	+	+	+
Bacillus sp	_	_	+	+	+	+
S. aureus	_	+	+	+	+	+
C. albicans	_	_	+	+	+	+

Key: + = Positive = Negative

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was carried out on the ethanol and the aqueous extracts and various compounds were identified. In the aqueous extract, 2H-1-Benzopyran-2-one-4-diethylphosphonate was found to be the most abundant compound with a percentage area of 28.09%. Hexa-methylcyclotrisiloxane and Decamethylcycl-opentasiloxane were found to be the second and third most abundant compounds with percentage areas of 17.69% and 12.39% respectively. The least abundant compounds include Benzo[h]quinoline, 2,4-dimethyl-, 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl- and 2-p-Nitrophenyl-oxadiazol-1,3,4-one-5 which have percentage areas of 0.18%, 0.26% and 0.25% respectively.

In the ethanol extract, Octamethylcyclotetrasiloxane and, Dodecamethylpentasiloxane were found to be the most abundant compounds with percentage areas of 10.98% and 6.64% respectively. The least abundant compounds include 13H-Dibenzo [a,i] carbazole, 2,5-Dihydroxybenzaldehyde, 2TMS derivative and Tris (tert-butyldimethylsilyloxy) arsane with percentage areas of 0.43%, 0.45% and 0.51% respectively.

Table 8: Gas Chromatography-Mass Spectrometry results for Aqueous extract of *C. zeylanicum* bark

Peak	RT (seconds)	Area%	Library/ID	Molecular weight (g/mol)	Molecular formular
1	5.482	17.69	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
2	6.569	0.33	Silane, triethoxymethyl-	178.30	$C_7H_{18}O_3Si$
3	6.638	0.23	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
4	7.118	0.26	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
5	7.633	0.61	2-Methoxy-2'-methyl-stilbene	224.30	$C_{16}H_{16}O$
6	7.713	6.53	5-Fluoro-8-quinolinol	163.15	C_9H_6FNO

7	7.822	1.48	4-Methylmandelic acid, di-TMS	310.53	$C_{15}H_{26}O_3Si_2$
8	8.160	1.61	Pentasiloxane, dodecamethyl-	384.84	$C_{12}H_{36}O_4Si_5$
9	8.314	28.09	2H-1-Benzopyran-2-one-4-diethylphosphonate	282.23	$C_{13}H_{15}O_5P$
10	8.818	0.35	Ethyl 3-amino-4-(1,3-benzodiazol-1-yl)benzoate	232.24***	$C_{11}H_{12}N_4O_2$***
11	10.105	0.21	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
12	10.151	0.64	2,4,4,6,6,8,8-heptamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocan-2-ol	298.59	$C_7H_{22}O_5Si_4$
13	10.225	0.27	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
14	10.529	0.39	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
15	10.780	12.39	Cyclopentasiloxane, decamethyl-	370.77	$C_{10}H_{30}O_5Si_5$
16	10.958	0.41	Tetrasiloxane, decamethyl-	310.68	$C_{10}H_{30}O_3Si_4$
17	11.364	0.28	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
18	11.759	0.29	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
19	12.188	0.46	1,2-Bis(trimethylsilyl)benzene	222.47	$C_{12}H_{22}Si_2$
20	12.514	0.34	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
21	12.897	0.28	2-Ethylacridine	207.27	$C_{15}H_{13}N$
22	13.121	0.30	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
23	13.229	5.73	Cyclohexasiloxane, dodecamethyl-	444.92	$C_{12}H_{36}O_6Si_6$
24	13.395	0.76	Cyclohexasiloxane, dodecamethyl-	444.92	$C_{12}H_{36}O_6Si_6$
25	14.791	0.49	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	282.29	$C_{17}H_{14}O_4$
26	14.997	0.25	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	207.14	$C_8H_5N_3O_4$
27	15.432	1.98	Pentasiloxane, dodecamethyl-	384.84	$C_{12}H_{36}O_4Si_5$
28	15.604	0.95	Cycloheptasiloxane, tetradecamethyl-	519.07	$C_{14}H_{42}O_7Si_7$
29	15.873	0.26	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	250.48	$C_{13}H_{22}OSi_2$
30	15.953	0.24	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
31	16.039	0.18	Benzo[h]quinoline, 2,4-dimethyl-	207.27	$C_{15}H_{13}N$
32	16.285	0.23	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
33	16.571	0.18	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
34	17.401	1.00	3,4-Dihydroxyphenylglycol, 4TMS de	458.9	$C_{20}H_{42}O_4Si_4$
35	17.567	0.57	(E)-2-bromobutyloxychalcone	359.3	$C_{19}H_{19}BrO_2$
36	19.100	0.59	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	503.07	$C_{14}H_{42}O_6Si_7$
37	19.289	0.78	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
38	20.622	0.52	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	264.43	$C_{15}H_{24}O_2Si$
39	20.840	0.32	Tris(tert-butyldimethylsilyloxy)arsane	468.7	$C_{18}H_{45}AsO_3Si_3$
40	22.018	0.92	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	503.07	$C_{14}H_{42}O_6Si_7$
41	22.276	0.34	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	264.43	$C_{15}H_{24}O_2Si$
42	23.289	1.54	Formic acid, 1-(4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-a]pyrimidin-5-yl)-, methyl ester	207.19	$C_9H_9N_3O_3$
43	23.540	0.35	4-tert-Amylphenol, TMS derivative	236.42	$C_{14}H_{24}OSi$
44	24.462	1.45	Cyclononasiloxane, octadecamethyl-	667.4	$C_{18}H_{54}O_9Si_9$
45	24.731	0.38	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	264.43	$C_{15}H_{24}O_2Si$
46	25.566	1.55	Cyclononasiloxane, octadecamethyl-	667.4	$C_{18}H_{54}O_9Si_9$
47	26.602	1.51	Cyclononasiloxane, octadecamethyl-	667.4	$C_{18}H_{54}O_9Si_9$
48	27.575	1.35	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	503.07	$C_{14}H_{42}O_6Si_7$
49	28.490	1.16	trans-3-Ethoxy-b-methyl-b-nitrostyrene	207.23	$C_{11}H_{13}NO_3$
50	29.491	0.99	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$

Table 9: Gas Chromatography-Mass Spectrometry results for Ethanol extract of *C. zeylanicum* bark

Peak	RT (seconds)	Area%	Library/ID	Molecular weight	Molecular formular
1	5.539	4.10	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
2	6.203	0.44	Pentasiloxane, dodecamethyl-	384.84	$C_{12}H_{36}O_4Si_5$
3	6.569	0.62	Tris(tert-butyldimethylsilyloxy)arsane	468.7	$C_{18}H_{45}AsO_3Si_3$
4	6.884	0.51	Tris(tert-butyldimethylsilyloxy)arsane	468.7	$C_{18}H_{45}AsO_3Si_3$
5	7.210	2.06	Benzene, 1-ethoxy-4-isothiocyanato	179.24	C_9H_9NOS
6	7.416	0.99	3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyltrisiloxane	324.63	$C_{12}H_{32}O_4Si_3$
7	7.628	1.15	(4H)1,3,2-Dioxaborin, 4-ethenyl-4, 6-diethyl-5-(1-methylethyl)-	208.11	$C_{12}H_{21}BO_2$
8	7.702	5.98	Arsenous acid, tris(trimethylsilyl) ester	342.49	$C_9H_{27}AsO_3Si_3$
9	7.817	1.87	1-Methyl-3-phenyl-3,4-dihydro-1H-quinolin-2-one	237.3	$C_{16}H_{15}NO$
10	8.097	6.64	Pentasiloxane, dodecamethyl-	384.84	$C_{12}H_{36}O_4Si_5$
11	8.160	0.34	Pentasiloxane, dodecamethyl-	384.84	$C_{12}H_{36}O_4Si_5$
12	8.297	10.98	Cyclotetrasiloxane, octamethyl-	296.61	$C_8H_{24}O_4Si_4$
13	8.589	3.25	Diethyl sulfate	154.19	$C_4H_{10}O_4S$
14	8.669	0.78	Pentasiloxane, dodecamethyl-	384.84	$C_{12}H_{36}O_4Si_5$

15	9.053	0.74	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetrasiloxan -1-ol	370.74	$C_{12}H_{34}O_5Si_4$
16	9.745	0.76	Cyclopentasiloxane, decamethyl-	370.77	$C_{10}H_{30}O_5Si_5$
17	9.922	1.76	2,4,4,6,6,8,8-heptamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocan-2-ol	298.59	$C_7H_{22}O_5Si_4$
18	10.151	0.43	13H-Dibenzo[a,i]carbazole	267.3	$C_{20}H_{13}N$
19	10.231	0.45	2,5-Dihydroxybenzaldehyde, 2TMS derivative	282.48	$C_{13}H_{22}O_3Si_2$
20	10.346	5.13	Cyclopentasiloxane, decamethyl-	370.77	$C_{10}H_{30}O_5Si_5$
21	10.535	1.54	Tris(tert-butyldimethylsilyloxy)arsane	468.7	$C_{18}H_{45}AsO_3Si_3$
22	10.775	5.12	Cyclopentasiloxane, decamethyl-	370.77	$C_{10}H_{30}O_5Si_5$
23	11.370	0.55	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_3Si_3$
24	12.114	0.72	Silane,dimethyl(dimethyl(dimethyl(2-isopropylphenoxy)silyloxy)silyloxy) (2-isopropylphenoxy)-	476.8	$C_{24}H_{40}O_4Si_3$
25	12.320	0.64	1,7-Di(3-ethylphenyl)-2,2,4,4,6,6- hexamethyl-1,3,5,7-tetraoxa-2,4,6-trisilaheptane	448.8	$C_{22}H_{36}O_4Si_3$
26	12.560	0.69	Cyclohexasiloxane, dodecamethyl-	444.92	$C_{12}H_{36}O_6Si_6$
27	13.001	1.17	Cyclohexasiloxane, dodecamethyl-	444.92	$C_{12}H_{36}O_6Si_6$
28	13.161	3.58	Cyclohexasiloxane, dodecamethyl-	444.92	$C_{12}H_{36}O_6Si_6$
29	13.310	2.39	Cyclohexasiloxane, dodecamethyl-	444.92	$C_{12}H_{36}O_6Si_6$
30	14.145	0.63	2-[(p-Trimethylsilyloxy)phenyl]-2-[(p-trimethylsilyloxyethylenoxy)phenyl]propane	416.7	$C_{23}H_{36}O_3Si_2$
31	14.729	0.50	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	428.92	$C_{12}H_{36}O_5Si_6$
32	15.393	2.49	Cycloheptasiloxane, tetradecamethyl-	519.07	$C_{14}H_{42}O_7Si_7$
33	15.501	1.02	Cycloheptasiloxane, tetradecamethyl-	519.07	$C_{14}H_{42}O_7Si_7$
34	15.759	0.32	Phenol, 2,5-bis(1,1-dimethylethyl)	206.32	$C_{14}H_{22}O$
35	17.178	0.59	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	503.07	$C_{14}H_{42}O_6Si_7$
36	17.373	1.72	Cyclooctasiloxane, hexadecamethyl-	593.2	$C_{16}H_{48}O_8Si_8$
37	17.458	0.89	Cyclooctasiloxane, hexadecamethyl-	593.2	$C_{16}H_{48}O_8Si_8$
38	18.895	1.12	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	503.07	$C_{14}H_{42}O_6Si_7$
39	19.146	1.90	Cyclononasiloxane, octadecamethyl-	667.4	$C_{18}H_{54}O_9Si_9$
40	20.445	0.49	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	503.07	$C_{14}H_{42}O_6Si_7$
41	20.663	1.72	Cyclodecasiloxane, eicosamethyl-	741.5	$C_{20}H_{60}O_{10}Si_{10}$
42	20.823	1.63	8-Methylnonanoic acid, ethyl ester	172.26	$C_{10}H_{20}O_2$
43	22.059	1.85	Cycloheptasiloxane, tetradecamethyl-	519.07	$C_{14}H_{42}O_7Si_7$
44	22.643	0.48	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_5Si_3$
45	23.341	2.08	Cyclononasiloxane, octadecamethyl-	667.4	$C_{18}H_{54}O_9Si_9$
46	24.531	2.18	Cyclononasiloxane, octadecamethyl-	667.4	$C_{18}H_{54}O_9Si_9$
47	25.549	6.30	Bis(2-ethylhexyl) phthalate	390.6	$C_{24}H_{38}O_4$
48	25.647	2.49	Cyclononasiloxane, octadecamethyl-	667.4	$C_{18}H_{54}O_9Si_9$
49	26.694	2.27	Cyclononasiloxane, octadecamethyl-	667.4	$C_{18}H_{54}O_9Si_9$
50	27.678	1.94	1,1,1,3,5,5,5-Heptamethyltrisiloxane	221.5	$C_7H_{21}O_2Si_3$

Discussion

The present study was done to determine the antimicrobial activity of cinnamon bark extract ethanol and water as solvents on microbial isolates (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus sp* and *Candida albicans*). Cinnamon was chosen for this study because it is a medicinal herb that has been used worldwide since antiquity. Dating roughly 4000 years ago, cinnamon has been used as a neuroprotective agent [13] and for the treatment of diabetes [14]. Ayurvedic literature shows that cinnamon has potent antiemetic, anti-diarrheal, anti-flatulent, and stimulant activities [15]. It also possesses potent antibacterial, antifungal, antitermitic, larvicidal, nematicidal and insecticidal properties [14, 16-18]. The bioactive properties of cinnamon compounds can affect the growth of *Escherichia coli*, which can cause common urinary infections [19].

From the results (Table 2 and Table 3), for both extracts, the minimum zones of inhibition were detected at the highest concentration of 200mg/ml and the lowest zones of inhibition were observed at the lowest concentration of 6.25mg/ml. This study is in line with the experiment carried out in the department of Pharmacology & Therapeutics in collaboration with the department of Microbiology, Mymensingh Medical College and Mymensingh, Bangladesh from July 2017 to July 2018 where the ethanol extract of *Cinnamomum zeylanicum* showed antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [20-21]. *Cinnamomum zeylanicum* showed strong antibacterial activity against *Pseudomonas aeruginosa*. The ethanol extracts of *Cinnamomum zeylanicum* were more effective against *Staphylococcus aureus* than *Escherichia coli.*

Upadhyaya *et al*. (2018) also reported ethanolic extract of cinnamon showed 14 mm and 11 mm inhibitory zone against *Staphylococcus aureus* and *Escherichia coli*, respectively [22].

The result of the minimum inhibitory concentration (MIC) for the aqueous and ethanol extract (Table 4 and Table 5), showed growth of all organisms on concentrations 12.5mg/ml and 6.25mg/ml. In order words, concentrations 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml were able to inhibit the growth of the organisms used.

The minimum bactericidal concentration (MBC) of the aqueous extract on the microbial isolates showed growth on all concentrations (Table 6). The results observed from the use of the ethanol extract on the microbial isolates showed growth on all concentrations except 200mg/ml for the organisms *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* For *Bacillus sp* and *Candida albicans*, no growths were observed at concentrations 200mg/ml and 100mg/ml but growth was observed at other

concentrations (Table 7).

In reference to Table 8 and Table 9, some compounds identified in the aqueous and ethanol extracts of *Cinnamomum zeylanicum* have been found to have medicinal purposes and possess anti-microbial characteristics. 5-Fluoro-8-quinolinol (synonym: 5-Fluoro-8-hydroxyquinoline) is a compound found in the aqueous extract of *Cinnamomum zeylanicum.* 8-quinolinol (8-hydroxyquinoline also known as oxine) is an organic compound derived from the heterocycle quinoline. Its fungicidal properties are used for the control of grey mould on vines and tomatoes. It has a role as an antibacterial, an iron chelator, an antiseptic drug and antifungal agrochemical.

4-Methylmandelic acid, di-TMS is a compound found in the aqueous extract of *Cinnamomum zeylanicum.* Mandelic acid has a long history of use in the medical community as an antibacterial, particularly in the treatment of urinary tract infections [23]. It has also been used as an oral antibiotic and as a component of chemical face peels analogous to alpha hydroxy acids [24]. The drugs cyclandelate and homotropine are esters of mandelic acid.

Phenol, 2, 5-bis(1,1-dimethylethyl) is a compound found in the ethanol extract of *Cinnamomum zeylanicum.* Phenol, 2, 5-bis(1,1-dimethylethyl) is a phenol with anti-bacterial activity [25], anti-fungal activity and antioxidant [26], anti-inflammatory activity [27] and anti-cancer activity.

The results of this study show that the ethanol extract of *Cinnamomum zeylanicum* bark has significantly higher antibacterial activity than the aqueous extract. This supports Abdulrasheed *et al.* [28] and Mukhtar and Ghori [29] findings that the antibiotic component of *Cinnamomum zeylanicum* bark is more soluble in ethanol, an organic solvent, than in water resulting in the release of an active antimicrobial component. The results of this work shows that antimicrobial property of the ethanol extract of cinnamon is higher than the aqueous extract following the mean zone inhibition results.

Conclusion

The study was carried out to evaluate the antimicrobial activity of ethanol and aqueous extracts of cinnamon bark on the microbial isolates *Candida albicans, Escherichia coli, Staphylococcus aureus, Bacillus sp* and *Pseudomonas aeruginosa*. It was demonstrated that both extracts have antimicrobial action at certain concentration levels as depicted by the inhibition zone diameter (IZD), mean inhibition concentration (MIC) and minimum bactericidal concentration (MBC) with the ethanol extract having a greater inhibitory effect on the selected microbes. For both extracts, it was noted that maximum microbial inhibition occurred at the highest concentration which was 200mg/ml and the least microbial inhibition occurred at the lowest concentration which was 6.25mg/ml. From the above results, it can be seen that ethanol and aqueous extracts of *Cinnamomum zeylanicum* have great bacteriostatic effect.

However, further research is needed in this field to verify cinnamon's efficacy *in-vivo* and to examine the plant's toxicity, medication interactions and adverse effects. Future *in-vitro* and *in-vivo* studies should be conducted to examine the cytotoxicity, medication interactions, plant side effects and the standardization of extraction methods. Further research should focus on the development of an appropriate form and route of administration of cinnamon so that therapeutic effects are maximized.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Alorkpa JE, Boadi NO, Badu M, Saah AS. Phytochemical screening, antimicrobial and antioxidant properties of assorted Carica papaya leaves in Ghana. Journal of Medicinal Plants Studies. 2016;4(6):193-198.
2. Almagboul AZ, Farroq AA, Tyagi BR. Antimicrobial Activity of Certain Sudanese Plants Used in Folkloric Medicine: Screening for Antibacterial Activity, Part II. Fitoterapia. 1985;56:103-109.
3. Yusha'u M, Garba L, Shamsuddeen U. *In vitro* inhibitory activity of garlic and ginger extracts on some respiratory tract isolates of gram-negative organisms. International Journal of Biomedical and Health Sciences. 2008;4(2):57-60.
4. Jakhetia V, Patel R, Khatri P, Pahuja N, Garg S, Pandey A, *et al*. Cinnamon: A pharmacological review. JASR. 2010;1:19–23.
5. Krishnamoorthy B, Rema J. End uses of cinnamon and cassia. In: Ravindran PN, Babu KN, editors. Cinnamon and Cassia: The Genus Cinnamomum. CRC Press; Boca Raton, FL, USA; c2004.
6. Griffith M, Postelnick M, Scheetz M. Antimicrobial stewardship programs: Methods of operation and suggested outcomes. Expert Rev Anti-Infe. 2012;10:63-73.
7. Yu VL. Guidelines for hospital-acquired pneumonia and health-care-associated pneumonia: a vulnerability, a pitfall, and a fatal flaw. Lancet Infect Dis. 2011;11:248-252.
8. Goossens H. Antibiotic consumption and link to resistance. Clin Microbiol Infec. 2009;15(3):12-15.
9. Pakyz AL, MacDougall C, Oinonen M, *et al*. Trends in antibacterial use in US academic health centers: 2002 to 2006. Arch Intern Med. 2008;168:2254–2260.
10. Aryal S. Mueller Hinton agar (MHA) – composition, principle, uses and preparation. Microbiology info, 2015.
11. Valgas C, De Souza SM, Smânia EFA, Smânia A Jr. Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 2007;38:369-380.
12. European Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology and Infectious Disease (September. Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution". Clinical Microbiology and Infection. 2000;6(9):509-515.
13. Khasnavis S, Pahan K. Sodium benzoate, a metabolite of cinnamon and a food additive, upregulates neuroprotective parkinson disease protein DJ-1 in astrocytes and neurons. Journal of Neuroimmune Pharmacology. 2012;7(2):424-435.
14. Kim SH, Hyun SH, Choung SY. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. Journal of Ethnopharmacology. 2006;104(1-

2):119-123.
15. Hossein N, Zahra Z, Abolfazl M, Mahdi S, Ali K. Effect of Cinnamon zeylanicum essence and distillate on the clotting time. Journal of Medicinal Plants Research. 2013;7(19):1339-1343.
16. Cheng S.-S, Liu J.-Y, Tsai K.-H, Chen W.-J, Chang S.-T. Chemical composition and mosquito larvicidal activity of essential oils from leaves of different Cinnamomum osmophloeum provenances. J. Agric. Food Chem. 2004;52:4395-4400.
17. Kong J-O, Lee S-M, Moon Y-S, Lee S-G, Ahn Y-J. Nematicidal activity of cassia and cinnamon oil compounds and related compounds toward Bursaphelenchus xylophilus (Nematoda: Parasita-phelenchidae) Journal of Nematology. 2007;39(1):31-36.
18. Moselhy SS, Ali HK. Hepatoprotective effect of cinnamon extracts against carbon tetrachloride induced oxidative stress and liver injury in rats. Biol. Res. 2009;42:93-98.
19. Harini S, Suneetha V. Disinfectant for Urinary Infection Caused by Escherichia coli by Using Natural Oils. J Biosci. Med. 2020;8:96-103.
20. Salma U, Saha SK, Sultana S, Ahmed SM, Haque SD, Mostaqim S. The Antibacterial Activity of Ethanolic Extract of Cinnamon (Cinnamomum zeylanicum) against two Food Borne Pathogens: Staphylococcus aureus And Escherichia coli. Mymensingh medical journal: MMJ. 2019;28(4):767-772.
21. Salma U, Sultana S, Saha SK, Ahmed SM, Salma MU, Nahar K, *et al*. *In vitro* Antibacterial Activity of Ethanol Extracts of Cinnamomum zeylanicum against Pseudomonas aeruginosa. Mymensingh medical Journal MMJ. 2020;29(2):248-253.
22. Upadhyaya S, Yadav D, Chandra R, Arora N. Evaluation of antibacterial and phytochemical properties of different spice extracts**.** African Journal of Microbiology Research. 2018;12(2):27-37.
23. Van Putten PL. Mandelic acid and urinary tract infections. Antonie van Leeuwenhoek. 1979;45:622–623
24. Taylor MB. Summary of Mandelic Acid for the Improvement of Skin Conditions. Cosmetic Dermatology; c1999. p. 26-28.
25. Kuppuswamy KM, Jonnalagadda B, Arockiasamy S. Gc-ms analysis of chloroform extract of Croton bonplandianum. Int J Pharma Bio Sci. 2013;4:4)
26. Raja Rajeswari, RamaLakshmi S, Muthuchelian K. GCMS Analysis of bioactive components from the ethanolic leaf extract of Canthium dicoccum (Gaertn.) Teijsm & Binn. J Chem. Pharm. Res. 2011;3(3):792-798.
27. Sanitha P, Mudiganti RKR, Prabhu K, Minu P, Kalaivani S, Aparna R, *et al*. Preliminary GC-MS analysis of an Ayurvedic medicine Kulathadi Kashayam". Chem. Pharm. Res. 2015;7(9):393-400.
28. Abdulrasheed M, Ibrahim I, Luka A, Maryam A, Hafsta L, Ibrahim S, *et al*. Antibacterial Effect of Cinnamon (Cinnamomum zeylanicum) Bark Extract on Different Bacterial Isolates. JEMAT. 2019;7(1):16-20.
29. Mukhtar S, Ghori I. Antibacterial activity of aqueous and ethanolic extracts of Garlic, Cinnamon and Turmeric against Escherichia coli ATCC 25922 and Bacillus subtilis DSM 3256. Int J Appl Biol Pharm Technol. 2012;3(2):131-6.

How to Cite This Article
Ahuokpeme Ifunanyachukwu C, Osuala Oluchi J, Ezemba Chinyere C, Ezemba Arinze S, Etikudike Victor O, Otolorin Isaac G. Antimicrobial effect of cinnamon bark extracts on microbial isolates. Journal of Advances in Microbiology Research 2020;1(2):42-50.