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Bioactive Effect of *Cinnamomum zealynicum* bark Extracts on Some Locally Isolated Pathogenic Bacteria

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Abstract

A series of experiments were conducted to evaluate the antibacterial effect of *Cinnamomum zealynicum* bark aqueous , methanol, and chloroform extracts against some gram positive and gram negative pathogenic bacteria which isolated from wound, throat infection, urine and stool during the period from December /2013 to February /2014 from Alkarama hospital in Wasit. All these isolates were identified by using VITEK2 compact system. Antibiotic sensitivity test of the bacterial isolates was determined for ten antibiotics. Chemical analysis showed that *Cinnamomum zeylanicum* bark extracts contained different active compounds (phenoles, alkaloids, tannins, glycosides, coumarins, saponins, resins flavones and essential oil). The laboratory tests of antibacterial activity , showed that *Staphylococcus aureus* was the most affected by the extracts under study then followed with *Enterococcus faecalis*, *Streptococcus pneumoniae, Escherichia coli and Klebsiella pneumoniae* respectively. Aqueous extract showed highest values in MIC (400 µg/ml) and MBC (450 µg/ml) for *S aureus*, followed by chloroform (350 µg/ml, MIC and 400 µg/ml, MBC), then methanol (335 µg/ml, MIC and 375 µg/ml MBC) while the lowest values were recorded for *K. pneumonia* (MIC 175, 200 and 250 µg/ml, and MBC 200, 250 and 300 µg/ml respectively). In other hand inhibition zones appeared at 200 µg/ml for *S. aureus* and *E. faecalis*, and at 300 µg/ml for *E. coli and S. pneumonia*, and at 400 µg/ml for *K. pneumoniae* for aqueous extract, while methanol extract inhibition zones started from 100 µg/ml for *S. aureus*, and at 200 µg/ml for *S. pneumonia and E. faecalis*, and at 300 µg/ml for *K. pneumonia* and *E. coli*. Chloroform extract showed inhibition zones for *S. pneumonia, E. faecalis* and *S. aureus* at 200 µg/ml and for *E coli* and *K. pneumoniae* at 300 µg/ml.

Keywords: *Cinnamomum zealynicum*, antimicrobial, pathogenic microorganisms

التأثير الحيوي لمستخلصات قلف نبات القرفة *Cinnamomum zealynicum* في بعض البكتريا الممرضة المعزولة محليا

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الخلاصة

أجريت سلسلة من التجارب لغرض تقييم الفعالية الحيوية للمستخلص المائي والميثانول والكلوروفورم لقلف نبات القرفة *Cinnamomum zealynicum* ضد بعض انواع البكتريا الممرضة الموجبة والسالبة لملون غرام والتي عزلت من مناطق مختلفة من (الجروح،والتهابات الحلق،والبراز ، والإدرار) للفترة من كانون الأول 2013 إلى شباط 2014 من مستشفى الكرامة في محافظة واسط والتي شخصت بجهاز ال VITEK2 compact . واجريت اختبارات الحساسية لهذه العزلات باستخدام عشرة انواع من المضادات الحيوية . بين التحليل الكيميائي للمستخلصات المذكورة احتوائها على عدد من المركبات الفعالة (فينولات، وقلويدات،

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وكلايكوسيدات، وكومارينات، وصابونين، وراتنجات، و فلافونات وزيوت أساسية) . أظهرت التجارب المختبرية للفعالية الضد بكتيرية للمستخلصات أن *Staphylococcus aureus* هي البكتريا الأكثر حساسية للمستخلصات ثم يأتي بعدها *Enterococcus faecalis* ثم *Streptococcus pneumonia* ثم *Escherichia coli* واخيرا *Klebsiella pneumoniae* . أظهر المستخلص المائي أعلى قيم للتركيز المثبط الأدنى (400 مايكرو غرام/مل) والتركيز القاتل الأدنى (450 مايكرو غرام/مل) لبكتريا *S. aureu* تبعها مستخلص الكلوروفورم (350 مايكروغرام/مل و 400 مايكروغرام/مل على التوالي) ثم الميثانول (335 و 375 مايكروغرام/مل على التوالي) ، بينما بلغت اقل قيم التركيز المثبط الادنى لبكتريا *K. pneumonia*e 175 و200 و 250 مايكروغرام/مل والتركيز القاتل الادنى 200 و250 و300مايكرو غرام / مل للمستخلصات الثلاثة على التوالي . من ناحية أخرى اظهر المستخلص المائي تثبيطاً عند التركيز 200 مايكروغرام/مل لبكتريا الـ *S. aureus* و *E. faecalis* وعند التركيز 300 مايكروغرام/مل لبكتريا الـ *E. coli* و *St. pneumonia* وعند التركيز 400 مايكروغرام/مل لبكتريا الـ *K. pneumonia* . *في حين* اظهر المستخلص الميثانولي تثبيطاً بدأ من التركيز 100 مايكروغرام/مل لبكتريا الـ *S. aureus* وعند التركيز 200 مايكروغرام/مل لبكتريا الـ *S. pneumonia* و *E. faecalis* وعند التركيز 300 مايكروغرام/مل لبكتريا الـ *K. pneumonia* و *E. coli* . واظهر مستخلص الكلوروفورم تثبيطاً عند وعند التركيز 200 مايكروغرام/مل لبكتريا الـ *S. pneumonia* و *E. faecalis* و*S. aureus* وعند التركيز 300 مايكروغرام/مل لبكتريا الـ *E coli* و *K. pneumonia* .

Introduction

In the last several years, the frequency and spectrum of antimicrobial-resistant infections have increased in both the hospital and the community due to the continued use of systemic and topical antimicrobial agents [1]. In addition, the side effects of overuse and misuse of antibiotics can harm vital organs [2]. Most important multidrug-resistant bacteria on the global scale include gram positive bacteria (Methecilline-resistant *Staphylococcus aureus*, vancomycin resistant enterococci) and gram-negative bacteria (members of enterobacteriaceae producing plasmid mediated extended spectrum beta lactamase (ESBL).

Plants produce large amounts of compounds known as phytochemicals, and each plant synthesizes a vast variety of these compounds, it's not only maintain the plant's physiological activities, but they also protect it against foreign agents such as bacteria, fungi, insects and animals that feed on them [3]. *Cinnamomum zeylanicum* tree belongs to the family, Lauraceae. Cinnamon has medicinal properties and has been used to treat gastrointestinal complaints and other ailments [4]. Cinnamon possesses antiallergenic, anti-inflammatory, anti-ulcerogenic, anti-pyretic, antioxidant, anesthetic activities [5]. Antioxidant studies with *Cinnamomum zeylanicum* bark showed better free radical scavenging capacity against a battery of free radicals [6]. The study on *Cinnamomum zeylanicum* indicated that the Cinnamon inhibits growth of several common bacteria, and therefore this study was aimed to investigate the bioactive effect of Cinnamon bark extract on some locally isolated pathogenic bacteria.

Materials and methods

Collection and characterization of bacterial isolates

In this study (70) clinical samples were collected from (out/in) patients (males and females) with different ages who suffered from different diseases such as urinary tract infection (UTI), diarrhea, wounds and throat infections. The patients were attended from AL-Karama hospital in Wasit city/Iraq during the period of December 2013 to February 2014. In case of wound and throat infection, samples were collected from patients by dry swab moisturized with little saline, in case of UTI and diarrhea, mid-stream urine and stool were generally collected in plastic universal sterile container. The stool samples were immediately inoculated in MacConky and XLD agar whereas the other samples were inoculated in MacConky, Mannitol salt agar, Nutrient agar and Blood agar and incubated for overnight at 37°C. The isolates were identified by using VITEK2 compact system.

Collecting of plant samples

Cinnamomum zeylanicum bark samples were collected from local market in Baghdad and identified by the herbarium of Biology Department, College of Science, Baghdad University. The bark of

cinnamon were cleaned with running water and dried at room temperature, then grounded into powder by electrical blender. The powdered parts were kept in plastic bags at 4°C until use [7].

Preparation of different plant extracts:

Hot water extract:

Aqueous extract of *cinnamomum zeylanicum* was prepared by adding 250 ml of hot distilled water to 50g of plant powder in flask, then stirred with a magnetic stirrer for two hours, and kept for three days. This mixture was filtered through filter paper (Watt man No 0.22). The supernatant was evaporated at 40◦C under reduced pressure in rotary evaporated, then the concentrated extract left at 40◦C temperature in oven to get powder [8].

Methanol extract:

A quantity of 50g *cinnamomum zeylanicum* powder was mixed with 250ml of methanol 70% and extracted by Soxhlet apparatus for 8 hours at 40-60°C. This solution was concentrated in rotary evaporater, then transferred to oven at 40°C to get powder [9].

Chloroform extract:

One hundred g of dried powder of *cinnamomum zeylanicum* was mixed with 500 ml of chloroform and placed in Soxhlet apparatus for 8 hours. The plant extract was filtered through whatt man No.1 filter paper, and transferred to rotary evaporator at 40◦C, then transfer to oven at 40°C to get powder [10].

Antibacterial activity of cinnamon bark extracts

Minimum Inhibitory Concentration (MIC)

The MIC of extracts was determined by preparing different concentrations as follows (100,200,300,400, and 500) µg/ml using the method described by [11]. Nutrient broth was used to prepare turbid suspension of the isolated bacteria, the dilutions was incubated at 37◦C for 30 minutes, until the turbidity become 0.5 which measured by vitek density check. At the point of the cells are assumed to be $1.5x10^8$ cfu/ml, 0.1ml of the cell suspension was inoculated into each of the tubes with the varied concentrations of extracts. All the tubes were incubated at 37°C for 24 hours. The tube with the lowest concentration which has no growth (turbidity) of the bacteria was represented the MIC.

Minimum bactericidal concentration (MBC)

The tubes of MIC that showed no growth of the bacteria were sub-cultured by streaking using sterile loop on nutrient agar plates or blood agar plates. The plates were incubated at 37°C for 24 hours. The MBC was represented the lowest concentration of extract that did not show any colony on plates [12].

Well diffusion agar

Bacterial suspension (1.5×10^8 cfu/ml) was spreaded on Mueller Hinton agar plates using sterile cotton swab, then wells with a diameter of 6 mm were made on the surface and filled with 100 microliter of extracts. Control wells were filled with DMSO and Cefixime (CFX) as negative and positive control respectively. Plates were incubated at 37ºC for 24 hr., after incubation period, the diameter of inhibition zones around wells were recorded in millimeters [13]. Tests were performed in triplicate.

Results and Discussion

Identification of bacterial isolates

Identification of 70 clinical bacterial isolates by vitek2 compact system apparatus revealed that 15 isolates were *S. aureus*, 15 isolates were *E. coli*, 15 isolates were *K. pneumoniae*, 15 isolates were *E. faecalis*, and 10 isolates were *S. pneumoniae*. Identification results of the bacterial isolates by Vitek2 were showed in Figures-1, 2, 3, 4, 5.

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	-
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Figure 1- Identification of *S. aureus* by vitek2 compact

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	+
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Figure 2- Identification of *E. coli* by vitek2 compact

Biochemical Details																
2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Figure 3- Identification of *K. pneumonia* by vitek2 compact

Biochemical Details																
2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	+	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Figure 4- Identification of *E. faecalis* by vitek2 compact

Biochemical Details																
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	+	16	BGAR	+	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	+	24	BGURr	-	25	AGAL	+	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	-	31	URE	+	32	POLYB	-	37	dGAL	-
38	dRib	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	dMAL	-	46	BACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	-	63	ADH2s	-
64	OPTO	-															

Figure 5- Identification of *S. pneumoniae* by vitek2 compact

Sensitivity test of bacterial isolates against antibiotics

The sensitivity of bacterial isolates was tested against ten antibiotics. Table-1 showed that *K. pneumoniae* was the most resistant to all antibiotics tested except imipenem and Trimethoprim/sulphomethaxozol, then *E. coli* which was resistant to 7 antibiotics. While *S. pneumoniae* was resistant to 6 antibiotics and other bacteria (*E. faecalis* and *S. aureus)* were resistant to 4 and sensitive to 6 antibiotics. Resistance maybe due to a spontaneous or induced genetic mutation and may be the acquisition of resistance genes from other bacterial species by horizontal gene transfer via conjugation, transduction and transformation [14].

Table 1- Antibiotic sensitivity test for bacterial isolates

Antibiotic name	*E. coli*	*K. pneumonia*	*E. faecalis*	*S. pneumoniae*	*S. aureus*
Amoxicillin	R	R	R	R	R
Gentamycine	R	R	S	R	S
Imipenem	S	S	S	S	S
Ciprofloxacin	S	R	S	S	S
Vancomycin	R	R	S	S	S
Erythromycin	R	R	R	R	R
Trimethoprim / sulphomethaxozol	S	S	S	S	S
Ampicillin	R	R	R	R	R
Ceftazidim	R	R	R	R	R
Tobramycine	R	R	S	R	S

S: sensitive, R: resistant

Active compounds in *Cinnamomum* zeylanicum bark extracts

Chemical analysis of cinnamon bark extracts revealed the absence of flavones in all extracts as shown in Table-2. The aqueous extract (hot water) contained glycosides, tannins, saponins, resins and phenoles, but did not contain alkaloids, cumarins, terpens, flavones and steroids because they are not soluble in water. On the other hand methanol extract contained all active compounds except flavones and steroids and this is due to the high polarity of methanol, while chloroform extract showed the absence of glycosides, saponins and flavones.

Table 2- Active compounds in *Cinnamomum zeylanicum* bark extracts.

active compounds	Cinnamon bark extracts		
	Chloroform	methanol	Hot water
Glycosides	-	+	+
Alkaloids	+	+	-
Tannins	+	+	+
Resins	+	+	+
Saponins	-	+	+
Coumarine	+	+	-
Flavones	-	-	-
Phenoles	+	+	+
Terpens	+	+	-
Steroids	+	-	-

+: existence, and –: absence of the active compound

Antibacterial activity of extracts

Antibacterial activity of aqueous extract

Table-3 showed the minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of aqueous extract for *Cinnamomum zeylanicum* bark.

A significant differences ($p \leq 0.05$) were noticed between the mean of MIC and MBC for bacterial isolates. The lower value of MIC&MBC showed for *S aureus* (250 & 300 mg/ml respectively), while the highest value of MIC&MBC was for *K. pneumoniae* (440 & 450 mg/ml respectively). The effect of cinnamon bark aqueous extract was clearly active against gram positive bacteria more than gram negative bacteria because of the difference in the cell wall structure, gram negative cell wall contain outer membrane which decreases the penetration of the bacteria cell while gram positive lack this outer membrane [15]. *S. pneumoniae* appeared less effect from than other gram positive isolates because this bacteria have capsule which consist from polysaccharides, the capsule give pathogenicity and resistance for antibiotics as well as *K. pneumonia*e which increase resistance of bacteria than other gram negative [16]. Inhibition zone for all isolates under study against different aqueous extract concentrations showed that there was no inhibition zone occurred at 100 mg/ml as well as at 200 mg/ml, except *E. faecalis* and *S aureus* which was 2.3 and 4.2 mm at 200mg/ml respectively Table-4.

The effect of aqueous extract was recorded at concentrations 300, 400 and 500 mg/ml which ranged between (0.0-5.6), (2.8-6.5), (4.3-8.0), (7.5-10.2) and (8.6-14.5) mm for the *K. pneumoniae, E coli, S. pneumoniae, E. faecalis* and *S .aureus* respectively. The presence of active compounds like tannins works on the inhibition of enzymes and transport of proteins in the cell membranes [17]. While Saponins is working to reduce the proportion of sugar within the bacteria that lead to bacterial cell death as well as for glycosides which have a similar but less effect [18]. The finding of this study was agreed with that of [19] and [20] who noticed the antimicrobial activity of aqueous extract of cinnamon on *S. aureus*. On other hand the results of the study was close to that results found by [21] when they detected the effect of aqueous extract of cinnamon on some gram positive and gram negative pathogenic bacteria in different concentrations.

Table 3- Minimum inhibitory concentration and Minimum bactericidal concentration of aqueous cinnamon bark extract (µg/ml).

Bacterial species	**MIC**	**MBC**
	Mean of inhibition zone diameter ± SE	
K. pneumoniae	400±0.0	450±0.0
E. coli	350±0.0	375±0.0
S. pneumoniae	300±0.0	350±0.0
E. faecalis	260±2.5	335±2.5
S. aureus	250±0.0	300 ±0.0
LSD	16.1	17.31

Significant difference P<0.05

Table 4- Inhibition zone of cinnamon aqueous extract against pathogenic bacteria.

Concentration of extract µg/ml / **Bacterial species**	**100**	**200**	**300**	**400**	**500**	**CFX 150µg/ml**
	Mean of inhibition zone diameter ± SE					
K. pneumoniae	0.0	0.0	0.0	3.0±0.0	5.6±0.2	2.0±0.0
E. coli	0.0	0.0	2.8±0.3	5.6±0.2	6.5±0.3	4.2±0.0
S. pneumoniae	0.0	0.0	4.3±0.7	7.3±0.1	8.0±0.0	5.3±0.1
E. faecalis	0.0	2.3±0.3	7.5±1.4	9.0±0.3	10.2±1.0	7.1±0.5
S. aureus	0.0	4.2±0.1	8.6±1.7	10.1±0.6	14.50	9.2±0.8
LSD	0.0	1.1	1.3	3.9	4.0	0.5

Zone of well (6mm), CFX (Cefixime) positive control. Significant difference P<0.05.

Antibacterial activity of methanol extract

The results of MIC, MBC and inhibition zones of methanol extract of cinnamon bark revealed more effective than aqueous extract. The MIC of the methanol extract for *K. pneumoniae, E. coli, S. pneumoniae, E. faecalis and S. aureus* were 335, 250, 210, 200 and 175 mg/ml while MBC were 375, 300, 250, 210 and 200 mg/ml respectively table (5). The differences in the effect of active compounds in cinnamon methanol extract on the different isolates could be due to differences in the structure of the cell wall between gram negative and gram positive bacteria. These results were confirmed by inhibition zones results (table 6), which showed that *S. aureus* isolate was more sensitive than other isolates followed by *E. faecalis S. pneumoniae, E. coli and K. pneumonia*, which ranged from (3.1-19), (0-16.8), (0-15), (0-11) and (0-7.6) mm for the concentrations 100- 500 mg/ml respectively. The mechanism of antibacterial action of alkaloids is attributed to their ability to intercalate with DNA, inhibition of enzymes (esterase, DNA-, RNA-polymerase), and inhibition of cell respiration [22].The mechanism of antibacterial activity of terpens is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. [23] Suggested that fairly high antibacterial activity of Coumarine is due to both its lipophilic character and planar molecular structure, which

contribute in penetration through bacterial cell membrane or cell walls. [24] Showed that alcoholic extract had active effect on Enterobacteriaceae.

Table 5- Minimum inhibitory concentration and Minimum bactericidal concentration of methanol extract cinnamon bark (µg/ml).

Bacterial species	MIC	MBC
	Mean of inhibition zone diameter ± SE	
K. pneumoniae	335±2.5	375±0.0
E. coli	250±0.0	300±0.0
S. pneumoniae	210±2.5	250±0.0
E. faecalis	200±0.0	210 ±2.5
S. aureus	175±0.0	200±0.0
LSD	11.8	12.9

Significant difference P<0.05

Table 6- Inhibition zone of cinnamon methanol extract against pathogenic bacteria.

Concentration of extract µg/ml / Bacterial species	100	200	300	400	500	CFX 150µg/ml
	Mean of inhibition zone diameter ± SE					
K. pneumoniae	0.0	0.0	3.6 ± 0.6	6.3±0.0	7.6±0.7	2.0±0.0
E. coli	0.0	0.0	5.0±0.3	8.3±0.2	11.0±0.8	4.2±0.0
S. pneumoniae	0.0	3.3±0.1	7.6±0.5	10.0±0.3	15.0±0.4	5.3±0.1
E. faecalis	0.0	4.2±0.2	9.7±0.3	13.3±0.4	16.8±0.7	7.1±0.5
S. aureus	3.1±0.1	6.5±0.0	12.0±0.1	14.8±0.6	19.0±0.9	9.2±0.8
LSD	0.3	2.57	1.33	3.1	3.7	0.5

Zone of well (6mm), CFX (Cefixime) positive control. Significant difference P<0.05

Antibacterial activity of chloroform extract

Table-7 showed that chloroform extract gave less effect than methanol extract. MIC values were 200, 210, 225, 335 and 350 mg/ml while MBC were 250, 260, 300, 350 and 400 mg/ml for *S. aureus*, *E. faecalis, S. pneumoniae* , *E. coli* and *K. pneumoniae* respectively, these results agreed with [25]. Table-8 showed decrease in inhibition zones compared with methanol extract for the same concentrations, chloroform extract did not give any inhibition zone at 100mg/ml for all isolates. This extract also showed no inhibition zone at 200 mg/ml for *E. coli* and *K. pneumoniae.* The highest inhibition zone recorded was at 500mg/ml which gave 6.6, 8.5, 11, 13.2 and 17.5 mg/ml for *K. pneumoniae*, *E. coli*, *S. pneumoniae*, *E. faecalis* and *S. aureus* respectively. Chloroform extract of cinnamon bark has less antibacterial effect on some gram positive and negative bacteria isolates than methanol extract, and that may refer to the low efficiency and concentration of active ingredients and weak polarity of chloroform extract [26]. The antibacterial effect of the extract could be attributed to the terpens which have the ability to rapture the cellular membrane by forming complexes with proteins on cell wall [27, 28].

Table 7-Minimum inhibitory concentration and Minimum bactericidal concentration of chloroform cinnamon bark extract (µg/ml).

Bacterial species	MIC	MBC
	Mean of inhibition zone diameter ± SE	
K. pneumoniae	350 ±0.0	400±0.0
E. coli	335 ±2.5	350±0.0
S. pneumoniae	225±0.0	300 ±0.0
E. faecalis	210 ±2.5	260 ±2.5
S. aureus	200 ±0.0	250±0.0
LSD	12.26	16.44

Significant difference P<0.05

Table 8- Inhibition zone of cinnamon chloroform extract against pathogenic bacteria.

Concentration of extract µg/ml / Bacterial species	100	200	300	400	500	CFX 150µg/ml
	Mean of inhibition zone diameter ± SE					
K. pneumoniae	0.0	0.0	2.1±0.0	4.0±0.0	6.6±0.2	2.0±0.0
E. coli	0.0	0.0	3.5±0.6	7.6±0.2	8.5±0.3	4.2±0.0
S. pneumoniae	0.0	2.0±0.10	5.1±0.4	8.3±0.1	11.0±0.	5.3±0.1
E. faecalis	0.0	3.3±0.3	8.3±0.3	10.0±0.3	13.2±1.0	7.1±0.5
S. aureus	0.0	5.2±0.1	9.4±0.1	11.1±0.6	17.50	9.2±0.8
LSD	0.0	1.33	2.33	3.1	3.7	0.5

Zone of well (6mm), CFX (Cefixime) positive control. Significant difference P<0.05

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