WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 2, 174-185. Research Article ISSN 2277– 7105

ANTIBACTERIAL ACTIVITY OF *CINNAMOMUM ZEYLANICUM* BARK OIL AND CINNAMALDEHYDE ON SOME LOCALLY ISOLATED PATHOGENIC BACTERIA

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Article Received on
04 Dec. 2016,

Revised on 25 Dec. 2016,
Accepted on 16 January 2017

DOI: 10.20959/wjpr20172-7707

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ABSTRACT

This study was conducted to evaluate the antibacterial effect of *Cinnamomum zealynicum* bark oil and cinnamaldehyde against some gram positive and gram negative pathogenic bacteria which isolated from wounds, throat infection, urine and stool during the period from December /2013 to February /2014 from Alkarama hospital in Wasit / Iraq. 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 oil extract contained different active compounds (phenols, alkaloids, tannins, glycosides, cumarins, saponins, resins and flavones). Cinnamaldehyde partially purified from cinnamon bark oil by using liquid-liquid system. High performance liquid chromatography (HPLC) showed concentration of cinnamaldehyde about 88% at retention time (2.587 min) comparison with standard (2.583 min). The laboratory tests of antimicrobial activity for cinnamon bark oil and cinnamaldehyde extracts showed that *Staphylococcus aureus* is the most affected by the extracts under study then followed by *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Escherichia coli and Klebsiella pneumoniae*. Cinnamon oil and Cinnamaldehyde showed best activity for *S. aureus*, which gave 80 µg/ml (MIC) and 110 µg/ml (MBC), and 35 µg/ml (MIC) and 50 µg/ml (MBC) respectively. While they gave 175 µg/ml (MIC) and 200 µg/ml (MBC), and 110 µg/ml (MIC) and 150 µg/ml (MBC) respectively for *K. pneumoniae*. Oil extract did not show inhibition zone at 50 and 100 µg/ml for *E. coli* and *K. pneumonia*, and at 50 µg/ml for *S. pneumoniae*, but showed inhibition zone for all isolates at other concentrations. In other hand cinnamaldehyde did not show inhibition zone at 50 µg/ml for *E coli* and *K.pneumoniae*, but showed inhibition zone for all isolates at other concentrations.

KEYWORDS: cinnamon oil, Cinnamaldehyde, HPLC, antimicrobial, pathogenic microorganisms.

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 (Rijnders *et al*., 2009). In addition, the side effects of overuse and misuse of antibiotics can harm vital organs (Bocanegra-Garcia *et al*., 2009). Most important multidrug-resistant bacteria on the global scale include gram positive such as Methicillin-resistant *Staphylococcus aureus*, and vancomycin resistant Enterococci and gram-negative bacteria such as 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 (Schultz, 2002). *Cinnamomum zeylanicum* tree belongs to the family, Lauraceae. Cinnamon has medicinal property and has been used to treat gastrointestinal complaints and other ailments (Cao and Anderson, 2011). Cinnamon possesses antiallergenic, anti-inflammatory, anti-ulcerogenic, anti-pyretic, antioxidant and anesthetic activities (Lin *et al*., 2003). Antioxidant studies with *Cinnamomum zeylanicum* bark showed better free radical scavenging capacity against a battery of free radicals (Varalakshmi *et al*., 2012). Essential oils are responsible for the distinctive aromas associated with individual plant species (Pengelly, 1996). The main constituent of cinnamon bark oil is cinnamaldehyde ((2E)-3-phenyle-2-propenal). Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Burt, 2004 & Kordali *et al.,* 2005). The bark oil is used in the food and pharmaceutical industries (Jayaprakasha *et al*., 2000). Therefore, this study was aimed to isolation of essential oil and cinnamaldehyde from cinnamon bark and study their antibacterial activity against 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 (Harborn *et al*., 1975).

Essential oils distillation

Essential oils (EO) were obtained by water steam distillation for 6 hours in according to (Senhaji *et al*., 2007). Fifty grams of cinnamon bark powder with 0.5 liter of distilled water (1:10 w:v) were extracted in the Clevenger apparatus at 100°C for 6 hours. Essential oil was stored at 4°C until use.

Chemical analysis of cinnamon oil

1. Detection of alkaloids and saponins were done according to (Sousek *et al*., 1999 and Stahl, 1969) respectively.
2. Detection of phenols and cumarins were done according to (Harborn, 1984).
3. Terpens and steroids, tannins, flavonoids, glycosides and resins were detected according to (Shihata, 1951).

Partial purification of Cinnamaldehyde

The cinnamon oil was transferred to a separation funnel, then about 5-10 ml of dichloromethane was added to the separation funnel, shacked and allowed to separate for 10 minutes, then dichloromethane was drained off. This step was repeated once more. The above layer which contain the cinnamaldehyde was dried by adding sodium sulfate until it is free flowing. The solution was transferred to a round bottom flask and the solid sodium sulfate

was rinsed with a little more dichloromethane, then evaporated by rotary evaporator, Cinnamaldehyde then collected in dark container.

Antibacterial activity of cinnamon oil and Cinnamaldehyde

Minimum Inhibitory Concentration (MIC)

The MIC of oil extract and cinnamaldehyde was determined by preparing different concentrations as follows (50,100,150,200 and 250) µg/ml and (25, 50, 75, 100 and 125) µg/ml respectively using the method described by Akinpelu and Kolawole (2004). Nutrient broth was used to prepare turbid suspension of the microbes, 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 cell is 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 microbes was represent the MIC.

Minimum bactericidal concentration (MBC)

The tubes of MIC that showed no growth of the microbes 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 represent the lowest concentration of extract that did not show any colony on plates (Spencer and Spencer (2004).

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 cinnamon oil and cinnamaldehyde. 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 (Lyudmila *et al.*, 2005). 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*.

Sensitivity test of bacterial isolates against antibiotics

The sensitivity of bacterial isolates were 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 may take the form of 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 (Anderson, 2003).

Table (1): Sensitivity test of bacterial isolates against antibiotics.

Antibiotic name	Sym	*E coli*	*K pneumonia*	*E faecalis*	*S pneumoniae*	*S aureus*
Amoxicillin	AX	R	R	R	R	R
Gentamycine	GN	R	R	S	R	S
Imipenem	IPM	S	S	S	S	S
Ciprofloxacin	CIP	S	R	S	S	S
Vancomycin	VA	R	R	S	S	S
Erythromycin	E	R	R	R	R	R
Trimethoprim / sulphomethaxozol	SXT	S	S	S	S	S
Ampicillin	AMP	R	R	R	R	R
Ceftazidim	CAZ	R	R	R	R	R
Tobramycine	TOB	R	R	S	R	S

S: sensitive, R: resistant.

Active compounds in *Cinnamomum* zeylanicum bark oil

Chemical analysis of cinnamon bark oil extract revealed absence of tannins and phenols and existence of other active compounds as shown in table (2).

Table (2): Active compounds in oil extract of *Cinnamomum zeylanicum* bark

Active component	Essential Oil
Glycosides	+
Alkaloids	+
Tannins	–
Resins	+
Saponins	+
Cumarine	+
Flavones	+
Phenoles	–
Terpens and steroids	+

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

Determination of cinnamaldehyde by using the High Performance Liquid Chromatography (HPLC) technique

The quality and quantity of partial purified cinnamaldehyde where determined by HPLC and the result were compared with standard cinnamaldehyde. Fig (1) showed that a curve of the sample was appeared at retention time 2.587 min which was closely compatible with standard cinnamaldehyde at retention time 2.583 min at absorbance 370 nm (fig -2). The concentration of cinnamaldehyde was reached 88% and these results were agreed with Al-Bayati and Mohammed (2009).

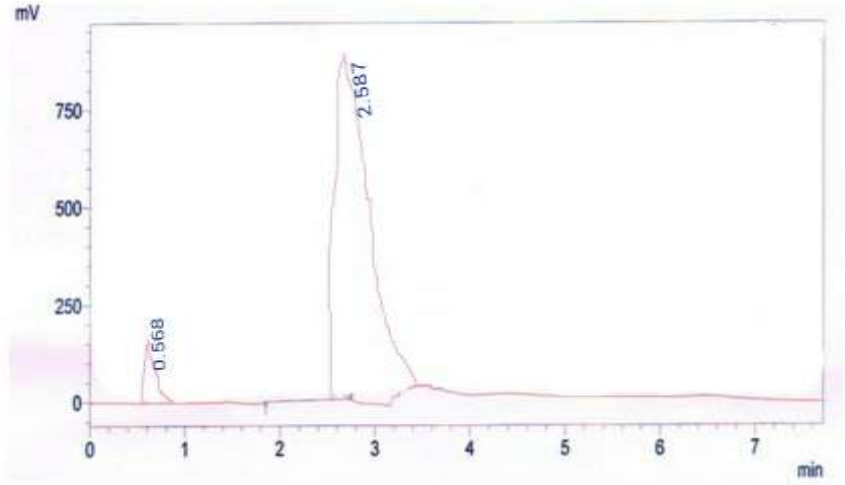

Figure (1): Determination partial of purified cinnamaldehyde by using High Performance Liquid Chromatography (HPLC) technique at absorbance 370 nm.

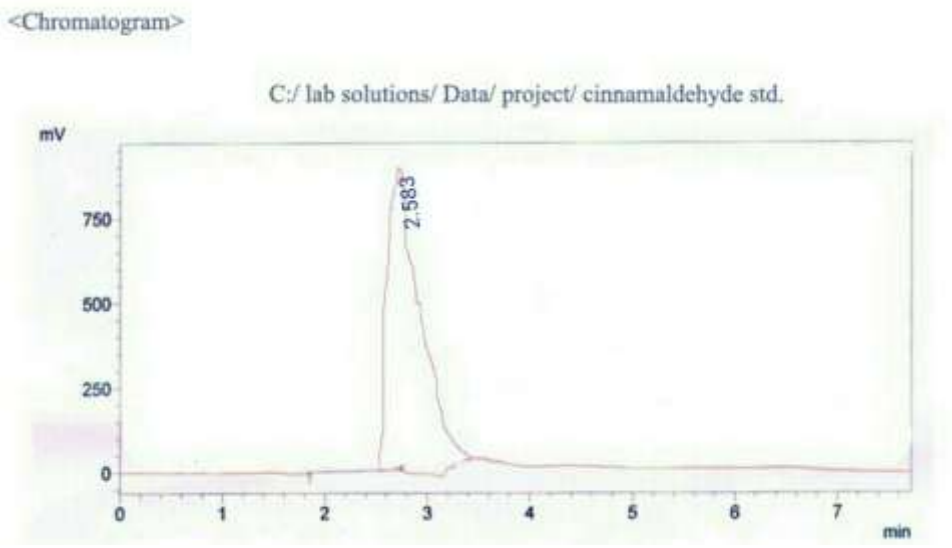

Figure (2): The cinnamaldehyde standard curve by using the High Performance Liquid Chromatography (HPLC) technique at absorbance 370 nm.

Antibacterial activity of cinnamon oil

The tables (3) and (4) showed the presence of significant differences at the level ($p \leq 0.05$) between the average of MIC, MBC and inhibition zone for *S. aureus* and each of the *E. faecalis, S. pneumoniae, E.coli* and *K. pneumoniae*. The effect of cinnamon oil was elucidated by using MIC and MBC tests which showed minimum values of MIC, and recorded 80 and 175 µg/ml, while maximum values for MBC were 110 and 200 µg/ml for *S. aureus* and *K. pneumoniae* respectively (table 3). Significant differences were recorded between the values of inhibition zones for all concentrations of oil extract. Table (4) showed that the diameter of inhibition zone increased with increasing of concentrations for all tested isolates, *S. aureus* was the most sensitive one followed by *E. faecalis, S. pneumoniae, E. coli and K. pneumonia*e which showed (20, 17.1, 15.5, 12.8, 9.6) mm respectively at 250 µg/ml. Cinnamon oil did not inhibit the growth of *E. coli and K. pneumonia*e at 50 and 100 µg/ml and did not inhibit the growth of *S. pneumoniae* at 50 µg/ml. The other isolates were sensitive for all concentration and the cinnamon oil gave inhibition zone diameter larger than Cefixime which used as control positive. The main reason for inhibiting the growth of bacteria is the active constituents in cinnamon oil, the variation in the concentration of the active compounds in each extract contribute to prevent the normal growth of the pathogenic bacteria (Skandamis and Nychas, (2001); Carson *et al*., (2002). Essential oil are potential source of novel antimicrobial activity especially pathogenic bacteria, cinnamon essential oil contain cinnamaldehyde and eugenol as major component (Ducke, 1994). Cinnamon oil also contain other active compounds which participate in antimicrobial activity as alkaloids, terpens, Cumarine and flavones (Alsengry, 2006). The MIC results in this study agreed with Shareef (2001), who mentioned that cinnamon oil gave (MIC) against some pathogenic bacteria (such as *E. coli, K pneumonia, S. aureus , P aeroginosa, Proteus spp and Brucella spp)* and observed that gram positive bacteria was more sensitive than gram negative to cinnamon oil. The result of this study were corresponding with Bowels *et al*., (1995) and Helander *et al*., (1998), they reported that *S. aureus, E. coli* and *salmonella typhimurium* were inhibited by essential oil of cinnamon. Friedman *et al*., (2002) found that essential oil of cinnamon was active against *E. coli* and *Campylobacter jejuni*. In another study (Houqe *et al*., 2008) recorded that essential oil of cinnamon showed high antibacterial activity against *S. aureus*.

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

Bacteria isolates	MIC	MBC
K. pneumoniae	175.0±0.0	200±0.0
E .coli	150.0±0.0	175±0.0
S. pneumoniae	125±0.0	15.0±0.0
E. faecalis	100±0.0	125±0.0
S. aureus	80±7.5	110±2.5
LSD	4.4	7.1

Significant difference at level P<0.05.

Table (4): inhibition zone of cinnamon oil against microorganisms.

Conc. / Bacteria	50	100	150	200	250	CFX 150µg/ml
K. pneumoniae	0.0	0.0	3.1±0.1	7.3±0.4	9.6±0.1	2.0±0.0
E. coli	0.0	0.0	5.3±0.3	9.3±0.2	12.8±0.5	4.2±0.0
S. pneumoniae	0.0	4.8±0.1	6.6±0.2	10.2±0.0	15.5±0.7	5.3±0.1
E. faecalis	2.3±0.0	5.3±0.3	8.3±0.4	11.0±0.1	17.1±2.1	7.1±0.5
S. aureus	3.1±0.1	6.8±0.0	10.8±0.3	13.7±0.5	20.0±1.3	9.2±0.8
LSD	1.6	1.9	2.0	2.3	2.6	0.5

Zone of well (6mm). DMS0 (control negative), CFX (control positive) Significant difference at level P<0.05.

Antibacterial activity of cinnamaldehyde

Cinnamaldehyde partially purified from cinnamon bark oil is found naturally in the bark and leaves of cinnamon tree of the genus cinnamomum. In *vitro* studies were conducted to evaluate the inhibitory effect of cinnamaldehyde. Results in table (5) revealed that MIC value ranged from 35 for *S. aureus* to 110 µg/ml for *K. pneumonia* while MBC value ranged from 50 to 150 µg/ml for the same isolates respectively. Zone of inhibition for cinnamaldehyde recorded significant results for all isolates except *E. coli* and *K. pneumonia*e which gave 0.00 diameter for 50 µg/ml, whilst diameter of inhibition zone increased gradually with increasing of concentrations which reached at 250 µg/ml to (10.7, 15.5, 19.3, 23.5 and 26.4) mm for *K. pneumonia, E. coli, S. pneumonia, E. faecalis and S. aureus* respectively (table 6). Our results were close to results achieved by Al-Bayati and Mohammed (2009) but disagreed with those who mentioned that cinnamaldehyde need high concentration to inhibit most pathogenic bacteria (Muthana *et al*., 2005), several studies have revealed that cinnamaldehyde detected by GC-MS was the major constituent and predominant active compound found in cinnamon (Baratta *et al*., (1998), Simic *et al*., (2004).

Table (6) showed the antibacterial activity of cinnamaldehyde. No inhibition zone was seen against gram negative bacteria at 50µg/ml, while gram positive bacteria under study and at each concentration used of cinnamaldehyde gave inhibition zone diameter larger than Cefixime which used as control positive. These results indicate to the bioeffect of cinnamaldehyde against bacterial isolates more than cinnamon oil. The results of this study agreed with Ead *et al.*, (2011) who observed antibacterial activity of cinnamaldehyde and best growth inhibition against gram positive bacteria than gram negative bacteria, this action of cinnamaldehyde could be through inhibiting of various cellular enzymes and proteins like amino acid carboxylase.

Table (5) Minimum inhibitory concentration and Minimum bactericidal concentration of cinnamaldehyde (µg/ml).

Bacteria isolates	MIC	MBC
K. pneumoniae	110 ± 2.5	150 ± 0.0
E. coli	100 ± 0.0	125 ± 0.0
S. pneumoniae	80 ± 7.5	100 ± 0.0
E faecalis	60 ± 2.5	80 ± 7.5
S. aureus	35 ± 2.5	50 ± 0.0
LSD	3.70	5.5

Significant difference at level P<0.05.

Table (6) inhibition zone of cinnamaldehyde against microorganisms.

Conc. / Bacteria	50	100	150	200	250	CFX 150µg/ml
K. pneumoniae	0.0	5.1±0.0	6.1±0.0	8.8±0.3	10.7±0.2	2.0±0.0
E. coli	0.0	6.6±0.2	7.1±0.2	12.5±0.1	15.5±2.2	4.2±0.0
S. pneumoniae	8.3±0.3	10.1±0.2	12.3±0.3	15.6±0.1	19.3±2.1	5.3±0.1
E. faecalis	11.3±1.4	14.0±1.1	17.0±0.7	21.0±0.3	23.5±1.1	7.1±0.5
S. aureus	13.6±1.2	15.0±2.2	18.7±2.1	23.3±2.6	26.4±3.1	9.2±0.8
LSD	1.1	1.3	1.9	2.1	2.4	0.5

Zone of well (6mm). DMS0 (control negative), CFX (control positive), Significant difference at level P<0.05.

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