WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 17, 81-91. Research Article ISSN 2277– 7105

CINNAMON'S ANTIBACTERIAL ACTIVITY ON THE BACTERIAL ISOLATES FROM URINARY TRACT INFECTIONS

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Article Received on
25 October 2017,

Revised on 15 Nov. 2017,
Accepted on 05 Dec. 2017

DOI: 10.20959/wjpr201717-10304

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ABSTRACT

Urinary tract infection is one of the most horrifying healthful problems that face enormous number of people yearly and because researchers are paying a great attention to carry out their researches on plants and herbs as an alternative for chemical drugs. This research has commenced to study the effect of cinnamon on bacterial isolates from urinary tract infections. During this study, 52 samples were taken from patients who were being questionable of urinary tract infection. Those patients ages are between 15 to 45 years of both gender and the study period was from September 2016 to may 2017. Samples were cultured on different media and it were diagnosed in routine methods depending on the cultural characteristics, microscopic examination, growth on the differential media and biochemical tests of each isolate. The results showed that females were more susceptible to the disease with 24 cases (54.5%) Compared to males which was 20 cases (45.5%), while growth did not occur in remaining samples. Results of bacterial isolation showed dominant of *E.Coli* (43.1%) as a major and important cause of UTI, particularly for age 15-45 years. Followed by *klebsiella pneumoniae* (25.5%)) then *Proteus mirabilis* (16.9%) and finally *Staphylococcus aureus* (%13.6). Sensitivity test of the bacterial isolates also has been conducted to aqueous extract of Cinnamon by using agar wells diffusion method. It was observed that the effectiveness of Cinnamon bark extract more than the effectiveness of the powder extract, as its noted that the extract effectiveness on the Gram negative bacteria was better than the Gram positive bacteria. The diameters of inhibition zone to the bark extract were (4.5 mm), (4.2 mm), (3.5 mm), (3 mm) for *E.Coli, klebsiella pneumonia, proteus mirabilis* and *Staphylococcus aureus* respectively, where as the The diameters of inhibition zone to the powder extract were (2.6 mm) for *E.Coli*, (2.5mm) for each *klebsiella pneumonia* and *Proteus mirabilis* and finally (2mm) for *Staphylococcus aureus*.

1. INTRODUCTION

Urinary tract infection (UTI) is the second most common infection next to respiratory tract in human body. The disease affects people of all ages and both gender, about 150 million people are diagnosed with UTI yearly. Different groups of microorganisms involved in UTI. Urinary tract infections were classified into uncomplicated or complicated.[1] Sometimes, the UTI is symptomatic or asymptomatic. Its clinical manifestations relying on the portion of the urinary tract involved, the etiologic organism(s), the severity of the infection and the patient's ability to create an immune response.[2] Signs and symptoms may include fever, chills, dysuria, urinary urgency, frequency and cloudy or malodorous urine. Infections are almost always ascending in origin and caused by bacteria in the periurethral flora and the distal urethra. These bacteria inhabit the distal gastrointestinal tract and colonize the perineal area.

The family of bacteria that most frequently cause UTIs is the *Enterobacteriaceae*, gram-negative facultative anaerobic bacilli commonly found in the large intestine.

The most common of these bacteria is *Escherichia coli* which forms about 90% of all Urinary tract infections. The other one is *Klebsiella* and *Proteus*, additionally, *Pseudomonas* that cause a complicated infections, especially in women. *Staphylococci* may cause 5-10% of UTIs in many populations. *E. coli* usually causes a child's first infection.[3] Staphylococcal infections, especially those due to *Staphylococcus saprophyticus* are common causes of urinary tract infection among female adolescent.

Recently, spices have attracted a great attention in their useful physiological functions and antimicrobial activity. Among these spices is the cinnamon which is one of the most popular and the oldest spices used for foods. It belongs to *Lauraceae* family and usually grows in South and South-East Asia.[4][5] The essential extracted oil from cinnamon is commonly used in the food industry because of its special aroma in addition to its medical properties.

In the recent years, some studies have reported that cinnamon oil had a broad range of antimicrobial activities against gram-positive and gram-negative bacteria.[6]

Therefore, the current study aimed to fulfill the following objectives:

1. Isolating of the bacterial isolates from the patient that infected with UTI.
2. Biochemical identification of bacterial strains.
3. To prepare aqueous extraction of cinammon (from bark and powder)

4. Evaluating the sensitivity of these bacterial isolates toward prepared extract by using of agar - well diffusion assay.
5. Studying of the age and gender impact on UTI.

2- MATERIALS AND METHODS

2.1 Collection of urine sample

Urine samples were collected from about 52 patients between the ages of 15-45 years, Clean -catch, midstream urine samples were collected in sterilized screw-capped universal after throughout preliminary cleaning of external genitalia with soap and water and those samples ought to be taken in early hours of morning.

2.2 Identification of pathogens

The bacterial isolates have been identified according to the below laboratory steps:

1. A loopful of urine specimen was streaked on various media; such as blood agar, macConkey agar, eosine methylene blue agar and mannitol salt agar. Cultural observation (color, size and colony morphology) were observed from the incubated plates.
2. During microscopic examination of urine specimen, the slides were prepared from each different colonies observed on the plates and Gram staining was performed. The results such as the gram positive or gram negative, motility, shape and arrangement of the bacteria were observed from this exam.
3. Biochemical Examination

The selected colonies have been subject to biochemical examination such as (catalase test, coagulase, triple sugar iron test, indole production test, citrate utilization test(S.C), urease test) for verifying of the pathogens.

2.3Aqueous extraction of spice

Spices including Powder and bark of Cinnamon *(Cinnamomum zeylanicum* were purchased from local market. The spices have been washed with distilled water thoroughly. A hundred gram of bark was crushed and sieved through screen mesh of cloth to get the fine powder. The powder was soaked in 200ml of distilled water and was kept at a room temperature for 24 hours and then it was filtered by using Whatman no. 1 filter paper. The filtration was heated up at 40-50°C using water bath, until thick paste is formed. The thick paste was considered as 100% concentration of extract. These extracts were stored at 4°C in refrigerator until it can be used.

2.4 Test microorganisms

Four bacterial isolates used in the present study and they are *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Staphylococcus aureus.*

2.5 Sensitivity test

The effect of extracts on the fourth bacterial isolates which mentioned above were carried out by agar well diffusion method. The minimum concentration of the plant extracts to inhibit the microorganisms were also determined by microdilution method.

2.5.1 Agar -well diffusion method

The available antibacterial in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly cultured with the test of bacterial isolates. Mueller Hinton agar plates were swabbed with a suspension of bacterial isolates, using sterile cotton swab then a sterile 4 mm cork borer was used to cut the wells at equidistance in each of the plates. 0.2 ml of each extract was added into each well and allowed to diffuse at room temperature for 20 minutes, then the plates were incubated aerobically overnight at 37ºC. The antibacterial activity was evaluated by measuring the diameter of zones of inhibition in millimeters.[7]

3- RESULTS AND DISCUSSION

In this study, a forty four samples out of 52 of urine samples have given positive results and have shown high cases of infection in female, unlike male gender. Meanwhile there was no grow in the balance samples as it shown in below table:

Table # 1: Isolation results according to gender.

Isolation results	Number	Percentage
Female	24	54.5%
Male	20	45.5%

Prevalence varies depending on geographic conditions and health conditions.[8]

All available research agreed that the pattern of incidence of urinary tract infection in females is more frequently than males.[9] This could be caused by urinary tract orifice near the anus in female making it easier for ascending infection than males as well as women who are infected with the disease were more prone to infection of other times. The studies have indicated the risk of re-infection among women for the presence of catalysts for bacteria in the cells lining the wall of the urinary system helps adhesion bacteria then

travels into the urinary tract.[10] In addition to the aforementioned factors anatomical differences between men and women played a major role in high casualties among women. Also, there are other factors that responsible for UTI such as urethra shortage in females and dryness of the area surrounding the external urethral orifice in males and antibacterial properties of prostate fluids.[11] Furthermore, the results harmonize with studies[12] &.[13]

Regarding the age factor, The study showed that the rate of infection is the highest in the age group 15-45 years, these results are coincided with many studies which indicate that cystitis is common in middle age Seema Rawat, (2015).[14]

Four different urinary pathogens were isolated from positive urine samples as shown in Fig. (1, 2, 3 & 4). These organisms were identified through different biochemical reactions (See Table # 2).

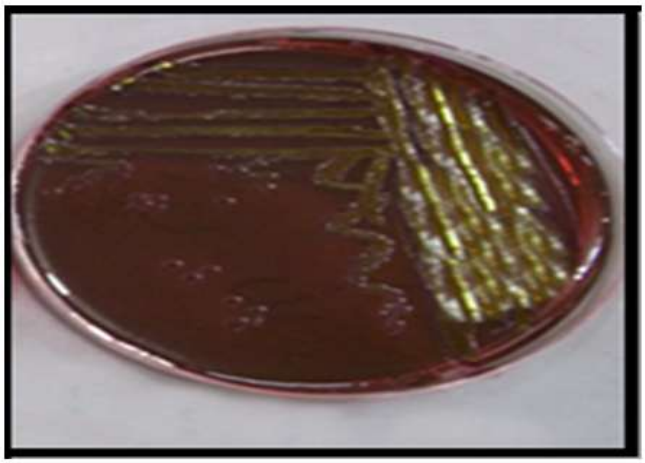

Fig. 1: *E.Coli* on Eosine methylene blue.

Fig. 2: *Klebsiella pneumoniae* on blood agar.

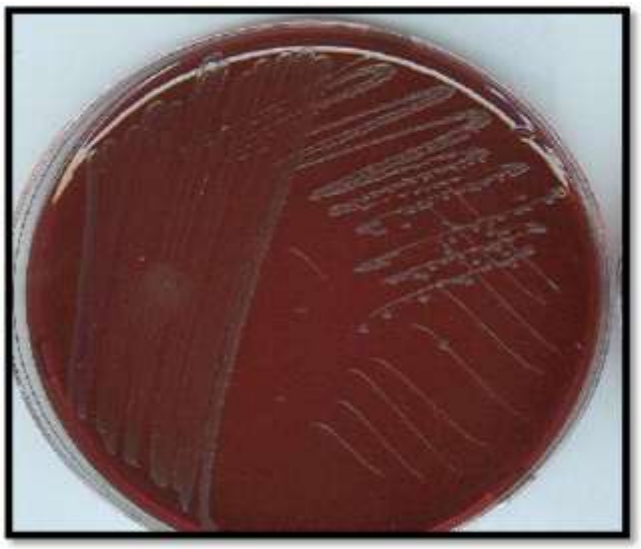

Fig. 3: *Proteus mirabilis* on blood agar.

Fig. 4: *Staphylococcus aureus* on blood agar.

Table # 2: Biochemical reactions for urinary bacterial isolates.

Bacterial isolates	Biochemical tests and Identification							
	Gram Stain	Catalase	S.C	Indole	Urease	Motility	TSI	Hemolysis
E.coli	-ve	+ve	-ve	+ve	-ve	+ve	A/A, Gas	ɤ
Klebsiella pneumoniae	-ve	+ve	+ve	-ve	+ve	-ve	A/A or K	ɤ
Proteus	-ve	+ve	-ve	-ve	+ve	+ve	K/A GAS, H2S	ɤ
Staphylococcus aureus	+ve	+ve	+ve	-ve	+ve	-ve	/	ß

+ve: positive /-ve: negative/ A : acidic / K:alkaline / ß: Beta / ɤ: gamma

Results showed that the most of the urinary tract infections caused by gram-negative bacteria like *E. coli* which was responsible for 19 (43.1%) of the cases. Followed by *Klebsiella*

pneumoniae 12 (27.2%), *Proteus mirabilis* 7 (15.9%) and finally by *Staphylococcus aureus* 6 (13.6%) as in Table # 3.

Table # 3: Bacterial isolates from urine samples.

Bacterial isolates	Number	Percentage %
Escherichia coli	19	43.1%
Klebsiella pneumoniae	12	25.5%
Proteus mirabilis	7	16.9%
Staphylococcus aureus	6	13.6%

Results of bacterial isolation is coincided with the findings of Beyene and Tsegaye (2011)[15], in his study he found that 90% of UTI cases caused by a gram-negative bacteria that belong to the family *Enterobacteriaceae*, while only 10% of the cases caused by gram-positive bacteria such as *Enterococcus, Staphylococcus and Streptococcus.*

The main cause of high incidence of the infection with *E coli* 19(44.1%) was due to the fact that these bacteria leave their natural place (micro flora of intestine) to urinary pathways causing inflammation of urinary tract, also it has numerous virulence factors as pili helping in adhesion to epithelia of urinary system. These results agreed with the findings of Beyene and Tsegaye (2011)[15] who showed that *E. coli* was responsible for (33.3%) of cases of urinary tract infection and Also coincided with the findings of (KİREÇCİ, *etal*, 2015).[16]

Antibacterial activity of the extracts were recorded as per diameters of the resulting inhibition zones of growth measured in (millimeters). Both extracts (bark and powder extracts) were tested against the four isolates, fig (5,6,7&8). The antibacterial activity of Cinnamon aqueous extract is summarized in below table # 4.

Table # 4: Diameter of inhibition zones of growth.

Bacterial isolates	Diameter of inhibition zone	
	Bark extract	Powder extract
Escherichia coli	4.5 mm	2.6 mm
Klebsiella pneumoniae	4.2 mm	2.5 mm
Proteus mirabilis	3.5mm	2.5 mm
Staphylococcus aureus	3 mm	2 mm

Most of the extracts exerted their antibacterial activity only at the highest concentrations. Results showed that the bark aqueous extract was more effective than the powder aqueous extract, The antibacterial activity of cinnamon has been attributed to the presence of some active constituents which may has lost their activity after grinding and exposure to the air or

due to its storage for long period. Also results showed that the antibacterial activity of the extract against Gram negative bacteria was more than of Gram positive bacteria and this might be related to the differences in the chemical structure of the bacterial cell wall.

The earlier studies suggested that the antibacterial activity of cinnamon was probably due to its oil as well as the pure cinnamaldehyde.[17] There were ten chemical components of cinnamon oil, The main components were eugenol (75.520%) and eugenyl acetate (4.403%). Previous studies reported that eugenol and eugenyl acetate were the major bioactive constituents of cinnamon oil. These volatile phenolic compounds could disrupt the membranes of bacterial cells, leading to the cell death.[18], [19], [20],[21] Also extract of cinnamon produce antibacterial activity against *Staphylococcus aureus* and these findings confirmed the observation of earlier studies of Fan *et.al*.[22], Yuste *et.al*.[23] Seeniva San *et.al*.[24]

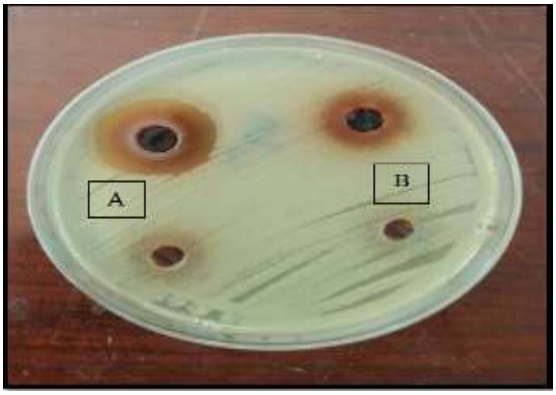

Fig. 5: Antibacterial effect of cinnamon aqueous extract on *Escherichia coli* (A: Bark extract, B: Powder extract).

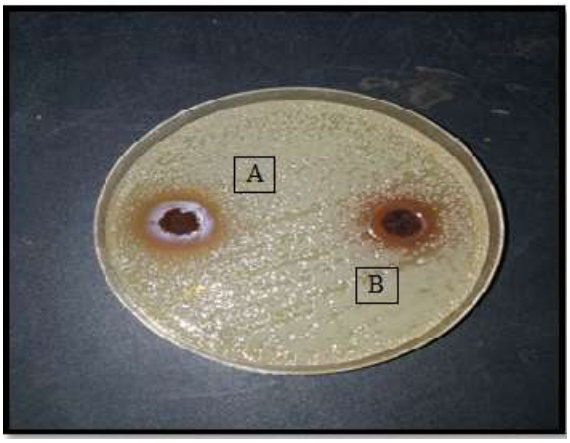

Fig. 6: Antibacterial effect of cinnamon aqueous extract on *Klebsiella pneumoniae* (A: Bark extract, B: Powder extract).

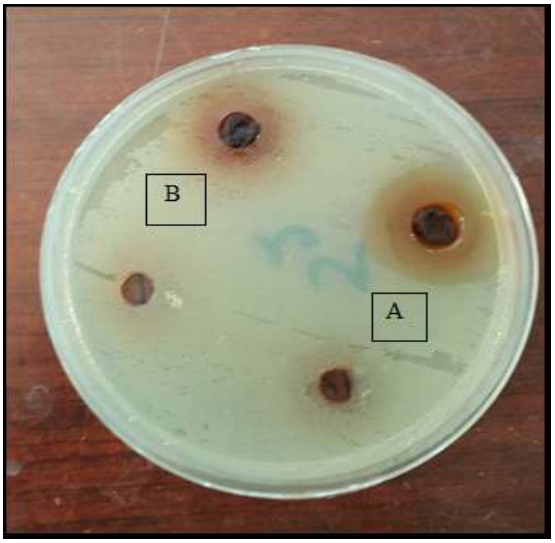

Fig. 7: Antibacterial effect of cinnamon aqueous extract on *Proteus mirabilis* (A: Bark extract, B: Powder extract).

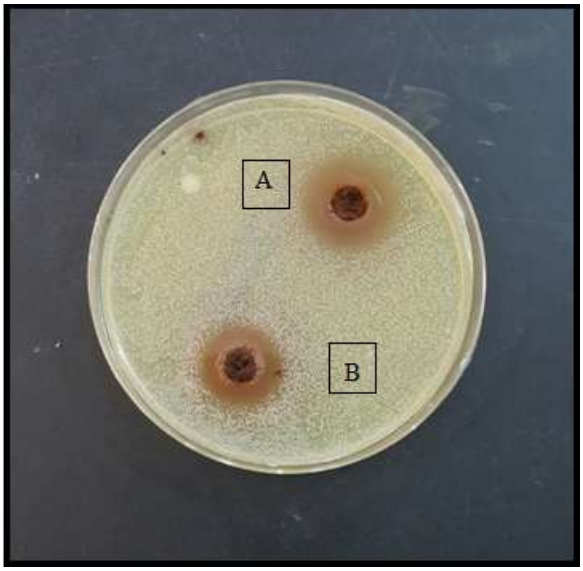

Fig.8: Antibacterial effect of cinnamon aqueous extract on *Staphylococcus aureus* (A: Bark extract, B: Powder extract).

CONCLUSION

It has been concluded that the Gram-negative bacteria (*Enterobacteraceae*) were responsible for urinary tract infections more than the Gram-positive and most of these isolates are sensitive to the aqueous extract of Cinnamon. The most common isolated bacteria from urinary tract infections were *E. coli,* followed by *klebsiella pneumonia, proteus mirabilis* and *Staphylococcus aureus.* Also, it was observed that the effectiveness of bark extract more than the effectiveness of the powder extract, as its noted that the extract effectiveness on the Gram-negative bacteria was better than the Gram-positive bacteria.

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