Antibacterial activity of aqueous extract *Cinnamomum zeylanicun* against of *Klebsiella pneumoniae* isolated from Bacteremic patients

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Abstract:
The study included collection of (70) blood samples of adults patients with Bacteremia and both Sex and different ages at the Imam AL-Hussain Teaching Hospital in the holy city of Kerbala, , the results showed of bacterial culture on media of MacConkey Agar , blood agar , diagnostic, Biochemical tests and confirmed the diagnosis using of Api20E , that 12 isolates belonging to bacteria *Klebsiella pneumoniae*. The results of the investigation of some of the virulence factors of *K. pneumoniae* showed that all isolates were surrounded by capsule ,and uncapable of producing Haemolycin enzyme while showed all isolates their ability to produce Urease enzyme and production Biofilm . Nine isolates (75%) showed ability to β-Lactamase enzyme ,The study also includes the qualitative detection of some active groups in *Cinnamomum zeylanicun* aqueous extract, The results showed that it is contained the Glycosides, Tannins, Resins, Saponins, and phenols, and is not containing alkaloids and Flavones .and it has pH (6.5). The Minimum Inhibitory Concentration (MIC) was determined *Cinnamomum zeylanicun* aqueous extract (6.25) mg / ml for the bacterial isolates used, The results showed that the different concentrations of the *Cinnamomum zeylanicun* aqueous extract showed a variation in the diameters. The concentration was 50 mg / ml the highest diameter was 33mm and the lowest diameter was 25 mm. The concentration (25 mg / ml) the highest diameter was 30 mm and the lowest diameter was18 mm. The concentration (12.5 mg / ml) had the highest diameter inhibition of 28 mm and the least diameter of inhibition of 16 mm, the concentration of (6.25 mg / ml) had reached the diameter of the inhibition of 25 mm and less diameter of inhibition 12 mm ,compared to the control factor containing sterile distilled water. The results were compared with the most used antibiotics, and through the results *Cinnamomum zeylanicun* aqueous extract showed a high inhibitory effect compared to the antibiotics used in the study.

Key words: Antibacterial activity , *Cinnamomum zeylanicun* , *Klebsiella pneumoniae* , Bacteremia .

Introduction :

Bacteremia signifies the presence of bacteria in the blood stream(1) **.** Bacteremia may be continuous or transient. In circulating blood microorganisms are present , whether continuously, transiently, or intermittently, are a threat to every organ in the body**.** They can have real consequences like shock, multiple organ failure, disseminated intravascular coagulation, etc**.** thence, the blood stream infections represent one of the most serious situations and, as a result, well timed detection and identification of blood stream pathogen is important (2)**.** Bacteremia has been reported increasingly in pediatrics age group. There are no dependable data on the incidence or prevalence of invasive bacterial infection introversion pediatrics age group. The bacterial isolation from blood cultures is usually significative of serious invasive infection that's require immediate antibiotictreatment(1).Bacteremia is a pathological condition with a high mortality rate that variesbetween 30 and 70 per cent and depended on several risk factors(3,4) The genus *Klebsiella spp*. is attributed to the intestinal family. These bacteria were first isolated by the world's Friedlander from pneumonia in 1882, And was identified as a satisfactory cause of epidemic and endemic infections in hospitals during the ' 50s of of the last century(5). These bacteria spread

widely in nature and are isolated from different habitats such as parts of the human body, animals , Sewage, soil, lakes, salt water and fresh water are generally opportunistic pathogens for humans and animals(6). The other habitat is the hospital environment and mucous surfaces in the Mammals , and the intestines are the most important places for the availability of these bacteria naturally(7) .Resulting in extensive use of broad spectrum antibiotics irregularly to the emergence of strains of *Klebsiella* spp. , bacteria have multiple resistance to antibiotics and may be one of the reasons is produced β-Lactamase enzymes(8).*K. pneumoniae* is the second cause of septicemia and septicemia in children and newborns, And acute diseases, especially in people with weakened immune system caused by diabetes,Chronic heart disease, stenosis of pulmonary vesseles, especially in elderly persons and newborn babies, and that most injuries to *K. pneumoniae* in hospitals are highly fatal if not treated properly(9). These bacteria also cause Otitis, Mucosistis , necrosis, and lung infections(10). *Klebsiella pneumonia* is an opportunistic pathogen that accompanies people with immunosuppression who suffer from various urinary tract infections(8).And resistant to phagocytosis because they contain the capsule(11). The virulence factors of the *Klebsiella* bacteria are antigen capsule(9) . lipopolysaccharides (LPS) , Bacteriocin and Biofilm(12). The capsule of *Klebsiella* spp. bacteria is one of virulence factors for human pathogenicity(9).Recent studies have confirmed the importance of urease as a pathogen and virulent factor causing many bacterial species and is produced by many bacterial strains that cause urinary tract infections such as *Pseudomonas*, *Klebsiella*, *Proteus*(13).There is a close relationship between the ability of bacteria to form biofilm and its ability to cause disease and cause chronic inflammation, The bacteria that produce biofilm have a greater ability to colonize and stay in the patient's body and become less sensitive to antibiotic treatment(14) .*K.pneumoniae* can form the biofilm ,Type 3 fimbriae plays a large role in the formation of the biofilm , which is a real problem in medical devices, especially urinary catheterization, which is the cause of many hospitals acquired infections (15).Plants have been used in alternative medicine since ancient times through the use of their extracts and active ingredients to treat many infectious and non-communicable diseases. Today, despite the effectiveness and efficiency of the systems of the manufacture of chemical medicines, medical plants are still the basic base in medicine and health system. Plants to take advantage of their medicinal and physiological properties and functions as health conservation requirements (16) .The cinnamon plant of the lauraceae family is characterized by its wide spread and its multiple uses as food or aromatic additives. *Cinnamomum* is one of the most widespread plant species. The bark was used to treat many diseases such asgastrointestinal disorders and urinary system problems and treatment of bad breath and treatment of colds and wounds and has seen in recent years his uses in the treatment of sugar(17 ,18).The effectiveness of the students because of the contents of the most effective substances, including polyphinols, including vanillic, caffeic, ferulic, coumaric, protocatechuic gallic and a group of volatile phenols,Which includes a group of hydrocarbons and oxygen compounds such as benzyl benzoate, cinnamyl acetate, eugeyl acetate, linalool, b- caryophyllene and cinnamaldehyde, It is the most effective and effective ingredient in the various extracts of the plant.(19).All these substances are known to have the ability and effectiveness against high blood sugar, insulin secretion or regulation of liver and fatty tissue(20).Some of the medical uses of the students that it uses food or preparations or flavors such as use in chewing gum also maintains the health of the mouth and recovery and clean the teeth and remove odors unpleasant and is also used to remove acne and diseases of the digestive tract and colon infections, which is anti-diarrhea and tonic of cellular activities and anti-hemorrhage and increases the flow of blood to the uterus and activates the growth

and reconstruction of damaged tissue and anti-bacterial and fungal effects and neuroprotective and It is tonic for the heart, liver and anti-vomiting (21).Due to the epidemiology of *Klebsiella pneumoniae* and the lack of studies related to it in the holy of Kerbala and the attempt to find effective compounds isolated from natural sources and use as a treatment to eliminate or reduce the growth of some pathogens, to reduce the side effects of antibiotics currently used and have serious effects on the health of the patient's body .

Materials and methods :

1. Collection of samples:

70 blood samples were collected from adult patients with Bacteremia and both Sexand different ages at the Imam AL-Hussain Teaching Hospital in the holy city of, before the start of blood withdrawal from the arm sterilizes the skin with ethyl alcohol (70%), then iodine (10%) and left for one minute to dry and then pull (7-10) milliliters of the right hand and carefully to avoid contamination(22). Wipe the lid of the bottle of alcohol to prevent potential contamination and inject blood into the special bottle. The bottles are transported as quickly as possible to the laboratory and placed in the blood Culture device (BacT / Alert 3D) for 1-5 days. In the case of positive result, a small amount of the blood-broth mixture is taken with a sterile syringe and cultured in blood and MacConkey agar and incubated at 37 ° C for 24 hours.

2. Diagnosis of Bacterial isolates:

Bacteriological isolates were identified by studying the general culture characteristics of the developing colonies on in Blood and MacConkey agar, And then examined the forms of colonies phenomenon and determined on the basis of textures, color, shape, size, as well as other general characteristics such as lactose fermentation or not, as well as the presence of blood analysis on blood agar or not(23.Microscopic and Biochemical tests were carried out for bacterial cells and APi 20 E kit was used to confirm the diagnosis of bacteria.

3. Detection of the virulence factors in *Klebseilla pneumoniae* :

- Detection of capsule presence using negative staining, Hemolysin and Urease test as described in(24).
- Detection OF Biofilm formation by Congo- red method(25 ,26).
- Detection of the production of β-Lactamase enzyme:• Preparation of bacterial suspension and Rapid iodine method(27).

4.Preparation of cold aqueous extract *Cinnamomum zeylanicun*:

• **Collecting plant samples**: *Cinnamon* bark samples were collected from local markets. The required parts were washed with distilled water, dried in the shade, then grinded with an electric mill and dried powders were kept in sealed containers until use.

• **Preparation of cold aqueous extract*Cinnamomum zeylanicun*:** using the method (28). by taking 50 g of dry powder in a 1000 ml flask and mixing it with 500 ml of distilled water and then mixing using the electric mixer and • Leave for 24 hours at room temperature. Then, filtered the mixture with several layers of medical gauze to remove the plankton. Centrifuge at 3000 rpm for 10 minutes.The filter was then filtered using the filter paper to obtain a clear solution. The extract was then dried using the oven at 40 ° C and the extract was kept in a dark bottle in the refrigerator until use.

5. Chemical Detection for cold aqueous extract of *Cinnamomum zeylanicun*:A series of chemical detection tests were carried out to detect some active groups of *cinnamon* extract. They included the detection of Glycosides, Phenols, Tannins, Saponins, Resins resins , Alkaloids, Flavones, and the pH(29).

6.Determination of the Minimum Inhibitory Concentration (MIC) cold aqueous extract of *Cinnamomum zeylanicun*:The Broth Dilution Technique(30), which relies Which is based on bacterial growth inhibition, in the preparation of a series dilution of aqueous extract for the purpose of determining MIC,dilutions (1.56, 3.12, 6.25, 12.5, 25, 50)

mg / ml for cold aqueous extract of *Cinnamomum zeylanicun*(31,32).

7.**Antibacterial activityfor *Cinnamon* extract:**

The method was used in well diffusion (33). The method included preparation of the Muller-Hinton Agar according to the instructions of the company equipped with the medium in the Petri dishes, After the concretion the medium was vaccination by suspension ,Spread the bacterial suspension using sterile swabs. Leave the dishes for 15 minutes to drink the residue in the medium of the plant and then work 5 well in each dish with a diameter of 5 mm by a sterile cork porer and add 10 µl of the plant extract at a concentration of 50 mg / ml, 25 mg / ml, 12.5 Mg / ml, 6.25 mg / ml of each concentration in four wells using a micropipette. The fifth well was filled with distilled water and returned to control. The dishes were incubated at 37 ° C for 24 hours and the results were recorded by measuring the diameters of the inhibition zone in millimeters.

Results and Discussion :

• **Isolation and diagnosis of *Klebsiella pneumonia*e bacteria:**Seventy bacterial samples were obtained from people with different bacteremia of different sex at different ages of the Imam Hussein Teaching Hospital in Kerbala.The results of the morphological and microscopic tests revealed that 12 (17.14%) isolates belonging to *Klebsiella pneumoniae* have a rod shape surrounded by capsule .There is a single or gorgeous pairs or short chains and distinguished from the remaining types being used are qualities and tests adopted Biochemical as shown in table (1).

Table (1): morphological,microscopic and Biochemical tests for diagnosis of*K. pneumoniae*.

tests	*K. pneumonia*
Gram stain	-
Motility test	-
Oxidase test	-
Catalase test	+
Indol production test	-
Methyl red test	-
Voges proskauer test	+
Ctrate utilization test	+
H_2S production test	-
Urea hydrolysis (urease test)	+
Gelatin laquification test	-
Lactose fermentation test	+
Growth in KCN media	+

Depending on the results of morphological ,Biochemical, and microscopic tests(6 ,34). the isolates are *K pneumoniae*. To confirm the diagnosis, the Api-20E, which is easy, fast and accurate, has been used to confirm results of the biochemical diagnosis.

- **Detection of the virulence factors in *Klebseilla pneumoniae* :**The current study of Detection a number of virulence factors associated with *K. pneumoniae* as shown in Table (2).

Table (2): virulence factors in bacterial isolates of *Klebsiella pneumoniae*:

No. isolate	Found of capsule	Production of hemolysin	Production of urease	Production of biofilm
k.pn1	+	-	+	+
k.pn2	+	-	+	+
k.pn3	+	-	+	+
k.pn4	+	-	+	+
k.pn5	+	-	+	+

k.pn6	+	-	+	+
k.pn7	+	-	+	+
k.pn8	+	-	+	+
k.pn9	+	-	+	+
k.pn10	+	-	+	+
k.pn11	+	-	+	+
k.pn12	+	-	+	+

Results showed that all the isolates study contained capsul were 100% by microscopic test and by using nigrosin stain. The cells appeared as bacillus surrounded by zone, And the laboratory test that all isolates contain a capsule , Results showed that all isolates *K. pneumoniae* has no ability to produce Hemolycin on the blood agar. Hemolycin is an important ferment factor for a wide range of negative and positive gram bacteria and contributes to pathogenicity. As well as the ability of bacteria to produce the enzyme urease. The results showed that all isolates of *K. pneumoniae* 100% have the ability to produce this enzyme, And all isolates had 100% availability to produce biofilm . All colonies were black with dry crystalline density. The positive result was that this study was close to(35).Bacteria that grow in the biofilm are tolerant to many antibiotics and are resistant to both opsonization, phagocytosis, various environmental conditions and also resistance to selective stress(36).Microorganisms that produce the biofilm increase antibiotic resistance more than 1,000 times more than they do not ,, And that the bacteria produced the biofilm are responsible for infections and pathogens that are difficult to treat because of the difficulty and restriction of the penetration of the antibiotic.(37).this study showed that nine of the 12 isolates of *K. pneumoniae* gave a positive result of the test (75%). There is a clear difference in the time of the positive result, ranging from several seconds to 2 minutes.β-Lactamase enzyme reduces the iodine to the iodide and the latter lacks the ability to interact with the starch and the formation of a complex violet directly converted to white, and can be explained at the time difference based on the concentration of of β-Lactamase enzymeproduct in the protoplasm.

• Chemical detection of some active groups and pH of the aqueous extractCinnamomum *zeylanicun*:

The results of the detection of the active groups in the extract of *cinnamomum zeylanicun* containing glycosides, tannins, resins, sapones and phenols in varying percentages and the absence of alkaloids and flavones. These results are consistent with what is mentioned by researchers who confirmed that cinnamon contains the above mentioned compounds in varying percentages,(33). the aqueous extract*Cinnamomum zeylanicun*contains active compounds such as glycosides, tannins, resins, and phenols. The presence of alkaloids and flavones was not observed. As shown in Table 4, it is clear that extract of *Cinnamomum zeylanicun* is distinguished by its active groups. This has high inhibitory effect against isolates Bacteria study Table (3).

Table (3): chemical detection of some active groups and the pH of the aqueous extract *Cinnamomum zeylanicun*:

Active groups	aqueous extract *Cinnamomum zeylanicun*
glycosides	+
alkaloids	-
tannins	+++
resins	+

soapiness	+++
flavones	-
phenols	++
pH	6.5

The sign (+) is a positive result of the test, while the reference (-) is a negative result of the examination.

• **Determination of (MIC) cold aqueous extract of *Cinnamomum zeylanicun* :**The results showed MIC was 6.25 mg / ml for the bacterial isolates of *K. pneumoniae* used in the study. The effect of this extract depends on the concentration used and its containment of the active substances. MIC The is due to Containing effective compounds, and extracts containing effective substances with low molecular weight have a more inhibitory effect, and may be due to differences in the sensitivity of bacterial isolates study againstextract of Cinnamon plant to the nature of the bacteria itself in terms of composition and thickness of the cell wall and the content of fat and protein.

• **Antibacterial activity of aqueous extract *Cinnamomum zeylanicun* against of *Klebsiella pneumoniae* isolated from Bacteremic patients:**The results of the test of the effect of aqueous extract of *Cinnamomum zeylanicun* against of *K. pneumoniae* by well diffusion, 12 isolates of the bacteria were used after confirming the isolation and diagnosis, And four different concentrations (6.25 - 12.5 - 25 - 50) mg / ml respectively of the extract of *Cinnamomum zeylanicun* using four wells and the fifth returned to control. Four concentrations were compared for the purpose of determining the inhibitory efficiency of each concentration of the concentrations mentioned , The results showed that the concentration of 50 mg / ml was the most affected bacterial isolates with the highest inhibition rate of 33 mm and 22 mm. The concentration of 25 mg / ml was the highest inhibition of 30 mm and 18 mm. The concentration of 6.25 mg / ml has a maximum diameter of 25 mm and a minimum of 12 mm in comparison to the control coefficient containing distilled water as in Table (4).

Table (4): Diameters of the inhibition zones (mm) for different concentrations of *Cuminomum zeylanicun* extract against *K. peumoniae* isolates from bacteremia patients

No. of isolate	6.25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml
k.pn1	22	24	27	30
k.pn2	25	28	30	32
k.pn3	14	18	21.5	26
k.pn4	14	17	20	25
k.pn5	22	24	27	30
k.pn6	17	21.5	24	28
k.pn7	24	28	30	32
k.pn8	12	16	20	25
k.pn9	12	16	18	22
k.pn10	25	28	30	33
k.pn11	14	17	20	25
k.pn12	17	20	24	28

It is clear from the above results that the best rate of inhibition diameters was in favor of 50 mg / ml concentration and increasing the diameters of the inhibition zones compared to the concentration of the extract. The *Cinnamomum zeylanicun* has high inhibitory effect against the pathogenic bacteria study. The inhibitory effect of Cinnamomum Because it contains effective compounds inhibitory.

Antibacterial activity for of *Cinnamomum zeylanicun* aqueous extract against of *Klebsiella pneumoniae* is increased by increasing its concentration. This is due to its effect on the active inhibitory compounds, which have a role in inhibiting the growth of bacteria, and may be due to its inhibition of growth of bacterial species because it contains some phenolic compounds such as thymol which has inhibitory effect against bacteria has an effect on the bacterial cell wall by dissolving the cell wall fat and forming the hydrogen atom with the water molecules and nitrogen amino acids inside the bacterial cell affecting the mechanism of action of the cell membrane of biology and thus inhibiting the growth of the organism .The difference in the effect of inhibitory activity of the extract depends on the concentration used and the bacterial isolation as well as the environmental conditions necessary for growth bacteria.and also to encourage the use of effective compounds isolated from natural sources and use as adjuvant treatment to eliminate or reduce the growth of some pathogens, to reduce the side effects of antibiotics used in Present time and which have serious effects on the health of the patient's body.

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