



INHIBITORY ACTIVITY OF AQUEOUS EXTRACT OF POMEGRANATE PEEL AND CAROB AGAINST BACTERIA ISOLATED FROM DIARRHEAL PATIENT IN KIRKUK HOSPITAL

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Abstract

This study was conducted to investigate the effect of some plant extracts (pomegranate peel and carob) on bacterial species, causing diarrhea. We collected 52 stool samples from diarrheal patients in Kirkuk hospitals. They were cultured in selective circles and were identified using morphological traits, biochemical tests and API 20E, which included more isolates of bacteria *E. coli*, then *Pseudomonas* spp., *Proteus* spp. and *Klebsiella* spp, of the total (52) samples. Antibiotic sensitivity tests were performed for isolates where *E. coli* was found more sensitive to anti-CTR and inhibition zone (22mm) compared to other antibiotics other bacteria are *Proteus* spp. sensitive to CIP inhibition (30 mm), *Pseudomonas*.spp is sensitive to CIP and inhibition (30mm), and the *Klebsiella* spp. are sensitive to IMP inhibition (40mm). The results of the water extracts of pomegranate peel and carob showed a clear inhibitory effect for the isolates of the studying as shown that the inhibitory effectiveness of aqueous extract of the carob more comparative with pomegranate peel extract against bacteria.

Key words : Diarrhea, Enterobacteriaceae, *Ceratonia siliqua* L., *Punica granatum* L. (pomegranate).

Introduction

Diarrhea is one of the most serious diseases widespread around the world particularly in developing countries which causing the loss of a large amount of body fluids leading to dehydration, and often leads to occurrence malnutrition especially in children with recurrent infections, and the situation is increasing, Especially in summer, with increased risk of drought, Diarrhea is particularly common in the summer, with hot weather and climate contributing to an increased incidence of the disease, with an increased risk of drought. More than 1.3 million cases of diarrhea are estimated annually in children under the age of five in Asia China), Africa and Latin America, and there are (6-4) million children of this age group dying each year from their injuries be a bacterial infection change in intestinal wall permeability that allowing the passage of molecules located in the intestinal cavity which causing immune disturbances as it stimulates the immune system to produce antibodies may have the ability to interact with body tissues which have antigenic determinants similar to those of molecules,

and multiple pathogens including bacterial and parasitic, these causes vary in their incidence and severity of pathogenicity in differentiated factors of virulence the involvement of more than one pathogen is found to cause infection As there is a large variation in the severity of the symptoms associated with diarrhea in the case of a pathogen, children develop diarrheal disease either as a result of exposure to pathogens entering the intestinal cavity through food or drinks or hands contaminated by these pathogens, fingers or other things such as tools Food or Toys (Adel, 2002; Amera, 2002).

Enterobacteriaceae is one of the most common types of gastrointestinal infections which includes Gram-negative bacillus, it is naturally colonized in the intestinal tract of humans and animals and has many species (*Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Serratia*, *Proteus*) and others, some of which, like *E. coli* are part of the normal flora and others such as *Salmonella* and *Shigella* are satisfactory to humans, these organisms have an optional aerobic or anaerobic life, moving (possessing peritrichous flagella) or non-

moving, fermented and oxidized glucose with gas production, positive for the catalase test, negative for the Oxidase test, Nitrates are reduced to nitrite, possess complex antigen structures, produces various toxins and pathogens (Rathore *et al.*, 2000). Recent research has increased the use of medicinal herbs and plants as therapeutic agents against pathogenic microbes and with limited side effects compared to chemical drugs. These include pomegranate peel and carob.

Punica granatum (Pomegranate) comes back to the *Punicaceae* family its native West Asia is cultivated in most Arab regions, especially the Mediterranean Basin, Iraq and the Levant, pomegranate has a wide range of medical functions and has been widely used in the field of traditional medicine in many countries of the world, several studies have indicated that the pomegranate peel has several therapeutic elements, including its antioxidant action, cancer, immune stimulating action and the deadly effect of harmful larvae, it is used in diarrhea treatment, rheumatism, gastric ulcers, skin lesions, sores and wounds and hypoglycemic effect and antimicrobial action through contain compounds [Alkaloids, Flavonoids, Glycosides, Polyphenol, Tannin] In particular Tannin, which accounts for about 25% of the crusts, has high anti-microbial activity through its association with proteins and a complex composition with the cell wall causing bacterial cell destruction, and it has an inhibitory effect against the negative and positive bacteria of the Gram strain (Saeed and Tariq, 2006; Sohaib, 2014).

Ceratonia siliqua L. (Carob) back to the genus *Ceratonia* which belong to the family of Fabaceae or Leguminosae spread naturally in the eastern Mediterranean, southern Europe and North Africa and live in semi-arid and wetland areas, it contains phenols, flavonoids, Ellagic acid and alkaloids that have a clutch effect, it also contains Coumarins and Saponins it is used to treat acute and chronic diarrhea for its holding effects it treats liver diseases, respiratory infections and skin it is anti-parasite, bacterial and fungal, it also inhibits cancer cells because it contains epigallocatechin-3-gallate and Epicatechin-3-gallat compounds that inhibit tumor cell growth, it is also a multi-purpose tree from which it is used in the food industry as an adhesive in the industry, and stamp paints and cosmetics therefore, our present study aimed at isolating and diagnosing a number of intestinal bacteria causing diarrheal cases and their response to many antibiotics commonly used to study the inhibitory activity of the water extract of pomegranate and carob plants on bacterial isolates (Zainab.A. Mohammed and Sabah, 2016; Rached *et al.*, 2016).

Materials and Methods

The study included the use of plants (carob and pomegranate peel) and obtained them from local markets and transferred to the laboratory washed with sterilized distilled water and dried on sterilized filter papers at laboratory temperature, crushed and grinded by an electric grinder and then stored in polyethylene bags in refrigerator 4°C (until use).

Plant extracts were prepared in a steeping manner (Sami and Jamil, 1988). 10 g of each studied plant powder was taken and placed in 100 mL sterilized distilled water and for a period of two hours and at the same time was steeping also for 24 hours the solids were disposed of by filtration of plant extracts via filtration paper then centrifuged at 3000 RPM for 10 minutes, then take the leachate and put in sterile bottles and save in the refrigerator until use, concentrations of each 100 ml extract of leachate were prepared in 100 ml of stilireized distilled water and the concentrations were sterilized using Millipore filter 0.45µm, through which the germs pass through.

The isolates were activated by growing on Nutrient Agar and incubating at 37°C for 24 hours, Under sterile conditions, 10 bacterial colonies were transferred to a test tube containing 5 ml of Nutrient broth, incubated 37 °C for (4-6) hours, and appropriate bacterial dilutions were performed so that the total cell count was approximately 10.77 ml (Al-abbasi, 2016).

The dishes containing Nutrient Agar were vaccinated according to the method (Fawzi, 1981) of 0.1 mL of bacterial suspension. The dishes were then left at room temperature for 15 minutes to absorb the vaccine. After that, the fertilized medium of the bacteria was drilled by two holes per dish with a sterile 7 mm diameter filter and 50 µL of all concentrations of the water extract prepared by a fine pipette inside the hole, then incubating the dishes at 37 ° C for 24 hours, then read the result by measuring the diameter of the inhibition area around the hole the antibiotic sensitivity test was performed on 15 Disks Diffusion Method Test according to (Connie *et al.*, 2011) (CFF), Cefixime (CFM), Piperacillin (PRL), Ceftioxone (CTR), Imipenem (IMP), Tetracyclin (TE), Gentamycin), Chloramphenicol (C), Clindamycin (CD), Erythromycin (E), Doxycycline (DO), Ciprofloxacin (CIP), Aztreonam (AT)

Results and Discussion

The results showed a difference between the types of bacteria under study, the most isolated isolates were *E. coli* (24) isolates, And *Proteus* spp. (16) isolation,

Pseudomonas spp. (12) isolation, *Klebsiella* spp. (12) isolation of the total (52) sample.

The results of the aquatic plant extracts showed a different effect depending on the type of bacteria under study, table 1 shows that the *E. coli* bacteria a significant effect of the carob extract compared to the extract of pomegranate peel in the inhibition area (30 mm), *Pseudomonas* spp. showed the effect of the carob extract and the inhibition area (25 mm) compared to the pomegranate peel extract, *Proteus* spp. was affected by carob extract and inhibition (25 mm) while its effect on pomegranate extract was negligible and *Klebsiella* spp. showed a clear effect of the carob extract and the inhibition area (30 mm) compared to the pomegranate extract, as shown in the pictures below (1-2-3-4).

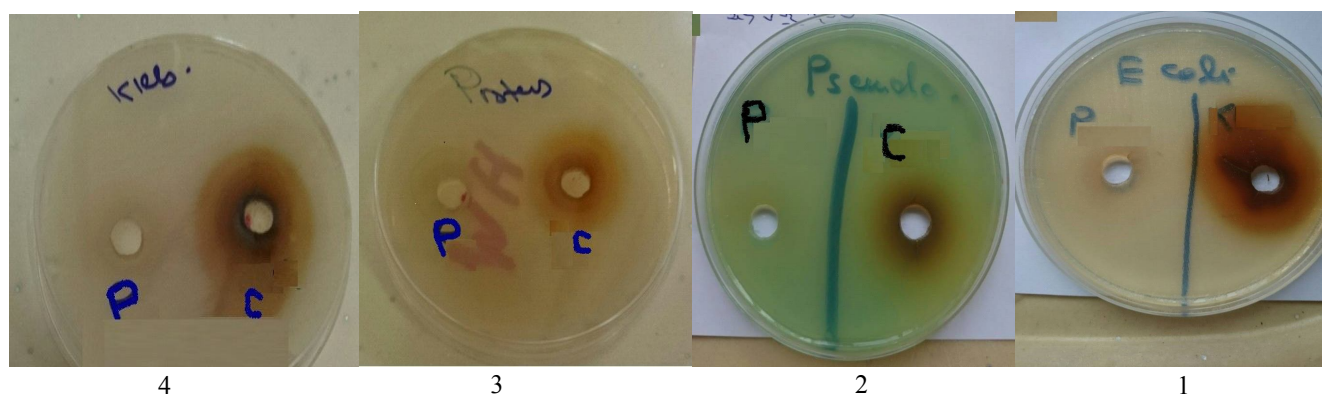
The results of the present study were consistent with all of these (Gil *et al.*, 2000), all of which showed sensitivity to the extracts of pomegranate husks used and to different degrees of effect from one type to another. The main reason for this effect was attributed to containing the extract on a wide range of secondary metabolites (Alkaloids, tannins, volatile oils, flavones, soaps, polyphenols, resins, alkaloids), For example, their effectiveness against bacteria is attributed to their ability to form a complex compound with melted cell proteins and are synthesized with the bacterial cell wall, with alkaloids where they interfere with the cell DNA, while tannins and phenolic compounds inhibit the work of enzymes and transport proteins either soaping interfere with the cellular membranes, especially with cell membrane sterols and work on the analysis of living cells.

An antibiotic sensitivity test for isolates showed clear results as *E. coli* showed sensitivity to antigens (CTR, IMP, CFP, PRL, CX, AK, AT) and *Proteus* spp. Sensitivity to (CIP, TOB, AK, CX) and *Pseudomonas* spp. Sensitive to (CIP, IMP, TOB, AK, GM) and *Klebsiella* spp. Sensitivity to (IMP, CIP, TE, AK, E, GM, DO, C, CD).

Table 1: The effect of the hydrolytic inhibitory effect of the pomegranate and Carob fruit on isolates of the intestinal family (measuring the inhibition area of one millimeter).

Bacteria	Pomegranate peel	Carob
<i>E.coli</i>	15	30
<i>Pseudomonas</i> spp.	5	25
<i>Proteus</i> spp.	13	25
<i>Klebsiella</i> spp.	18	30

Intestinal bacteria are highly capable of developing antibiotic resistance, so the use of antibiotics on a large scale and in a non-specific manner leads to the detection of a group of antibiotic-resistant internal bacteria that can transmit resistance genes to infectious pathogens and other germs in polluted environments, bacteria use many mechanisms to resist antibiotics. It is possible to have more than one mechanism in one organism, antibiotic resistance may be due to alterations in the pumping process as the entry of the antibiotic into the bacterial cell stimulates the production of the transport proteins this effectively transfers the antibody (energy) from inside the germ cell to the outside, leaving it with a low concentration cannot influence the cell's effectiveness and examples of antibiotics that can resist this way: tetracycline and erythromycin,. The antibiotic may not be able to enter the bacterial cell due to a change in the outer membrane proteins that leads to a change in the antibiotic transfer sites for example, B-lactam, is transported through the outer membrane of intestinal bacteria through membrane proteins called porins in the event of any change of these proteins, these antibiotics cannot enter the germ cell There may be a change in the binding sites to which antibiotics are associated, such as a change in the proteins associated with penicillin for positive and negative Gram-positive bacteria, Changes in ribosomes cannot be associated with tetracycline and erythromycin, In addition, antibodies that work on the



P: Pomegranate peel
C: Carob

cellular wall may be resistant by the bacterial cell resulting from the formation of alternative proteins in the cell wall (Sami Hashem Majeed and Jamil, 1988).

The study showed that isolates resistant to several antibiotics may all be sensitive water extract pomegranate peel and carobs may have a negative effect on the growth of bacteria to one or more of the chemical components contained in the extract. The presence of flaxseeds such as Tannine may affect the protein layer in a bacteria, killing or may affect the plasma membrane, thereby altering its functional properties and this leads to inhibition of bacterial growth, and the presence of compounds (Polyphenol, Flavonoids, Ellagic acid and Triterpens) in water extracts of pomegranate crusts in its antimicrobial action (Ahmed, 1985; Shaymaa, 2016).

Conclusions

Based on the results of the currently study showed the water extract of the carob more effective and effect to the intestinal bacteria of the extract peel pomegranate fruit, the study should extend the inhibitory activity of these plants to other bacterial isolates for the purpose of large-scale treatment rather than chemical use.

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