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Inhibition Effect of Pomegranate Peel Extract and Orange Peel Extract on *E.Coli* Bacteria Growth from Chees

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ABSTRACT: The fruits of different plant species (pomegranates and oranges) were collected from the local markets of the city of Nasiriyah and dried their crusts and attended alcoholic and aquatic extracts of these fruits and studied the effectiveness of inhibition in the growth of E.coli bacteria. A moral difference (P≤0.05) was observed between the effect of the alcoholic and watery extract of pomegranate peels compared to the effect of the alcoholic and watery extract of orange peels as well as the inhibitory effect of antibiotics.

The effect of the pomegranate peel alcohol extract on E.coli bacteria was found to be more effective in inhibition if the high inhibitory concentration (30mm) followed by the hydropode extract of pomegranate peels if the inhibitory concentration (25 mm) the inhibitory concentration extracted was orange peel (18 mm) and the water extract For orange peels it was (9 mm) and if compared with the antibiotics used where it was observed that the highest efficacy of antibiotics (Gentamycin) where the diameter of inhibition (18 mm) and the least inhibition was for the antibiotic (Trimethoprim) where the diameter of inhibition (7 mm)was approximate.

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INTRODUCTION

Confirms the future view of the use and development of antibiotics where pathological microbiology will continue to find the vital means available to it to resist the effect of these antibiotics and stop their effect, which leads to the emergence of strains resistant to the effect of these antibiotics and no matter how new antibiotics scientists have devised Misuse and collateral damage will remain a multifaceted and globally widespread scientific and behavioral problem and the interest in discovering new types of antibiotics will remain in which the side effects on health decrease and have effectiveness against pathogenic microbes a goal and purpose sought by scientists diligently.

Resistance to these antibiotics is considered a dangerous indicator of health by affecting the increase in infection and the spread of diseases and increased mortality as well as the high costs of making antibiotics, so there was a need to find safe drugs to use from natural sources badly plant or animal, The plants are rich in compounds Biological Bio compounds that are effective anti-microbiology such as Volatile oils, Alkaloids, Tannins, Phenols, Glycosides, Saponins, and saponins. Microbiology towards different plant extracts according to the differences like peptidoglycan in positive and negative bacteria of the pigment of the crone as well as the effect of teichoic acid in addition to the effect of the nature of polysaccharides and layers of fat and protein; These three compounds keep the cell from entering the cells (Singh *et al*,
2020). Belitz *et al* (2009). These together gain negative bacteria resistance different from positive bacteria and studies indicate a difference in the anti-bio-organism effectiveness of plant extracts depending on plant type and microorganism, this study aimed to choose the inhibitory effectiveness of crust extracts of Pomegranate and orange peels on *E.coli* bacteria.

Escherichia coli

It is one of the most important types of bacteria that live in the intestines of mammals, discovered by the scientist Theodor Ishersh, also known as the bacterium of the large intestine.

Escherichia coli is a gram-negative bacterium and makes up about 80% of its aerobic flora structure. Anaerobe anaerobic germs are predominant lye in the intestines of mammals. The presence of this bacterium in the surrounding environment refers to fecal

contamination, so it is often used as evidence of water contamination and judging whether it is drinkable or not drinkable from a bacterial point of view, it should be noted that the addition of chlorine gas to water eliminates *E.coli* bacteria (Jawets *et al*., 2004). This bacterium in its main medical effects is non-pathogenic and is a source of vitamin K, but many of its strains cause different patterns of the intestinal and urinary tract and other diseases such as internal bleeding, bleeding, blood poisoning, urinary inflammation, meningitis, and pneumonia.

TRANSMISSION METHODS

1-By mouth, these bacteria enter and settle the human intestine and begin to cause various damage within the human body, the acidity of the intestines and stomach and some types of antibodies found in the human body are the first lines of defense against this type of bacteria but the symptoms begin to appear on the body Man within(24-72)hours.

2- Drinking contaminated water, especially non-chlorinated tap water in some countries.

3- People traveling to countries that do not care about the cleanliness and sterilization of drink and food.

4- Eating contaminated food, such as properly sterilized foods, especially meat, unpasteurized milk, juices, and non-sagas and cheese vegetables.

5- *E .coli* bacteria can be transmitted from those infected with it who work in restaurants without washing their hands thoroughly after entering the toilet.

- Direct contact with animals may also transmit bacteria.

Diagnosis

Such cases are diagnosed from the pathological biography and the nature of the symptoms suffered by the patient, and the diagnosis is confirmed by a transplant to confirm the presence of these types of bacteria.

syndrome

1 - Dehydration, especially in children.

2- In cases where the patient's body is unable to maintain fluids due to vomiting and frequent diarrhea, it is recommended to go to the hospital for intravenous treatment.

3 -It is not recommended to use any antihistamines or vomiting before consulting a doctor, especially if diarrhea is accompanied by blood.

4- Usually the doctor does not need to dispense any antibiotic because these types of bacteria are controlled inside the body within two or three days and the body returns to its normal state and condition.

5 - The main idea in the treatment of these types is to maintain body fluids.

Pomegranate Peel

It belongs to the Pomegranate Tree Punicagranatum and is known as the pomegranate of the Pomegranate family Punicaceae and is native to south-west Asia and northwest India and its cultivation is commercially spread in most Arab countries, especially Mediterranean basin, Iraq, and the Levant (Saadi *et al*, 1988), It contains many active substances such as volatile alkaloids and the antioxidants such as low-density lipoproteins carrying cholesterol and phenolic compounds (Hamid *et al*,2009), the outer shell of the pomegranate fruit contains tannic acid, and crusts contain many alkaloid substances including pelletierine. Isopellefierine, ethylpelleticrin, pseudopelleticrin, N-, methylisopellelierine ethyl, in the roots and stems of the pomegranate tree. It has been observed in many studies that pomegranate fruit has a deadly and inhibitory act of the growth of negative and positive microbes of the pigment of the crème dethandaine. For urine and beneficial for the stomach. The pharmacological value of pomegranate peels is due to its presence of tannin, acolytes of volatile and antioxidants such as phenolic compounds, which inhibit the oxidation of low-density lipoproteins carrying cholesterol (Hamid *et al*, 2009) as well as the presence of pectic compounds. Compounds, Tannins, phenolic compounds, and Saponins.

Orange peel

Citrus fruits are one of the important foods for humans, with crusts amounting to 10-20% of the weight of fruits (Dhanoun Agha & Daoud ., 1991) citrus plants are characterized by a high percentage of volatile oils in addition to the presence of other compounds in smaller quantities vary in proportions according to chemical factors And physics, biological and environmental. Citrus oil is found in the outer shell of many phenolic compounds, including flavones, flavanones, and Cinnamic acid (John,2004) The orange leaf crust contains colored substances that gain the fruit's color and extract the volatile oils in addition to the presence of pigments including carotenoids and is distributed in the outer layer of the plant a group of oil glands containing volatile oils and the amount of oil that can be extracted from Crusts is about(0.08% -0.5%)

Peel oil in citrus fruits is an important volatile compound associated with smell and taste, including terpene hydrocarbons and oxygenated compounds that include organic acids, aldehydes, alcohols, esters, and ketones.

Sampling

Samples of *E. coli* bacteria were collected. The samples were collected in refrigerated boxes until they were delivered college laboratories. It was examined in the laboratory of the Faculty of Agriculture, University of Sumer.

Diagnosis of isolations with API-*E. coli*-20

The API-E.COLI-20 System Was Used to confirm isolates' diagnosis as (collee *et al.*, 1996). This system contains (20) tests, and the results were read of the AP-*E.coli* tape test after adding the reagents listed below to the tests for each of them and as instructed by the manufacturer and as follows :

1. Voges-Proskaure tests added a drop of both VP1 and VP2 detectors and the result was positive when purple or pink appeared 10 minutes later.
2. Nitrate reduction tests one drop of n1T1 and N2T2 detectors were added and the result was positive when red appeared after 10 minutes.
3. The alkaline phosphatase test detector added one drop of zymbh and ZYMA and the result was positive when violet appeared 10 minutes later.

The numerical profile of the tests was formulated in totals of three tests indicated in numbers (1.2.4) and by giving the corresponding number of the positive interaction for each group, this leads to a seven-digit model for the 20 tests. In the kit bar and reference to the analytical profile index, the type of bacterial isolation is diagnosed.

Preparation of aqueous and alcoholic extract

The pomegranate peels and orange peels were collected from Dhi Qar local markets, It was washed with cold water and completely isolated the crusts, then cut lengthways in the form of slices and dried the pomegranate husks at room temperature for seven days and then they were milled using an electric mill to get powder, then prepared the extract by blending. Then 10 g of each was taken and placed in flasks containing 100 ml sterile distilled water, and the solution was exposed to a temperature of 60°C /1 hour after leaving for 24 hours, then filtered with filter paper then the aqueous extracts of pomegranate and orange peels were kept in purified containers to be used ;The alcoholic was prepared on a soxhlet system consisting of a 5-liter flask attached to a heater blanket containing a cartridge which was placed about 1 kg of crushed pomegranate peel Subsequently three liters of an extraction solution of ethanol (80%) were transferred, resulting in a ratio of extraction 1:3. The process was conducted at 78 °C and was interrupted when 8 siphoned been completed.

Testing the vital potency of extracts

Samples of bacteria at the age of 6 hours and the number of cells with 610 cells / cm3 and the concetraton (30%) was used implanted in the center of nutrient broth and inoculated by Petri dishes container on the center of Muller Hinton agar, then the dishes were cuddled at a temperature of 37 m and for a period of 24 hours, it was observed then an aura around the tablets containing the water extract and alcohol, this halo represents the diameter of inhibition (mm), then compared with the antibiotic

.

THE RESULTS AND DISCUSSION

By studying the efficacy of extracts to inhibit the growth of bacteria (*E.coli*), it was found that the alcohol extract of pomegranate peels was more effective in inhibition if the highest inhibitory concentration (30mm) followed by the hydropode extract of pomegranate husks if the inhibitory concentration (25 mm) the inhibitory concentration extracted was crusted. Alcohol orange was (18 mm) and the water extract for orange peel was (9 mm) if compared with the antibiotics used it was shown to be the highest effectiveness of antibiotics (Gentamycin) where the diameter of inhibition (18 mm) and the least inhibition was for the antibiotic (Trimethoprim) where the diameter of inhibition (7 mm) was Almost.

It was noted that there is a moral difference(p>005) in the effectiveness of plant extracts and their effect on the growth of bacteria where we note that the actual microbial results of the alcoholic hydro extract of pomegranate peels towards bacterial isolates and this is consistent with the findings (Voravuthikunchail *et al*., 2004)

That alcoholic extracts the aquatic pomegranate husks were the most efficient among 38 plant extracts against *E.coli* isolation. It is noted from the results that the alcohol extract of pomegranate peels was more efficient than the water extract in the event against microbial and this is consistent with what (Pradeep *et al*. 2008) stated that the alcohol extract was more efficient than the raw extract of the ripe and immature fruit as (Nimri *et al* .,1999) concluded that it is efficient Alcohol extract pomegranate peels against a number of bacteria including Paerginosa, Berceuse *E.coli* were the most resistant bacteria of plant extracts (15 plant extracts) effective against microbes in the study conducted among 45 plant extracts was the peels of pomegranate extract from the vegetable

extracts Efficacy against a number of nurses and the alcohol extract was enough of the hydroponic extract if the diameters of the inhibition areas ranged from (31 - 40mm) to *staph. aureus* bacteria and (12- 30mm) bacteria *B. cereus* against microbial pomegranate contain several antimicrobial-effective compounds such as Alkaloid, tannin, polyphenol, glycosides, flavonoid tannin compounds of about 25% . It is highly effective against microbes through its association with proteins and is complex with cell wall caused by the crash of bacteria cells (Cowan,1999) as well as the plant contains a number of phenolate compounds such as caffic which has been shown to be effective against bacteria and against fungi (Cowan1999).

The table shows that there is a difference between the types of plants and their effect on the growth of bacteria, where the alcohol extract of pomegranate peels has the highest effectiveness on the bacteria used in the study compared to the rest of the extracts and may be due to the efficacy of the anaphylactic extract to the group of chemical compounds such as Phenols, flavonoids, and fluoride in the extract and other side chains, which gives him flexibility in working on many targets of the germ cell (Hugo & Russell., 1987) indicated the ability of these compounds to deposit proteins due to the formation of bonds hydrogen between ring hydroxide aggregates and proteins thus inhibiting the action of enzymes necessary for microscopic cayenne metabolism.

He concluded from this study that the alcohol extract of pomegranate has a wide effect on microscopic biology and from the observation of the living studied shows that they have a difference between them, which is positive for the dye of the dignity and the other negative to dye the dignity and some cause several diseases for humans and plants so the use of pomegranate in the possibility of The production of therapeutic substances (antimicrobial antimicrobial agants) thus reducing the incidence of resistance that appears among bacteria as a result of the widespread use of commonly used antibiotics as well as can also be used in the treatment of some plant diseases caused by fungi We recommend further studies on pomegranates, extraction of active substances and isolation to increase their impact on microbiology.

Table 1: Demonstrates the effectiveness of antibiotic sup pall on inhibition of the growth of *E.coli* bacteria estimated (mm).

Types of antibiotics (comparison)	mm R1	mm R2	mm R3
Cephalosporin	10	9	9
Tobramycin	9	10	10
Norfloxacin	11	10	12
Gentamycin	18	18	17
Pipracillin	13	12	11
Kanamycin	11	11	13
Oxacillin	12	12	13
Methicillin	8	10	9
Trimethoprim	7	8	7
Ciprofloxacin	8	8	9
Tetracycline	16	15	15

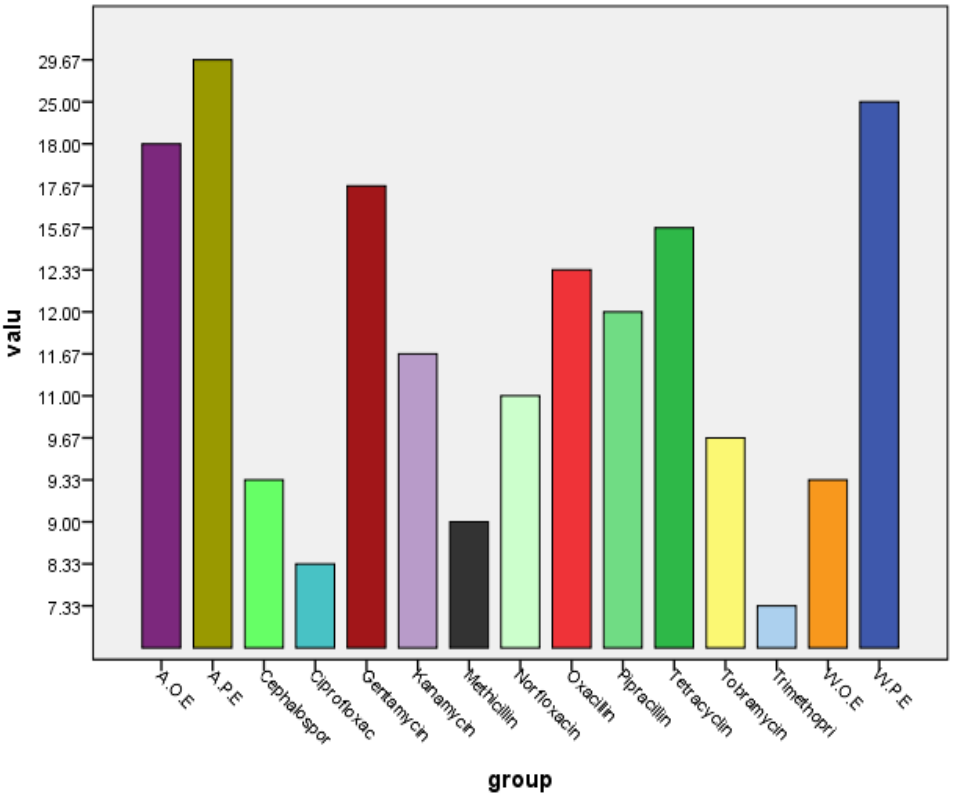
Table 2: Demonstrates the effectiveness of extracts to inhibit the growth of *E. coli* bacteria estimated b (mm).

Type of extract	mm R1	mm R2	mm R3
Alcoholic extract for pomegranate peels	30	30	29
Water extract for pomegranate peels	25	23	27
Alcoholic extract for orange peels	15	19	20
Water extract for orange peels	10	9	9

Table (3) :Average effect of extracts and antibiotics on bacterium *E.coli* Standard Error

gradation	Name of antibiotic ± standard error	Medium ± standard error
1.	Alcoholic extract for pomegranate peels	**0.33 ±29.6667**
2.	Water extract for pomegranate peels	**0.154 ±25.00**
3.	Alcoholic extract for orange peels	**0.52±18.00**
4.	Gentamycin	**0.34 ±17.6667**
5.	Tetracycline	**0.38 ±15.6667**
6.	Oxacillin	**0.36 ±12.3333**
7.	Pipracillin	**0.56 ±12.0000**
8.	Kanamycin	**0.63 ±11.6667**
9.	Norfloxacin	**0.57 ±11.0000**
10.	Tobramycin	**0.23 ±9.6667**
11.	Water extract for orange peels	**0.33±9.333**
12.	Cephalosporin	**0.32 ±9.3333**
13.	Methicillin	**0.87 ±9.0000**
14.	Ciprofloxacin	**0.72 ±8.3333**
15.	Trimethoprim	**0.63 ±7.3333**

Curve (1) :show rate of inhibition of E.coli by antibiotic and alcoholic and aqueous extracts of pomegranate peels and orang peels



- **A.P.E Alcoholic extract for pomegranate peels**
- **A.O.E Alcoholic extract for orange peels**
- **W.P.E Water extract for pomegranate peels**
- **W.O.E Water extract for orange peels**

CONCLUSION

This study concluded both extracts had a complete inhibition property of *E.coli* but the potential rate of inhibition in pomegranate peels was more than in orange peels and the alcoholic extracts had a high rate of inhibition of aquas extract for both them (30%)concentration used .

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