

Isolation and Diagnosis of Phenolic Compounds in Pomegranate Peel and Their Use in Inhibition of Intestinal Pathogenic Bacteria Isolated from Human Intestine and Stomach

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Abstract

Background: Pomegranate (*Punica granatum* L.) has gained commercial importance in food and health industries due to increasing scientific evidence linking its consumption to better health outcomes. **Objective:** This study aimed to detect the active substances (phenols, tannins, flavonoids, alkaloids, and saponins) in the pomegranate peel and their effectiveness against bacteria isolated from intestine and stomach which included *Salmonella* and *Escherichia coli*, which are responsible for most gastrointestinal diseases. **Materials and Methods:** The experiment was designed randomly and was statistically analyzed using the least significant difference at $P < 0.05$. The plant extracts were obtained by alcoholic extraction using Soxhlet. The compounds were diagnosed qualitatively and quantitatively using reference methods. **Results:** The results showed that peels contained high concentration compounds of alkaloid, tannic acid, and saponins. A 15% alcohol extract gave a high inhibition rate compared to the water extract and alcohol at a rate of 40 mm corresponds to 19 mm in the chloroform extract and 20 mm in the water extract at the concentration of 15%. A 15% concentration of alcoholic extract with antacid ampicillin and chlorophyll was compared with high efficacy compared to effective anti-ampicillin.

Keywords: Antioxidants, *Escherichia coli*, phenolic compounds, pomegranate peel, *Salmonella*

INTRODUCTION

Pomegranate tree very branchy and different species fruits are sweet taste, including sour and moderate. The pomegranate was mentioned at the Pharaohs and used to treat intestinal worms. Al-Razi said that the pomegranate peel was used to treat stomach ulcers and was used to treat chronic diarrhea.^[1,2]

Pomegranate contains a lot of antioxidants, and pomegranate peel, which occupies about 26% to 30% of the weight of the fruit contains large amounts of antioxidants, such as phenolic compounds such as flavonoids (anthocyanin's) and catechins and compounds Other flavonoids) as well as tannins. The medicinal value of pomegranate peels is due to their containment of tannin and phenols as antioxidants that inhibit the oxidation of low-density lipoproteins in addition to the presence of aminoacids.^[3] The most important causes of intestinal infections and infectious ulcers are opportunistic and pathogenic bacteria which represent a group of species

which are distinguished by biochemical tests such as bacilli (*Salmonella* and *Escherichia coli*) which is a major cause and assistant in most infections, infectious ulcers, and diarrhea and may go beyond that to infections in the urinary system.^[4]

The use of plants and medicinal herbs in the field of health protection does not cause side effects, as the patient adhered to the terms of treatment, and because of the high prices of medicines and industrial cheating that is currently associated with the negative repercussions on the body such as increased blood circulation and other diseases, which the goal of the study to pay attention to medicinal plants and use as a

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treatment alternative to shed light on the most important chemical ingredients in scaling, quantification, and testing of the biological efficacy of pomegranate extract in the growth of some isolated bacteria from intestinal infections.

MATERIALS AND METHODS

Pomegranate fruit collection

The fruits of pomegranates were obtained from a farm in Diyala province, Iraq. Fruits were harvested after ripening and the grains were taken away. Peels with pulp dried at 25°C for 7 days and then placed in plastic bottles for use.

Qualitative detection of chemical components in raw extract of pomegranate peel

To obtain the most important chemical components of the raw extract of pomegranate peel, perform a qualitative chemical analysis by taking 100 g of pomegranate peel and grinding them and adding them to 200 ml of distilled water. Then, dehydrate at 50 mL temperature for 12 hours and solvent deposition in 200 ml of distilled water again and used in qualitative tests for both tannins, alkaloids, steroids, flavonoids, glycosides, and soaps.^[5,6]

Quantification of chemical components in pomegranate peel extract

Determination of phenols

5 ml of crude extract has been added that recorded in the previous paragraph and the solution consisting of 2 ml of ammonium hydroxide, 10 ml of distilled water, 5 ml of alcohol, and then left for 1 h after that, sample was read by spectrophotometer at 505 nm. Prepare standard solution of ammonium hydroxide and alcohol for comparison.^[7]

Determination of alkaloids

Dissolve 5 g of sample in 200 ml of acetic acid mixture and ethanol by 15:1. Gradually, add the ammonium hydroxide to the precipitate and spray the solution and the solvent and precipitate again, which represents alkaloids and is weighed.^[6]

Determination of tannins

500 mg of the sample was dissolved in 50 mL of distilled water in a vibrator holder for 1 hour and then filtered. The water was then added again and filtered, then 2 ml of dissolved iron chloride was added in hydroxide acid. Potassium cyanide is measured at 120 nm.^[8]

Determination of saponin

20 g of sample was taken and 80 ml of ethanol was added to the precipitate and it was reextracted from 20 mL ether. The process was repeated several times and then 60 mL of butanol was added, filtered, and washed with 5% sodium chloride. The weight of precipitation that represents the soap content in the sample.^[9]

Determination of flavonoids

Ten gram of plant sample is taken and 100 ml of methanol alcohol is added to the solution. The solution is filtered with filter paper (Whatman No. 41), which evaporates the extract,

and the residue weight, which represents the flavonoids, are present in the sample.^[7]

Preparation of different concentrations of crude extract

The water extract of the pomegranate husks was prepared with a 10% and 15% concentration of dissolve 10 and 15 g of sample in 100 ml of distilled water after being extracted by the Soxhlet using three solvent (ethyl alcohol, chloroform, and water).^[10]

Preparation of the isolates

Bacterial isolates from the Yarmouk General Hospital were taken from patients with digestive health problems and were isolated from stool and vomiting samples. Laboratory tests were conducted at the Ministry of Science and Technology/Food Contamination Research Center.

Biological efficiency of extract (alcohol, water, and chloroform) against intestinal bacteria

The diffusion method around the drill was used to measure the biological efficacy of pomegranate extract against the growth of some bacterial isolates. The salinity of the nutritious medium in the bacterial larvae, containing (4×910 units), formed a colony/ml by means of a cotton swab sterilized in a 90 mm diameter dish and drilled with a 4 mm central diameter on the middle surface of the plant with the cork hole and placed 200 µl of the preprepared extract in each hole. The dishes were incubated for 5 days at 37°C and the results were recorded by measuring the inhibition diameter in millimeters around each hole. The drilling control represents water, chloroform, and alcohol only.^[11]

Comparison of biological efficacy of extract of alcohol 15% with the ampicillin and chloramphenicol against pathogenic bacteria

15% of the alcohol extract was tested in comparison with ampicillin and chloramphenicol (obtained from SDI, Iraq) with absolute concentration (without adding any other substance), and a concentration of 50 mg/100 ml perform the test as in the preceding paragraph.^[12]

Statistical analysis

The collected data were calculated and analyzed by using the Statistical Package for Social Sciences (SPSS) 20th version (IBM SPSS Software, Chicago, Illinois, U.S.A). for windows.

Table 1: Detection of the most important chemical components of the raw pomegranate peel extract

Alkaloids	Tannins	Phenols	Flavonoids	Glycosides	Saponins
+	+	+	+	-	+

+: Positive, -: Negative

Table 2: Percentage of chemical components in raw extract of pomegranate peel

Alkaloids (%)	Tannins (%)	Phenols (%)	Flavonoids (%)	Saponins (%)
1.7	18.9	1.0	0.5	3.5

Table 3: Bioactivity of alcohol, chloroform, and water extracts of pomegranate peel against intestinal bacteria

Isolates	Diameter inhibition of bacteria (mm)									Average
	Alcoholic extract			Chloroform extract			Water extract			
	0	10%	15%	0	10%	15%	0	10%	15%	
<i>Escherichia coli</i>	15	22	40	13	17	19	0	17	20	18.1
<i>Salmonella</i>	17	30	30	12	16	20	0	11	16	16.8
LSD 5%	Among the three concentrations=1.5									

LSD: Least significance difference

RESULTS AND DISCUSSION

Qualitative detection of chemical components of crude extract of pomegranate peel

The results show the qualitative chemical detection of the active ingredients in the pomegranate extract, which shows that the raw extract contains alkaloids, tannins, phenols, flavonoids, and soap shown in Table 1 which are inhibitory substances for the growth of bacteria. Tannins and alkaloids inhibit the growth of isolated bacteria from pathogenic infections from multiple parts of the body.^[12] Most medicinal herbs are effective because they contain active substances and ingredients. Most pharmacists have used them to extract and purify these materials to make them an effective independent substance. Pomegranate husks are an ideal reservoir for most materials. Tannins and alkaloids which are effective because they contain these substances and other amino acids and organic substances.^[13]

Pomegranate peel contains a variety of active substances that give pomegranate its medical and therapeutic importance.^[14]

Quantitative detection of chemical components of the extract of pomegranate peel

Table 2 shows the percentage of the chemical components of the crude extract for pomegranate peels, which shows that the tannins were the most common in the extract.^[15] Active substances are important in increasing the biological effectiveness of the herbs in which they exist and work to inhibit the types of bacteria and both works (tannins and alkaloids) to treat diarrhea and intestinal colic. Pomegranate acid is best in treating patients with gastrointestinal problems where they have demonstrated efficacy against intestinal bacteria such as *E. coli*, an opportunistic organism that has the ability to attack the body and contribute to the increase of the disease.^[16] Quantitative substance dependence in medicinal plants determines the therapeutic efficacy of these plants in eliminating the growth of pathogenic microorganisms.^[17]

Bioactivity of alcohol, chloroform, and water extracts of pomegranate peel against pathogenic bacteria

The results of the study showed the effect of the alcoholic, chloroform, and water extracts prepared from the pomegranate peel on the growth of some intestinal bacterial strains of the family Enterobacteriaceae and isolated from the patients who are lying in Yarmouk General Hospital. The results showed a significant inhibition of both types of bacteria, and this study is in agreement with other studies in the same field.^[18]

Table 4: Effectiveness of 15% alcoholic extract compound with ampicillin and chloramphenicol against pathogenic bacteria

Bacteria	Alcohol extract 15%	Ampicillin	Chloramphenicol
<i>Escherichia coli</i>	13	11	10
<i>Salmonella</i>	21	21	14
LSD 5%	2.366	1.566	1.78

LSD: Least significance difference

Table 3 shows that the 15% alcohols extract gave a high inhibition rate compared to the water extract, and alcohol at a rate of 40 mm corresponds to 19 mm in the chloroform extract and 20 mm in the water extract at the concentration of 15%. This may be due to the quantity and quality of active substances released, and alcohol free is greater compared to chloroform and water. Alcohol is an excellent solvent for most medicinal herbs to be studied. Alcoholic extract in most researchers in the field of biological best effectiveness on water extracts and chloroform. Because the active substance that affects bacteria dissolves in organic solvents rather than dissolved in water.^[19,20]

The efficacy of the alcohol extract was compared to 15% with antibiotics ampicillin and chloramphenicol, which is widely used in the treatment of most bacterial infections; Table 4 shows that the alcohol extract has no effect in inhibiting it in the case of bacteria *Salmonella* where the extract was given an effective similar to ampicillin at a rate of 21 mm each, taking into account that the concentration used for antimicrobial is high and is not used in regular and routine medicines and this is consistent with^[11] who confirmed the superiority and efficacy of plant medicinal extracts on antibiotics.

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Conflicts of interest

There are no conflicts of interest.

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