Antimicrobial Activity of Punica granatum L. against Diarrheagenic E.coli

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Abstract

The present investigation evaluates the antimicrobial activity of crude extracts from the rinds of Punica granatum against gram negative, diarrhea-genic E.coli. in this study we investigated in vitro antimicrobial activity of dry rinds of Punica granatum in different solvent in 3 different concentra-tions, the solvent used are alcoholic ethanol and aqueous, in this study we found that the activity of Punica granatum extract against diarrheagenic E. coli increased by increasing concentration, and watery solvent is better in its action than alcoholic solvent.

Introduction

Nowadays researchers are increasingly turning their attention to folk medicine, looking for new discovery to develop better drugs against micro-bial infections caused by various pathogens(1). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogenic strains(2). It is important to discover new antimicrobial compounds with diverse chemical structures and with novel mechanisms of action for new and re-emerging infectious disease(3). The antibiotic resistance and failure of chemotherapeutic exhibited by pathogenic microbial infectious agent has led to the screening of several medicinal plants for their potential antimicrobial activity(4).

The pomegranate (Punica granatum L) is one of the oldest edible fruit which has a long history as a medicine fruit and has been used extensively in the folk of many cultures(5). All plant organs of the pomegranate tree have been used to ameliorate on array of common diseases(6). The fruit was seen by ancient Egyptian, as a symbol of prosperity and ambition, and parts of the tree were used as treatment for tapeworm and other parasitic infections(7). According to ancient Chinese, the red juice was regarded as a soul concentrate homologous to human blood and capable of coffering a person longevity or even immortality(8). Nowadays pomegranate is an important commercial fruit crop that is widely cultivated in parts of Asia. North Africa, the Mediterranean, and the Middle East(9). In addition to the medicinal use of dried products, the fruit is consumed directly as fresh arils or juice and is used in the food industry in the manufacture of jellies, concentrates and flavoring and coloring agents. In particular, there has been renewed global interest on the functional and nutriceulical benefits of pomegranate fruit(10). The phytochem scals like ellagic acid, gallic acid, punicalagine extracted from pomegranate revealed antimicrobial activity when assayed against Escherichia coli, Pseudomonas aeruginosa, methicillin-resistant Staphyl-ococcus aureus, and other harmful bacteria(11). Braga et al(7) evaluated the effect of the whole pomegranate fruit methanol extract on S. aureas and subsequent enterotoxin production they suggested that pomegranate extracts could be considered a potential antibacterial therapeutics

with the additional ability to inhibit enterotoxin production. They added that the antibiotic properties of the extract are of extreme interest in light of the on growing threat of bacteria strains developing resistance to conventional antibiotics.

Pomegranate rind is rich in polyphenols including ellagitannins, gallotannins, ellagic acid, gallic gueratine. These polyphenols exhibit various biological activity, such as eliminating free radicals, inhibiting oxidation and microbial growth, and decreasing the risk of cardio- and cerebrovascular diseases and some cancers. Researchers have shown that preparations containing pomegranate rind extract can be used to prevent and for cure atherosclerosis, diarrhea, gastric ulcer, venereal disease and estrogen-related diseases(11).

Aim of the study: Is to determine the effect of extract of Punica granatum on gram negative diarrheagenic E.coli.

Materials and Methods

1. Bacterial Isolates and Culture Media

The diarrheagenic E.coli which are diagnosed by using of molecular methods was used for testing of plant extract effectivity. The isolates were subcultured from glycerol mixed broth media that was stored at -80°C, on fresh MacConkey agar plates for 24hours prior to plant extract test, then testing of plant extract was done on Muller Hinton agar.

2. Punica granatum L. Extraction

i. Plant Extraction for Aqueous Extract

Twenty five grams of air-dried powder were inserted in 100 ml distilled water and boiled on slow heat for 2 hours. It was then filtered through filter paper and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected. This procedure was repeated twice. After 6 hours, pooled together, and concentrated to make the final volume. It was then autoclaved at 121°C and stored at 4°C, for further use as antimicrobial agents(13).

ii. Plant extraction for alcoholic extract

Twenty five grams of air dried powder were taken in 100ml of organic solvent (70% ethanol) in a conical flask, plugged with cotton, and then kept on rotary shaker at 190-200 rpm for 24 hours. after 24 hours, it was filtered through filter paper and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and the solvent was evaporated to make the final volume and stored at 40°C in airtight bottles. For farther, use(13).

This final volume of both aqueous and ethanolic extraction made the stock volume of 25%, then the other concentration is made which is 20%, 15% of both water and alcoholic extraction, and filter paper was used for preparation of disks which saturated in different concentration for using in the antibiotic sensitivity test.(13)

3. Study of Antibacterial Activity by Using Agar Disc Diffusion Method for Aqueous Plant Extract and Alcoholic Extract

The Mueller Hinton Agar medium was prepared and sterilized at autoclaves for 15min, the sterilized agar medium was poured into sterile petri dishes under aseptic conditions and allowed to solidify. The 24 hours-old cultures of pathogenic E.coli strains were inoculated and evenly spread on the surface of the agar by sterile swab to get uniform lawn culture of the organism(13).

The antimicrobial activity of the extracts and control was evaluated by the disc

diffusion method described by Bauer et al., (1966) with modifications(14).

Filter paper disc which had previously been sterilized in an oven at 100°C for two hour were soaked with the three extract at 3 concentration (15%, 20%, 25%) of both water and alcoholic extract discs were placed on MH agar and inoculated with the test bacteria and incubated at 37°c for 18-24 hours.

After incubation, inhibition zone were measured to determine which concentration of the extract inhibited bacterial growth. The resulting haloes were compared with control discs, which composed of filter paper disk soaked with distal water and considered as control negative(14).

4. Statistical Analyses

The program of spss version 18, was used to analysis data by T. test to determine the statistical significance of the data. P value of <0.05 was considered significant.

Results

The effect of three different concentration (15%, 20%, 25%) of each alcoholic and aqueous extract of Punica granatum were examined against E.coli species by using of disk diffusion method, and compared with control negative in this case the control was distilled water, we observed that antibacterial activity of plant extract increased with increasing the extract concentration, the inhibition zone of each aqueous and alcoholic at (15%) concentration was 5.8 mm, 5.6 mm respectively, when extract concentration increase to (25%) the inhibition zone of each aqueous and alcoholic extract also increase to 6.9 mm, 6.8 mm respectively as shown in table (1). also it was noticed that there was difference between the aqueous and alcoholic extract in antibacterial activities that were in the same concentration , the aqueous extract was better in action than alcoholic extract as shown in table (2). statistical analysis appears that there was significant differences between the concentration of 15% and 25% of both alcoholic and aqueous extract($p < 0.05$).

Discussion

Punica granatum Extract

From the current result, there was clear effect of antibacterial activity of plant extract by increasing the concentration. And the aqueous extract showed better action than the alcoholic extract.

The present result was agreement with study of Muhammad in Mosul(15) who found that the water extract had a better effect on the growth of bacteria than alcoholic extract, and with the study of Saeed,(16) who found that aqueous extract was better in action than alcoholic extract, and the action increased with increased concentration, and this may be related to presence of many chemicals in the aqueous extract of Punica granatum such as tannine polyphenol, flavonoids, and ellagic acid etc.. that may effect on protein synthsis and protein natures of bacteria that lead to kill it or it may effect on the plasma membrane that lead to suppression of bacterial growth(17), and the less efficiency of alcoholic extract in comparism with aqueous extract may be due to the effect of ethanol on the nature of chemicals in this plants, that leads to loss of its efficiency in killing of this bacteria (18) found that both of alcoholic extract and aqueous extract have apotent inhibitory effect of EAEC.

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Table(1) Inhibition zone of bacteria treated with extract of *Punica granatum*

No.	No. of sample	15%		20%		25%	
		W/mm	ALC/mm	W/mm	ALC/mm	W/mm	ALC/mm
1.	2	6	7	7	7	7	8
2.	4	6	5	7	6	7	6
3.	5	7	6	7	6	8	7
4.	9	7	6	7	7	8	7
5.	11	6	7	7	7	7	8
6.	13	6	5	7	6	7	6
7.	25	5	5	6	6	6	6
8.	38	6	6	6	7	7	8
9.	40	5	6	6	6	7	7
10.	44	5	5	5	5	6	6
Total		58	56	63	62	69	68
Mean		5.8	5.6	6.3	6.2	6.9	6.8

Table (2) Statistical Analyses of the results of plant extract

	No. of strains	*Mean diameter (mm) ± SD*	*S.E.**	*T-test (df=9)*	*95% C.I.***	*P- value*
15% *Punica granatum* in Water	10	5.9 ± 0.74	0.1	11	-1.33 to -0.87	<0.0001***
***25% Punica granatum* in Water**	10	7 ± 0.67				

**S.E. = Standard Error / **C.I. = Confidence Interval / ***Highly Significant*

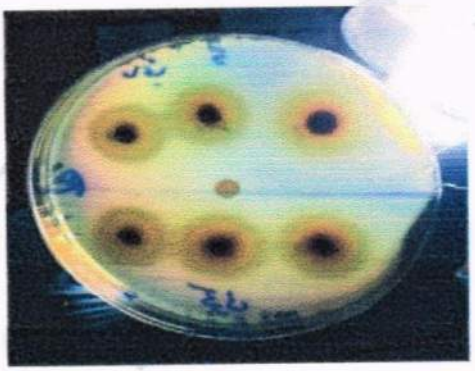

Figure (1) Disc Diffusion Method for *Punica granatum* Extract