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Research Article

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In-vitro antibacterial effect of *Punica granatum* peel extracts despread in Syria on clinically isolated *Pseudomonas aeruginosa*

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ABSTRACT

Pomegranate is one of medicinal plants which were used for along time ago in treating many diseases. Lots of the studies carried out on the fruits of pomegranate peel extract showed that its content of antibiotics is capable of inhibiting the growth of bacteria and some species of parasites and fungus. In this study three extracts of sweet Pomegranate(Punica granatumL.)peels and three extracts of sour Pomegranate peels were prepared by using three solvents (Methanol – Ethanol – Distilled water). The antibacterial effect of the previous extracts was studied against Pseudomonas aeruginosa by using agar well diffusion method, also it was compared to Ciprofloxacin as a reference drug. The results showed that the Methanolic extracts of Sweat and sour pomegranate peels possess a high efficiency close to the potency of Ciprofloxacin against Pseudomonas aeruginosa.

Keywords: *Punica granatum*, antibacterial effect, *Pseudomonas aeruginosa*, pomegranate peel, ciprofloxacin, Syria.

INTRODUCTION

The common name of *Punica granatum* is *pomegranate. Its belongs* to the family of *Punicaceae*. Iran is the origin country of *pomegranate* and then it has spread in the Himalayas of northern India. It is grown mainly in the Mediterranean region of Asia, Africa, Europe and some parts of the United States of America [1].*P. granatum* is considered a medicinal plant and one of the most promising in treating various infectious diseases. It is one of the most important plants at the grassroots level because of its medical and religious significance. It was used hundreds of years ago in the natural treatments as an anthelmintic drug, in addition to its usage in tanning-purposes.

Peels of *P. granatum* are useful in treating gastrointestinal problems such as diarrhea [2],but its use as an anti-microbial is rare despite the fact that many researches have proved its effectiveness against fungi and both of gram positive and gram negative bacteria [3].Peels *of P. granatum* contain many phenolic compounds (tannins, flavonoids, anthocyanin and Gallic acid), which are the effective substances against many bacteria and other microorganisms [4].

Pseudomonas aeruginosa is widely spread in nature, found in soil, animals and plants environments. The source of this bacteria is usually the intensive care units in hospitals, also *P. aeruginosa* is one of the internal flora of

the human intestine. There are also small numbers in the skin [5]. These germs are characterized to resist antibiotics and the ability to acquire resistance to all antibiotics [6].

P. aeruginosa is an opportunistic pathogen possess a high ferocity in patients who suffer from some low defense mechanisms and discouraged immunologically as well as children and infants, it can invade the bloodstream and cause blood poisoning Septicemia [7]. The increasing resistance of microbes to antibiotics is caused by misusing them. Some antibiotics are not sufficient to treat certain bacterial infections, as well as they may cause a negative impact on human health as a result of killing normal flora in the intestines, According to these facts researchers try to discover new bacterial antibiotics do not cause the killing of this flora.

The Aim of Study was to Investigate the antibacterial effect of methanolic, ethanolic and aqueous extracts of both sweet and sour *Punica granatum* peels against *Pseudomonas aeruginosa* and then compare the antibacterial efficiency between them.

EXPERIMENTAL SECTION

1- Collection of samples and Extraction:

The samples of both sweet and sour *Punica granatum* were collected from a field in Deer Atiyah area in the countryside of Damascus during the period of maturity, The fruits were separated to a sweet taste and sour taste and were washed with distilled water to get rid of dust and impurities. Peels were removed and dried at room temperature 25C° for a week to get dry weight, and then 6 extracts were prepared from the peels using three types of solvents (methanol, ethanol, and distilled water) for each Sort of pomegranate (sweet and sour) . The operation of extraction was done using Soxhlet apparatus , and then the extracts were dried using a rotary evaporator. The dried extracts resulting were then preserved in opaque sterile glass tubes in temperature -20 Celsius in order to avoid the oxidizing of active substances.

2- Prepare Serial dilutions of extracts of *Punica granatum*

Serial dilutions of extracts were prepared at concentrations: (10- 5- 2.5- 1.25) % for each solvents (methanol, ethanol, and distilled water).

3- Prepare Bacteria

Pseudomonas aeruginosa clinically isolated and obtained from the laboratory of Al Assad Hospital in Damascus in Syria was used to make suspensions of in tryptic soy broth at concentrations $^{6}10$ CFU/ml (Colony Forming Units) based on Standard McFarland [8].

4-investigate the efficiency of *Punica granatum* against *Pseudomonas aeruginosa*

Well diffusion method in petri dishes which contain Muller Hinton Agar(MHA) (The diameter of well is 7 mm)[9] was used in the study according to these steps:

Well diffusion method in petri dishes which contain Muller Hinton Agar (MHA) (The diameter of well is 7 mm) [9] was used in the study according to these steps:

1- The suspensions of Bacteria were speared on (MHA) plate, and put in room temperature for 15 minutes.

2- Three wells were done borer in (MHA) plate using sterile cork borer.

3- 15 microliters of each concentration were put in the wells .After that the previous plates were left in the room temperature for 30 minutes allowing the extract to diffuse into the agar . The plates were observed after incubation for 24 hrs at 37 ^{o}C. If the antibacterial activity was present on the plates, then an inhibition zone surrounding the well containing the extracts of *P. granatum* will appear. Each test was done triplicate and the Means of the diameters were calculated.

4- Positive control was prepared (by using discs of ciprofloxacin / Bioanalyse/ Turkey 5 mcg) against *P. aeruginosa*. Negative control was also prepared using three solvents: (Methanol, Ethanol and distilled water) and then put in the wells.

RESULTS AND DISCUSSION

Table 1.Efficiencyofthemethanolic, ethanolic and aqueous extracts of both sweet and sour *P. granatum* against *Pseudomonas aeruginosa*

Concentration %	Methanolic Extract		Ethanolic Extract		Aqueous Extraction	
	Sweet	Sour	Sweet	Sour	Sweet	Sour
	Mean± SD of DIZ					
1.25	0	12±1.0	0	11±1.2	0	0
2.5	11±2.5	16±0.5	10±1.5	14±1.0	0	0
5	13±1.5	18±0.5	11±1.5	15±2.5	0	11±0.5
10	17±2	22±1.0	16±1	18±2.0	12±1.5	15±0.5

***Mean± SD**:Three times of DIZ ±Standard deviation.*
***DIZ:** Diameter of the inhibition zone.*

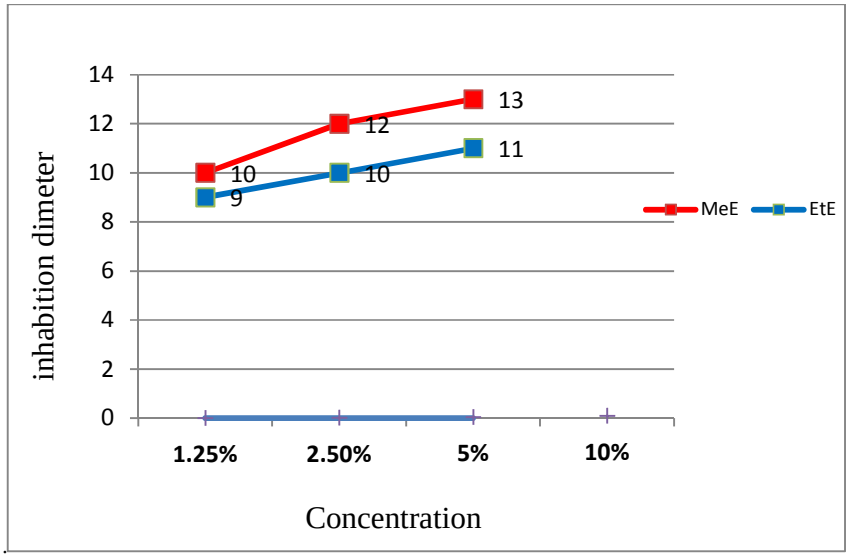

Figure 1. Efficiency of the methanolic, ethanolic and aqueous extracts of sweet *P. granatum* against *Pseudomonas aeruginosa.*

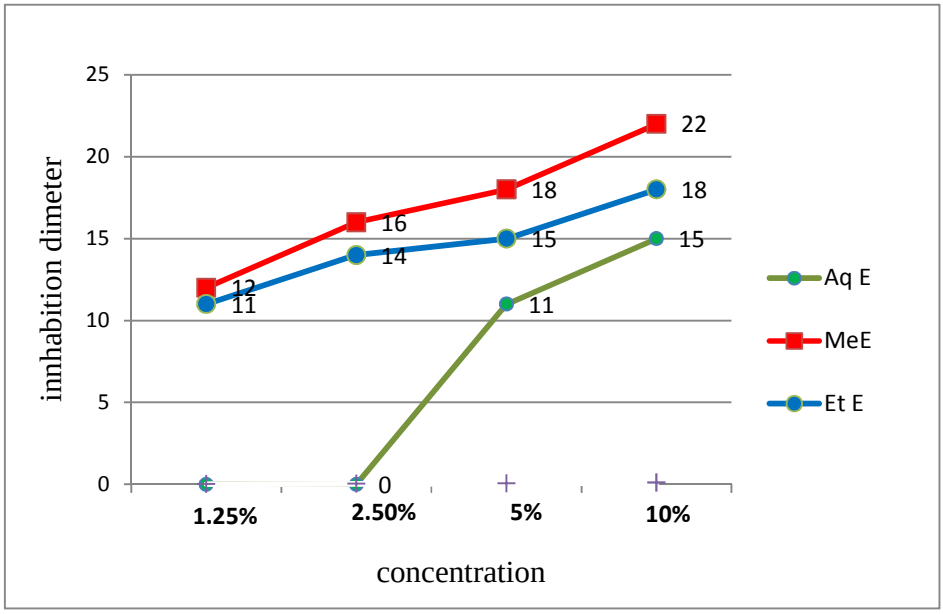

Figure 2. Efficiency of the methanolic, ethanolic and aqueous extracts of sour *P. granatum* against *Pseudomonas aeruginosa*

The results showed that all applicable extracts have an inhibitory effect against the bacteria studied.

There was a better effectiveness of *P. granatum* extracts applied to *P. aeruginosa* at high concentrations, due to an increase in the concentration of active substances and that was compatible with [3].

Results explained that the effect of aqueous extract of sweet and sour *P. granatum* varieties was less (DIZ) (15±0.5, 12±1.5) than the effect of both methanolic and ethanolic extracts against *P. aeruginosa*, That was compatible with (Dahham *et al*., 2010) [10], Because the solubility of total Phenolic compounds in aqueous solution is very low compared to the solubility in organic solvents and this is closely to the study [11].

All coefficient of variation values (p value) confirm on the presence of statistically significant indication when the comparison is done between the averages of diameters of Inhibition zones (DIZ) (17±2, 16±1, 12±1.5) mm that results from the application of (methanolic, ethanolic and aqueous) extracts of sweet *P. granatum* with the averages of diameters of Inhibition zones (DIZ) (22±1.0, 18±2.0 , 15±0.5) mm that results from the application of previous extracts of sour *P. granatum* (p value <0.01), in other words, the results of the study showed that the extracts of sour *P. granatum* peels had a better efficiency against *P. aeruginosa* compared with the extracts of sweet *P. granatum* peels. This may be due to the fact that the extracts of sour *P. granatum* is richer in active compounds.

CONCLUSION

The Study showed that the efficiency of sour *P. granatum* peel extracts is higher than the efficiency of sweet *P. granatum* peels extracts, while the efficiency of methanolic extracts of both sour and sweet of *P. granatum* is higher than ethanolic extracts against *P. aeruginosa*.

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