EVALUATION OF IN-VITROANTIBACTERIAL ACTIVITY OF AQUEOUS AND ALCOHOLIC EXTRACTS OF THE PEELS PUNICA GRANATUM AND OLEA EUROPAEA LEAVES

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ABSTRACT

While we are faced with increasing numbers of cases of infections with microorganisms that are multi-antibiotic resistant, we must continue to research into alternative therapies. Many of these are traditional medicines that were abandoned following the introduction of antibiotics and, without doubt, antibiotics have proven time and time again to be invaluable in the war on microbes. The present study was carried out to determine the antibacterial potential of the cold and hot aqueous extracts and ethanolic extract of the peels of Punica granatum and Olea europaea leaves against Staphylococcus aureus (Gram positive bacteria), Escherichia coli and Pseudomonas aeruginosa (Gram negative bacteria) by agar disk diffusion method. The results showed the absence of any activity of Olea europaea aqueous extracts against the three pathogens and its alcoholic extract exhibited a weak activity on E. coli and S. aureus only. All of the Punica granatum extracts showed a strong activity against the three microorganisms, P. aeruginosa was more sensitive with zone of inhibition of 29mm in the alcoholic extract, and the inhibition zone decreased with the decrease of extract concentration. These results suggest that peels of Punica granatum have interesting antibacterial activities.

المستخلص

بينما نواجه أعدادًا متزايدة من حالات العدوى بالكائنات الدقيقة التي تكون مقاومة متعددة للمضادات الحيوية ، يجب أن نستمر في البحث عن علاجات بديلة. والكثير من هذه الأدوية هي الأدوية التقليدية التي تم التخلي عنها بعد إدخال المضادات الحيوية ، ومن المؤكد أن المضادات الحيوية أثبتت مراراً وتكراراً أنها لا تقدر بثمن في الحرب على الميكروبات. أجريت الدراسة الحالية لتحديد القدرة المضادة للجراثيم من المستخلصات المائية الباردة والساخنة والمستخلص الايثانولي من قشور

Punica granatum وأوراق Olea europaea ضد بكتيريا (البكتيريا إيجابية الجرام Staphylococcus aureus) ، Escherichia coli و Pseudomonas aeruginosa (البكتيريا سالبة الجرام) عن طريق طريقة نشر آجار القرص. وأظهرت النتائج عدم وجود أي نشاط من مستخلصات Olea europaea المائية ضد مسببات الأمراض الثلاثة ومستخلصاتها الكحولية أظهرت نشاطًا ضعيفًا في E. coli و S. aureus فقط. أظهرت جميع مستخلصات بانيكا جراناتوم (Punica granatum) نشاطًا قويًا ضد الكائنات الحية الدقيقة الثلاثة ، وكان P. aeruginosa أكثر حساسية مع منطقة تثبيط 29 مم في المستخلص الكحولي ، وانخفضت منطقة التثبيط مع انخفاض تركيز المستخلص. هذه النتائج تشير إلى أن قشور Punica granatum لديها أنشطة مضادة للجراثيم مثيرة للاهتمام.

Keywords: Antibacterial activity; *Punica granatum*; *Olea europaea*; Aquatic extract; Alcoholic extract.

1. INTRODUCTION

In ancient time, several herbs and spices were used in food, not only as a flavoring agent and food preservative but also as a folk medicine [1]. The study of biologically active compounds from natural sources has always been of great interest to scientists looking for new sources of useful drugs for treating infectious diseases. Infectious diseases caused by bacteria, fungi, viruses, and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance [2]. It is known that the olive fruit, its oil and the leaves of the olive tree (*Olea europaea*) have a rich history of nutritional and medicinal uses [3]. Oleuropeosits (oleuropein), flavones, flavonols and substituted phenols (tyrosol, hydroxytyrosol) are some phenolic compounds in the olive leaf extract [4]. It has been reported by many researchers that the olive leaf extract has an antimicrobial activity because of its high phenolic content [5-7]. The compounds are found in various plant parts such as stems, roots, leaves, bark, flowers or fruits and seeds and include alliin/allicins, isothiocyanates, and plant pigments. *Punica granatum L*. has been widely used by traditional medicine in America, Asia, Africa and Europe for the treatment of different types of diseases [8,9]. In the ancient Egyptian culture, the pomegranate fruit (*Punica granatum*) was regarded as a symbol of prosperity and ambition,

making it common practice to decorate sarcophagi with depictions of the plant [9]. The aim of the present study was to assess the in vitro antibacterial activity of different medicinal plants extracts of *Punica granatum* peels and *Olea europaea* leaves.

2. MATERIAL AND METHOD

1. **Sample collection and preparation:** The present study was carried out in Microbiology Laboratory at Al-Asmarya Islamic University-Zliten, Libya. Peels of *P. granatum* and the leaves of *O. europaea* were collected in fresh condition from local farms. Collected samples were washed thoroughly with distilled water. The cleaned peels and leaves were air dried for two weeks with constant monitoring to avoid fungal contamination. Dried samples were ground to fine powder and stored in sterilized air tight container at room temperature for further analysis.
2. **Preparation of Cold and Hot Aqueous Extracts:** Cold Aqueous extract of *P. granatum* peels and *O. europaea* leaves was prepared by soaking of 10g of the prepared powder in 100 ml of distilled water for 24 hours in the room temperature and filtered. This step was repeated three times, to insure that all the compounds in the powder dissolved. The filtrates of the three days mixed together and kept at 50 ˚C to evaporate all the solvent, leaving behind the Cold Aqueous Extract (CAE). Hot Aqueous Extract (HAE) for both plants was prepared with same procedure of the cold aqueous extract with keeping the mixture flask before the filtration in 80°C water bath.
3. **Preparation of Ethanolic Extracts (EE):** Ethanolic extract (EE) of all the samples was prepared by soaking of 10g of the prepared powder in 100ml of 75% Ethanol for 24 hours in the room temperature and filtered. This step was repeated also three times. The filtrate of the three days joins together and then concentrated and used for testing antimicrobial activity.
4. **Microorganisms:** Bacterial strains used in the present study including *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were collected from Zliten Teaching Hospital Laboratory.

Each of the bacterial specimens was incubated in liquid culture dilutions and incubated at 37°C for 20 minutes to reach the logarithmic stage, then measured to a 0.5 McFarland dilution which delivered a final concentration of approximately 105 CFU per ml.

5. **Antimicrobial activity:** The antimicrobial potency of Aqueous and Ethanolic extracts of *P. granatum* peels and *O. europaea* leaves on the selected three pathogens was studied using disk inhibition method [10]. In disk inhibition zone method, the Mueller-Hinton agar medium was inoculated with freshly prepared cells of each bacteria to yield a lawn of growth. After solidification of the agar, a number of sterilized disks were dipped into extract solutions of different concentrations, and placed on the plates. After incubation at 37˚C for 24 h, the antimicrobial activity was measured as diameter of the inhibition zone formed around the disc [11]. At the same time, a comparison antibiotic control test was made using commercial disks, Ciprofloksasin and Gentamicin discs.

3. RESULTS

In this study, *Punica granatum* peels and *Olea europaea* leaves extracts was tested for antibacterial activity against three bacterial species and it was presented in Table (1). The diameters of inhibition zones of the *P. granatum* peels cold aqueous extract was observed 28 mm against *S. aureus* and 21 mm against *E. coli* and 25 mm against *P. aeruginosa*. There was a similarity between the inhibition zones of the hot and cold aqueous extracts of *P. granatum*. The Ethanolic extract showed a relatively stronger activity against the three pathogens, with a maximum inhibition zone of 29 mm against *P. aeruginosa.*

Table (2) present the antibacterial activity of Cold and Hot Aqueous and Ethanolic Extracts of *Punica granatum* peels at different concentration. However, *Olea europaea* leaves aquatic extracts didn't show any activity against any bacteria, in reverse of that, the Ethanolic extract showed a weak activity only against *S. aureus* and *E. coli* with an inhibition zone of 10 and 11 mm against *S. aureus* and *E. coli* respectively. The diameters of inhibition zones of Ciprofloksasin discs are larger than the

diameters of inhibition zones of *Olea europaea* leaves extracts and Gentamicin discs, but it was closely similar to *Punica granatum* peels extracts as shown in Figure (1).

Table (1):

Antibacterial Activity of the Cold and Hot Aqueous Extracts (CAE and HAE) and Ethanolic Extracts (EE) of *Punica granatum* peels (Pg) and *Olea europaea* leaves (Oe) by Disc Diffusion Method (Inhibition zones in millimeter).

Test Organisms	COLD AQUEOUS EXTRACT		HOT AQUEOUS EXTRACT		ETHANOLIC EXTRACT		POSITIVE CONTROL	
	Punica granatum	*Olea europaea*	Punica granatum	*Olea europaea*	Punica granatum	*Olea europaea*	Cip 5µg	Gen 10µg
S. aureus	28	-	21	-	25	10	26	16
E. coli	21	-	21	-	23	11	22	15
P.aeruginosa	25	-	26	-	29	-	28	16

Table (2):

Antibacterial activity of Cold and Hot Aqueous Extracts and Ethanolic Extracts (EE) of *Punica granatum* peels at different concentration (Inhibition zones in millimeter).

C [%]	MO								
	COLD AQUEOUS EXTRACT			HOT AQUEOUS EXTRACT			ETHANOLIC EXTRACT		
	S.aureus	*E.coli*	*P.aeruginosa*	*S.aureus*	*E.coli*	*P.aeruginosa*	*S.aureus*	*E.coli*	*P.aeruginosa*
75	**28**	**21**	**25**	**21**	**21**	**26**	**25**	**23**	**29**
50	**19**	**18**	**21**	**20**	**21**	**23**	**24**	**23**	**29**
25	**18**	**20**	**15**	**19**	**17**	**22**	**23**	**20**	**26**
10	**16**	**17**	**22**	**19**	**15**	**20**	**20**	**19**	**21**
5	**17**	**19**	**16**	**16**	**16**	**15**	**24**	**19**	**20**
2.5	**15**	**12**	**12**	**13**	**17**	**11**	**16**	**14**	**18**
1	**11**	**10**	**10**	**11**	**14**	**10**	**14**	**13**	**17**
0.1	**8**	**7**	**8**	**8**	**7**	**8**	**8**	**9**	**12**

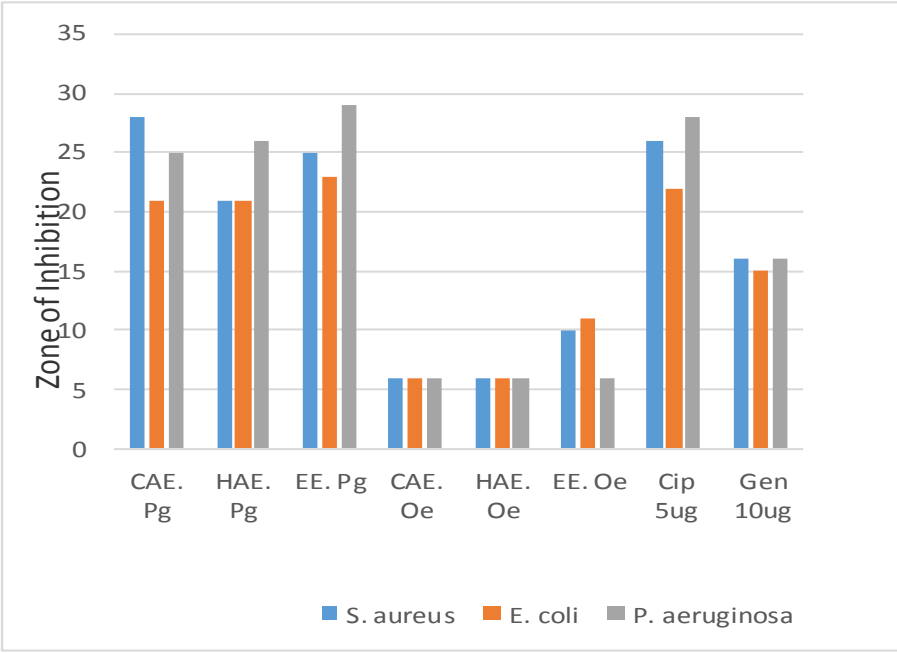

Figure (1): Antibacterial Activity of the Cold and Hot Aqueous Extracts (CAE and HAE) and Ethanolic Extracts (EE) of *Punica granatum* peels (Pg) and *Olea europaea* leaves (Oe) by Disc Diffusion Method.

4. DISCUSSION

Nearly 80% of the world populations depends on the traditional medicine for primary health care, mainly including the use of natural products [12]. Researchers have extensively studied the biological properties of *Punica granatum* and their results showed that this plant is ethno medically valuable [13]. *P. granatum* peel extracts are currently used for treatment of respiratory diseases and in the preparation of therapeutic formulae. The tannin rich ellagitannins and phenolic acids of Punica granatum have antibacterial, antifungal and antiprotozoal activity

[14,15]. In the current study the cold aqueous, hot aqueous and ethanolic extracts of *P. granatum* showed zone of inhibition of at least 25mm against *P. aeruginosa.* Which was greater than that of Gentamicin 16 little lesser than Ciprofloksasin, 21mm against both of *S. aureus* and *E.coli* which was also greater than the first positive control Gentamicin 16 and 15mm on *S. aureus* and *E.coli* respectively, and mostly similar to Ciprofloksasin 26 and 22mm. Ethanolic extract of Punica granatum was greater than that of standard Gentamicin and Ciprofloksasin. This was in agreement with Ahmad *et al*. who found alcohol as a better solvent for extraction of antimicrobial active substances compared to water and hexane [16]. The antibacterial activity of peels of *P. granatum* may be indicative of presence of metabolic toxins or broad spectrum antimicrobial compounds [17,18]. In addition, this work reveals that aqueous leaves extract of the plant *Olea europaea*, didn't show any inhibition. Many studies confirm positive role of *O. europaea* extracts in inhibitory pathogenic bacteria. Markin *et al*, also reported that water extract of olive (*Olea europaea*) leaf with a concentration of 0.6% (w/v) killed *E.coli, P. aeruginosa, S. aureus* and *K. pneumonia* in 3h exposure [19], which not agree with the present study. In study Owen *et al*, Phenolic compounds within *O. europaea* leaf extract have shown antimicrobial activities against several microorganisms including: *E. coli, S. aureus, K. pneumoniae, B. cereus, S. typhi* and *V. parahaemolyticu* [20]. In our study, *O. europaea* leaves ethanolic extract showed weak Antimicrobial abilities and highest inhibition of 10 and 11 mm against only *S. aureus* and *E. coli*. The results obtained in this study revealed antimicrobial efficacy of the aqueous and ethanolic peels extract of *Punica granatum* and only the ethanolic extract of *Olea europaea* on test isolates. The observed antimicrobial effects of this medicinal plants on the organisms tested, though in-vitro appear interesting and promising and may be effective as a potential source of novel antimicrobial drugs.

5. CONCLUSION

Overall, it can have concluded that Pomegranate (*Punica granatum*) peel is a good source of antibacterial compounds against

pathogenic and drug resistance bacterial strains and it can be use as drug against pathogens. The study reveals that different extraction with different solvents has antimicrobial activity, this plant can be used in preparation of effective natural medicines and it should be further investigated.

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