

A chemotherapeutic efficacy of some antibiotics and *Punica grantum* L. extracts against *Propionibacterium acnes* isolated from acne vulgaris cases

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ABSTRACT

The study aimed to isolate Propionibacterium acnes as a bacterial agent of acne vulgaris, study the antibacterial activity of extracts of Punica granatum L. (leaves, fruits, and crusts) against P. acnes, and compare these extracts with the selected antibiotics commonly used in the treatment of acne vulgaris.

A total of 143 patients (72 males: 71 females) aged between 14 and 18 years suffering from acne vulgaris were included in the study; specimens (swabs) were collected from these patients (both sex, various degree of acne) in the dermatology unit of Al-Basrah General Hospital and Alsader Teaching Hospital in Basrah City during October 2013–March 2014.

From a total of 147 cases of acne vulgaris, 104 cases (72.7%) reflected positive cultures. The double mode of isolation was found in 47 cases (45.2%) followed by other modes. The present study found that P. acnes is a more effective bacterial pathogen isolated from 143 cases of acne vulgaris followed by other bacterial pathogens. Three extracts (crust, leaves, and fruits) from P. grantum and eight standard antibiotics [Penicillin G (P), Chloramphenicol (C), Cephalexin (CE), Bactrim (Sxt), Tetracycline (TE), Erythromycin (E), Kanamycin (K), and Gentamicin (CN)] were used to determine the antibacterial activity against P. acnes isolated from patients with acne vulgaris. The crust extract was found more effective, which gave 28 mm of inhibition zone with a minimum inhibitory concentration (MIC) ranging 600–750 mg/ml; leave and fruit extracts gave 20 mm (MIC: 700–900 mg/ml) and 14 mm (MIC: 1000–1200 mg/ml) of inhibition zones, respectively; and the antibiotics Gentamicin and Cloxacillin gave 28 mm of inhibition zone followed by other antibiotics. The study evidenced the antibacterial activity of extracts of P. granatum L. (leaves, fruits, and crusts) against P. acnes in comparison with the selected antibiotics commonly used in the treatment of acne vulgaris.

Key words: Pomegranatum, antibiotics, acne, P. acnes

INTRODUCTION

Acne is a major skin problem among the youth, and is usually initiated by androgen and propagated by bacterial flora of hair follicles such as *Propionibacterium acnes* and *Staphylococcus epidermidis* (1). Pomegranatum extracts are extensively used throughout the world as a traditional beverage, which contain many chemical compounds like tannins, mainly catechins, flavonol, and alkaloid substances. Pomegranatum extracts are used as an antibacterial, antioxidant, and immune-modulating agent (2).

Punica granatum L. (Pomegranate) is a member of the family Punicaceae, which is a deciduous spreading shrub or small tree bearing thorns. This plant is found all over Iraq. Pomegranate peel is an inedible part obtained during the processing of pomegranate juice. Pomegranate peel is a rich source of tannins, flavonoids,

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FIGURE 1: Punica granatum fruit

polyphenols and some anthocyanins such as Delphinidins, Cyanidins, and so on (3,4). Antioxidant and antibacterial properties of pomegranate peel in in vitro model systems have been reported. All the compounds of pomegranate peels are reported to have therapeutic properties (5) Extracts of pomegranate peels show antibacterial property against bacterial strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (2,3). Pomegranate juice (PJ) has become more popular because of the attribution of important biological actions (6). Numerous studies over the past decade have shown that PJ contains higher levels of antioxidants compared with other fruit juices and beverages (7). Researchers evaluated the commercial polyphenol-rich beverages available in the US market for their total phenolic compounds, antioxidant activity, and antioxidant function on cholesterol (8). Pomegranate and its products also exhibit a strong activity against some species of bacteria such as *Bacillus subtilis*, *Shigella*, *Salmonella*, *S. aureus*, *Vibrio cholera*, *E. coli*, and *Yersinia enterocolitica*, which justifies its use as a biopreservative in food (9,10). The PJ consumption also helps keep the prostate specific antigen (PSA) levels stable in men and even slows its rise by extending the PSA doubling time. PJ is also helpful against heart disease, Alzheimers disease, and some types of cancer such as prostate and colon cancers (11).

Pomegranate Chemical Composition: (12)

Food Value Percentage Minerals and Vitamins Concentration in mg

Moisture: 78.0%, Calcium: 10 mg

Protein: 1.6%, Phosphorus: 70 mg

Fat: 0.1%, Iron: 0.3 mg

Minerals: 0.7%, Vitamin: C 16.0 mg

Fiber: 5.1%, Vitamin B complex: Trace amounts

Carbohydrates: 14.5%

100% Caloric value 65

Values Per 100 g of edible portions.

The aims of the study were to isolate *P. acnes* as a bacterial agent of acne vulgaris, study the antibacterial activity of extracts of *P. granatum* L. (leaves, fruits, and crusts) against *P. acnes*, and compare these extracts with the selected antibiotics commonly used in the treatment of acne vulgaris.

MATERIALS AND METHODS

Patients and Sample collection

A total of 143 patients (72 males: 71 females) aged between 14 and 18 years suffering from acne vulgaris were included in the study; specimens (swabs) were collected from these patients (both sex, various degree of acne) (13) in the dermatology unit of Al-Basrah General Hospital and Alsader Teaching Hospital in Basrah City during October 2013-March 2014.

Bacteriological study

A sterile swab was used to collect the sample and inoculate in brainheart infusion and then streak on mannitol salt agar, blood agar, MacConkey agar, and Nutrient agar (Oxoid). All cultures were incubated aerobically and anaerobically (candle jar with catalyst) for 24 h at 37°C, all media were sterilized

by autoclave (1.5 pond/cm³) for 15 min, and all glassware were sterilized in an oven at 180-200°C for 2 h.

The bacterial agents were identified according to routine laboratory techniques (14).

Antibiotics and plant extract

Eight standard antibiotics (as antibiotic disks) were used for comparing their effects against plant extracts: Penicillin G (P) 10 unit, Chloramphenical (C) 30 g, Cephalexin (CE) 30 meg, Bactrim Sxt (25 meg), Tetracycline TE (30 g), Erythromycin E (15 mg), Kanamycin K (30 g), and Gentamicin CN (10 g) were used in this study in comparison with aqueous extracts of leaves, fruits, and crusts of *P. grantum* (100, 500, and 1000 mg/ml).

Antibiotics susceptibility

Two techniques were used to determine the antibacterial activity of plant extracts and the standard antibiotics (14).

(1) Plate (agar diffusion) method: To determine the growth inhibition zones by using MullerHinton Agar.

(2) Tube (dilution) method: To determine minimum inhibitory concentration (MIC) by using brainheart infusion.

Ethical improvement

The present study was carried out with the improvement and agreement of Ethical and Medical Committee in the College

TABLE 1: Modes of isolation of bacterial types isolated from 143 patients with acne vulgaris.

Mode of isolated bacterial types	No. of cases	% from the +ve cases
Single	33	31.8
Double	47	45.2
Three	17	16.3
Over than three	7	6.7
Total no. of cases	143	
No. of positive cultured cases		104 (72.7 %)

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RESULTS

The modes of isolation are shown in Table 1.

From a total of 143 cases of acne vulgaris, 104 cases (72.7%) reflected positive cultures.

The double mode of isolation was found in 47 cases (45.2%) followed by other modes.

The present study found that *P. acnes* is a more effective bacterial pathogen isolated from 143 cases of acne vulgaris followed by other bacterial pathogens as shown in Table 2.

TABLE 2: Bacterial types isolated from 104 positive cultured cases of patients with acne vulgaris.

Bacterial types	No. of cases	% from the +ve cases (104)	% from total isolates (167)
E. coli	20	19.2	11.9
Proteus	13	12.5	7.7
Klebsiella	18	17.3	10.7
Staphylococcus . epidermidis	23	22.1	13.7
Propionibacterium acnes	35	33.6	20.9
Pseudomonas	21	20.2	12.5
Staphylococcus aureus	29	27.9	17.3
β.hem streptococci	8	7.7	4.7
Total no. of isolates	167		
No. of positive cultured cases	104		
Isolation rate/case	1.6 isolates/case		

TABLE 3: Effects of Punica granatum extracts on Propionibacterium acnes isolated from acne vulgaris.

Extract	Concentration of aqueous extracts µg/ml diameter of inhibition zones (mm)			MIC µg/ml
	100	500	1000	
Fruits	NE	4	14	1000–1200
Leaves	6	11	20	700–900
Crust	8	15	28	600–750

NE: Not effective.

TABLE 4: Diameters of inhibition zones of antibiotics against Propionibacterium acnes.

Antibiotics	Diameter of inhibition zone (mm)
Pencillin G (10)	NE
Methicillin (30)	14
Cephalexin (30)	20
Tetracycline (30)	18
Neomycin (30)	20
Gentamicin (30)	28
Cloxacillin (30)	28
Chloramphenicol (30)	16

Table 3 shows that the crust extracts of *P. granatum* were more effective than other extracts and gave an inhibition zone of 28 mm in comparison with 20 mm and 14 mm for a concentration 1000 mg/ml of leave extract and fruit extract, respectively.

However, the MIC of these extracts were 600, 750, 700–900, and 1000–1200 µg/ml for crust, leave, and fruit extracts, respectively.

We found that Gentamicin and Cloxacillin were more effective antibiotics that gave 28 mm of inhibition zone in comparison with another antibiotics as shown in Table 4.

DISCUSSION

Many drugs and preparations are used in the treatment of acne vulgaris, which include 1) topical treatment such as erythromycin, clindamycin, benzyl peroxide, azelaic acid, and retinoic acid and 2) systematic therapy such as oral tetracycline, erythromycin, co-trimoxazole, oral retinoid, and hormonal therapy (15–18).

Pomegranatum (*P. granatum*) has been used in traditional medicines for treating many diseases (systemic, burns, and skin diseases). In our study pomegranatum extracts succeeded in affecting *P. acnes* isolated from acne vulgaris. These results may be due to the presence of many active ingredients like tannin (catechin), alkaloid, flavonol, enzymes, and amino acids (19). The phenolic groups of tannin are responsible for the antibacterial effects of pomegranatum (20). One of the etiological factors in the pathogenesis of acne vulgaris was bacteria (13–15). The antibacterial activity of the peels of *P. granatum* may be indicative of the presence of metabolic toxins or broad spectrum antimicrobial compounds that act against both gram +ve and gram ve bacteria (21–25). Ethanolic extracts exhibited a higher degree of antibacterial activity as compared to other extracts tested against bacteria causing gut infection, stomachache, and diarrhea (21, 26–31). Other studies reported that *P. granatum* contains a large amount of tannins (25%), and antibacterial activity may be indicative of the presence of secondary metabolites (32, 33).

In conclusion, pomegranatum extracts are effective and may be used in future to treat acne vulgaris. The potential therapeutic properties of pomegranate are wide ranging and include the treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and protection from ultraviolet radiation.

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