



ENHANCEMENT OF ANTIBACTERIAL EFFECTIVENESS OF AMOXICILLIN TRIHYDRATE AND AMPICILLIN-SULBACTAM

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ABSTRACT

The improvement of antibacterial activity of antibiotics is an important issue because of the increase of multiple antibiotic resistant pathogen microorganisms. In this study, the synergistic effects of *Punica granatum* L. acetone fruit peel extract and antibiotics (Ampicillin-Sulbactam and Amoxicillin trihydrate) combinations were investigated. The disc diffusion method was used for the determination of the synergistic effect. Pomegranate acetone fruit peel extract and Amoxicillin trihydrate combination showed a synergistic effect against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium*, *Enterococcus faecalis*, and *Bacillus subtilis*. Besides, the combination of *P. granatum* acetone fruit peel extract and Ampicillin-Sulbactam exhibited synergistic effect against *S. epidermidis*, *E. faecalis*, and *B. subtilis*. According to the results of this study, *Punica granatum* acetone fruit peel extract highly improved the antibacterial efficacy of Amoxicillin trihydrate and Ampicillin-Sulbactam.

Keywords: Synergy, *Punica granatum*, Amoxicillin trihydrate, Ampicillin-Sulbactam, Antibiotic

1. INTRODUCTION

The extensive use of antibiotics has caused resistance of pathogen microorganisms and this is a serious medical problem [1]. The synergistic enhancers derived from plants may not possess any antimicrobial properties when used alone. However, when they are used concurrently with the standard drugs, they enhance the activity of the drugs [2]. Synergy was derived from the Greek word “syn-ergo” meaning working together. The resulting effect may be defined as a combination that is significantly greater than the sum of its parts. In the antimicrobial synergy concept, the formulation may enhance efficacy, reduce toxicity, decrease adverse side effects, increase bioavailability, lower the dose and reduce the advance of antimicrobial resistance. Recently, new antimicrobial combination drugs have become a research priority [3].

The World Health Organization (WHO) determined that traditional medicine is used by 80% of the people living in developing countries. The traditional medicinal drugs derived directly or indirectly from plants, constitute 25% of the pharmaceutical armamentarium [4]. *Punica granatum* L. (Punicaceae) which is a shrub or a small tree [5], is rich in phytochemical compounds [6], like gallotannins, ellagic acid, gallic acid, punicalins and punicalagins [7]. It has been used in several systems of medicine for a variety of ailments [8].

Pomegranate is native to Persia and it has been spread into Asia, North Africa and Mediterranean Europe, including Turkey [9]. Pomegranate peel extracts have markedly antioxidant capacity. Also, *Punica granatum* fruit peel extracts have been reported that they possess a wide range of biological actions including anti-cancer, antimicrobial, antidiarrheal, anti-tyrosinase, anti-inflammatory and anti-diabetic activities [10]. *Punica granatum* fruit peel, consisting about 50% of the total fruit weight is one of the major by-products of the food processing industry and it is discarded as waste [10, 11].

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In this study, the synergistic interaction between *Punica granatum* L. fruit peel extract and antibiotics (Ampicillin-Sulbactam and Amoxicillin trihydrate) was investigated against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella typhimurium* ATCC 14028, *Enterococcus faecalis* ATCC 29212, and *Bacillus subtilis* ATCC 6633.

2. MATERIALS AND METHODS

2.1. Materials

Punica granatum L. fruits were collected from Çiçekpınar village, Düzce, Turkey in October 2017. Test microorganisms used in this study were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella typhimurium* ATCC 14028, *Enterococcus faecalis* ATCC 29212, and *Bacillus subtilis* ATCC 6633 which were supplied from Microorganism Culture Collections of Microbiology Research Laboratory of Biology Department, Sakarya University, Turkey.

2.2. Preparation of Fruit Peel Extract and Antibiotic Solution

Punica granatum L. fruit peels were dried in shade for 7 days and the dried fruit peels were ground by using an electric mill. The fruit peel powder of *Punica granatum* L. was kept in acetone for 3 days in a dark place at a rate of 1:10 (w/v). The solvent in the obtained extracts was evaporated by using a rotary evaporator (Heidolph Laborota 4000 efficient) under vacuum at 40–45°C for 10 minutes. The determined concentration (6.4 mg/30 µL) of the prepared extract was obtained by adding DMSO. 5 mg of antibiotic (Ampicillin-Sulbactam, Amoxicillin trihydrate) was dissolved in 5 mL of DMSO.

2.3. Preparation of Overnight Bacterial Culture

Test microorganisms used in the study were inoculated to Tryptic Soy Broth (Merck) and were incubated at 37°C for 24 hours. Bacterial suspension used in the experiments was prepared from the overnight culture and was adjusted to 0.5 McFarland by using a densitometer (Biosan).

2.4. Determination of Synergistic Interaction Between Fruit Peel Extract and Antibiotic

The disc diffusion method was used to determine the synergistic effect of the fruit peel extract and antibiotics. 1 mL of fruit peel extract (6.4 mg/30 µL) and 1 mL of antibiotic solution (1 mg/mL) were mixed. Sterile discs (6 mm in diameter, Rotilabo) were impregnated with the 30 µL of prepared fruit peel extract-Ampicillin-Sulbactam and fruit peel extract-Amoxicillin trihydrate. DMSO impregnated discs were used as negative control. Also, fruit peel extract (3.2 mg/30 µL) impregnated discs and antibiotic (0.5 mg/mL) impregnated discs were used in the experiments to compare with the combination of fruit peel extract-antibiotic. The impregnated discs were allowed to dry for 24 h. 0.5 McFarland bacterial suspension was inoculated to Mueller Hinton Agar by using a sterile swab. The impregnated discs were slightly pressed onto the Agar and were incubated at 37°C for 24 hours. The diameters of the inhibition zone (IZs) were measured by using an electronic digital caliper. The synergy tests were performed three times. The flow-chart of the experiment was given in Figure 1.

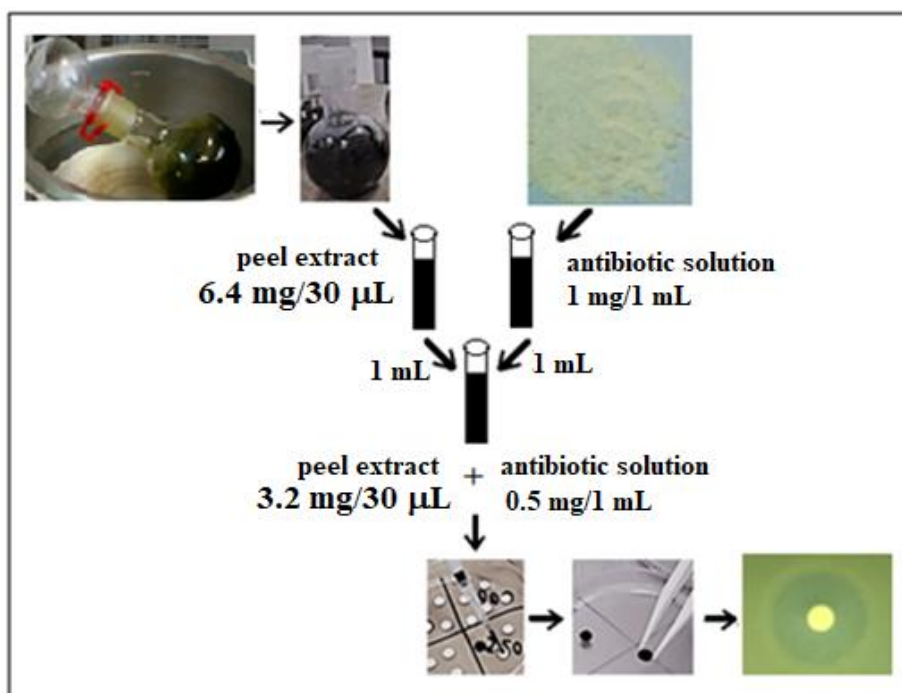


Figure 1. Flow-chart of the experiment

3. RESULTS AND DISCUSSION

This study analyzed the synergistic interaction between the acetone fruit peel extract of *Punica granatum* and the antibiotics (Ampicillin-Sulbactam and Amoxicillin trihydrate) against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella typhimurium* ATCC 14028, *Enterococcus faecalis* ATCC 29212, and *Bacillus subtilis* ATCC 6633. In the experimental process, 1 mL of *Punica granatum* fruit peel extract (6.4 mg/30 µL) and 1 mL of Amoxicillin trihydrate (1 mg/1 mL) were mixed and named as P+A1. Also, 1 mL of *Punica granatum* fruit peel extract (6.4 mg/30 µL) and 1 mL of Ampicillin-Sulbactam (1 mg/1 mL) were mixed and named as P+A2. Sterile discs (6 mm in diameter) were impregnated with 30 µL of prepared P+A1 and P+A2 separately. DMSO impregnated discs were used as control. Also, 30 µL of fruit peel extract (3.2 mg/30 µL) impregnated discs and 30 µL of antibiotic (Ampicillin-Sulbactam, Amoxicillin trihydrate, 0.5 mg/mL) impregnated discs were used in the experiments to compare with the combinations of acetone fruit peel extract and antibiotics. The synergistic effect of the fruit peel extract and the antibiotics was determined by using the disc diffusion method. The obtained diameters of the inhibition zone (IZs) of the fruit peel extract (3.2 mg/30 µL), Amoxicillin trihydrate (0.015 mg/30 µL) and combination of fruit peel extract and Amoxicillin trihydrate against *S. typhimurium* were presented in Figure 2.

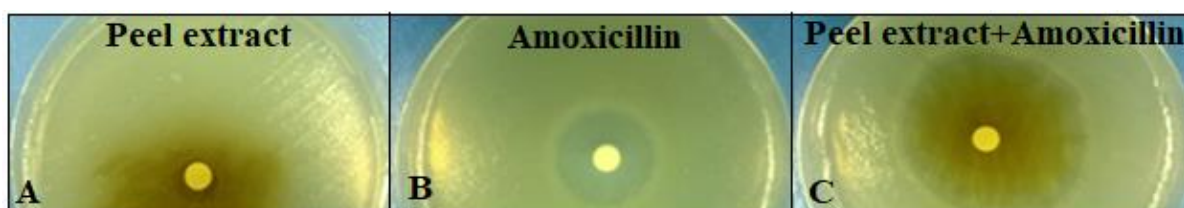


Figure 2. Diameters of the inhibition zone (IZs) against *S. typhimurium*, A) Fruit peel extract (3.2 mg/30 µL), B) Amoxicillin trihydrate (0.015 mg/30 µL), C) P+A1(Combination of fruit peel extract and Amoxicillin trihydrate)

The measured diameters of the inhibition zone (IZs) by using an electronic digital caliper were presented in Table 1 and Table 2. Experimental studies were performed two times under aseptic conditions and the diameters of IZs were the average of the two replicates.

Table 1. Synergistic interaction between *Punica granatum* L. fruit peel extract and Amoxicillin trihydrate

Microorganism	Diameters of the inhibition zone (IZs) (mm)			Synergy
	Peel extract (3.2 mg/30 µL)	Amoxicillin trihydrate (0.015 mg/30 µL)	P+A1*	
<i>S. epidermidis</i>	11.0	30.0	55.3	+
<i>S. typhimurium</i>	10.3	23.3	46.2	+
<i>S. aureus</i>	32.5	20.2	56.4	+
<i>E. coli</i>	10.1	10.7	27.0	+
<i>E. faecalis</i>	0	13.2	25.2	+
<i>B. subtilis</i>	0	27.7	37.5	+

*P+A1 : Combination of fruit peel extract + Amoxicillin trihydrate

Table 2. Synergistic interaction between *Punica granatum* L. fruit peel extract and Ampicillin-Sulbactam

Microorganism	Diameters of the inhibition zone (IZs) (mm)			Synergy
	Peel extract (3.2 mg/30 µL)	Ampicillin-Sulbactam (0.015 mg/30 µL)	P+A2*	
<i>S. epidermidis</i>	11.0	27.9	43.0	+
<i>S. typhimurium</i>	10.3	24.7	32.1	-
<i>S. aureus</i>	32.5	24.1	46.6	-
<i>E. coli</i>	10.1	21.0	22.4	-
<i>E. faecalis</i>	0	21.4	28.6	+
<i>B. subtilis</i>	0	27.9	50.6	+

*P+A2 : Combination of fruit peel extract + Ampicillin-Sulbactam

There are very few studies about the synergistic effects of *Punica granatum* extract and antibiotics in the literature. Malik *et al.* carried out the synergic interaction between *P. granatum* ethyl acetate extract and antibiotics (amikacin, ampicillin, ciprofloxacin, erythromycin, lincomycin, nitrofurantoin, tetracycline and trimethoprim) against *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermidis* (MTCC 3086), *Staphylococcus hominis* (MTCC 4435), *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 1885), *Klebsiella pneumonia* (MTCC 4030) and *Pseudomonas aeruginosa* (MTCC 7453). The results of the study showed that good synergistic interaction between the ethyl acetate extract and the antibiotic (lincomycin, tetracycline) was occurred. MICs of lincomycin and tetracycline were declined from 150 to 5 µg/mL and 250 to 2.5 µg/mL in combination with the peel extract [12].

The interaction between *Punica granatum* methanolic extract and the antibiotics (chloramphenicol, gentamicin, ampicillin, tetracycline, and oxacillin) against 30 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) was investigated by Braga *et al.* It was reported that *Punica granatum* methanolic extract enhanced the activity of all antibiotics tested [13]. In another study, the synergistic effect of methanolic extract of *Punica granatum* fruit pericarp and ciprofloxacin was determined against extended-spectrum β-lactamase (ESBL) producing *Escherichia coli*, *Klebsiella pneumoniae*, and metallo-β-lactamase (MBL) producing *Pseudomonas aeruginosa* [14]. Lakshmi *et al.* reported that the MIC of antibiotic (Tetracycline, Amoxycillin, Erythromycin, Cefuroxime, Cephalosporin, Penicillin G, Oxacillin and

Methicillin)-methanolic extract of *Punica granatum* combinations significantly decreased compared to the MIC of antibiotics [15].

4. CONCLUSION

The enhancement of antibiotic effectiveness has been gained great attraction owing to the increase in the occurrence of multiple antibiotic resistant pathogen microorganisms. This study is the first study examining the synergistic effect of *Punica granatum* fruit peel extract and the antibiotic (Ampicillin-Sulbactam and Amoxicillin trihydrate) against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella typhimurium* ATCC 14028, *Enterococcus faecalis* ATCC 29212, and *Bacillus subtilis* ATCC 6633. The results of the present study proved that *Punica granatum* fruit peel extract highly improved the antibacterial efficacy of Amoxicillin trihydrate against *E. coli*, *S. aureus*, *S. epidermidis*, *S. typhimurium*, *E. faecalis*, and *B. subtilis*. However, the combination of *P. granatum* fruit peel extract and Ampicillin-Sulbactam exhibited synergistic effect against *S. epidermidis*, *E. faecalis*, and *B. subtilis*. This synergistic effect enhanced the antibacterial activity of Ampicillin-Sulbactam and Amoxicillin trihydrate. Thus, the antibiotics can be used at lower doses for the treatment of bacterial infection diseases. The use of lower doses of antibiotics may cause the reduction of the side effects. Moreover, pomegranate peel is low cost raw material since it is an agro-industrial waste. The results of the present study may be beneficial for further studies in academic world and pharmaceutical industry to improve the antibacterial efficacy of antibiotics and to develop new antibacterial therapy combinations.

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