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Antibacterial Potential of Pomegranate Peel Extracts on Escherichia coli Isolated From Benha Hospital in Egypt

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Abstract

Introduction. Pomegranate is an old fruit which has many antimicrobial compounds. Impact of pomegranate peel extracts on Escherichia coli clinical isolate from Benha general hospital was investigated in this study. Material and Methods: pomegranate peel extraction was primed and GC/MS chromatogram analysis was carried out using the GC Agilent Technologies gas chromatography fitted with HP-5 fused silica column (25mm × 0.25mm, film thickness of 0.25 µm) and interfaced to the flame-ionization detector (FID). The antibacterial potential of Pomegranate peel extractions against E.coli sample was done using well diffusion method. Results: According to (GC/MS) technique, methanol and aqueous extracts of pomegranate peel have inhibitory effects on E. coli isolate. The minimum inhibition concentrations (MICs) of methanolic and aqueous extracts were 100 and 60 mg/L, respectively. In this study , the MICs values of Aqueous extract of pomegranate peel were higher than of methanolic extract of pomegranate peel ($P<0.05$). Zones of inhibition and values of MICs indicated that the aqueous extract of pomegranate peel had more effect on E. coli isolate than methanolic extract of pomegranate peel. Conclusion: In this study, the extraction of pomegranate peel has antibacterial effect on E.coli isolate. However, the aqueous extract had a higher effect on E.coli than the methanolic extract.

Keywords: E.Coli, pomegranate peel , ESBLs.

1. Introduction

Antimicrobial drug resistance in pathogenic bacteria is a global concern [7]. E. coli is an essential pathogen, which causes multidrug resistance [10]. Infections which caused by these pathogenic isolates were increased through the last eras in hospitals [13]. The spread of pathogenic E. coli in unitcare surroundings are difficult to control due to the presence of antimicrobial resistance mechanisms specifically ESBLs [12].

In particular, fruit peels have antimicrobial compounds which have antimicrobial and antioxidant effects that can protect from pathogenice bacteria. These compounds are secondary metabolites (phenols, steroids and alkaloids) which are important for human health [17]. Pomegranate (Punica granatum L.) is a fruit that is widely used for its medicinal properties [5].

Researchers have shown the effect of pomegranate peel extracts on a host of pathogenic or drug-resistant bacterial strains utilising in vitro techniques, such as Agar Disk diffusion assays or MICs [4]. The pharmacological effects of pomegranate have been reported and many researches on pomegranate have been done due to its great medicinal uses and nutritional values [2]. Many studies found that the extractions of pomegranate have many effects on bacteria, fungus, viruses and some other activities [6].

The aim of this work was to assess the impact of pomegranate peel extraction on E.coli isolated from Benha hospital,

2. Materials and Methods

Extraction

The preparation steps of both aqueous and alcoholic pomegranate peel extracts were carried out as previously reported [8]. Fresh fruit of pomegranate were bought from the local market. Pomegranate peels were removed manually. The peels were cooled then washed and left 5 days to get dehydrated beneath the sunlight.

Later, dehydrated peels were cut into lesser parts and ground using an electric grinder till converted to well powder. The powder was sieved using a (75 µm × 20 cm) sieve. Weight about 20 g of powder and melted in a suitable quantity of deionized water and the mixture was heated until boiling at that point cool for 24 hr at 37 ^{o}c. The mixture was filtered several times to extract the identical product and then heated in the extraction concentration method. The collected extract was located in a 250 ml volumetric bottle and then diverse concentrations (20, 40, 60, 80, and 100) ppms were prepared from the standard solution.

Gas chromatography-mass Spectrometry (GC-MS) for Identification of Pomegranate Peel Extract Compounds

GC-MS analysis of the major important constituents of the extraction of Pomegranate Peel was done using the GC Agilent Technologies (Santa Clara, CA) using 6890 N apparatus equipped with the split-splitless injector attached to HP-5 fused silica column and interfaced to the flame-ionization detector (FID). About 1 µl of ethanol diluted oil was injected under the following operational conditions: carrier gas was He (1ml/min). The temperature degrees were adjusted as follows: detector at 280°C and injector at 250°C, while the column temperature was linearly programmed from 40°C to 260°C at 4°C/min. Constituents were determined by comparisons of their spectral outcomes with those provided on MS libraries (NIST/Wiley), and by comparison of their retention profile (calibrated AMDIS), with the data from the literature [1].

Antimicrobial Activity Assay

Antimicrobial activities of pomegranate peel extracts were evaluated using agar disk-diffusion. Antimicrobial activities of the extracts were assessed by the agar well diffusion technique [14]. Approximately 20 ml of purified Muller Hinton Agar were poured into a sterilized Petri plates. Following solidification, 100 µl of freshly cultured pathogen was flushed onto particular plates. The wells were cut over the agar plates using a sterile gel puncher at different concentrations (20, 40, 60, 80, and 100) against replicates for one isolate of E. coli ; and all plant extracts were added to the wells. The plates were incubated for 24 hours at 37^{o}C. Then the diameter of inhibition zones around each well was measured in mm and documented.

Statistical Analysis

The mean and standard deviation (SD) of the clear zone diameter in agar well diffusion method as well as the MIC of pomegranate peel extracts were determined. Data were analyzed using Statistical Package for Social Sciences for Windows, version 19.0 (SPSS Inc.). Statistical significance was identified at the 95% confidence level ($P < 0.05$).

3. Results

GC/MS profiles of main phytochemicals identified in the pomegranate peel extract (PPE).

GC/MS chromatograph recognized and assessed the existence of the compounds in the extract of PPE Fig.(1). The distinguished compounds are combinations of terpenes and related alcohols. Chief compounds recognized in the extracts of the pomegranate peel extract are shown in Table 1. Thirteen compounds were detected in the extract like Geranyl vinyl ether, Linalool, benzoic acid, Benzaldehyde, Oleic Acid, Phenol, 2-methoxy-3 (2-propenyl), and Eicosapentaenoic acid.

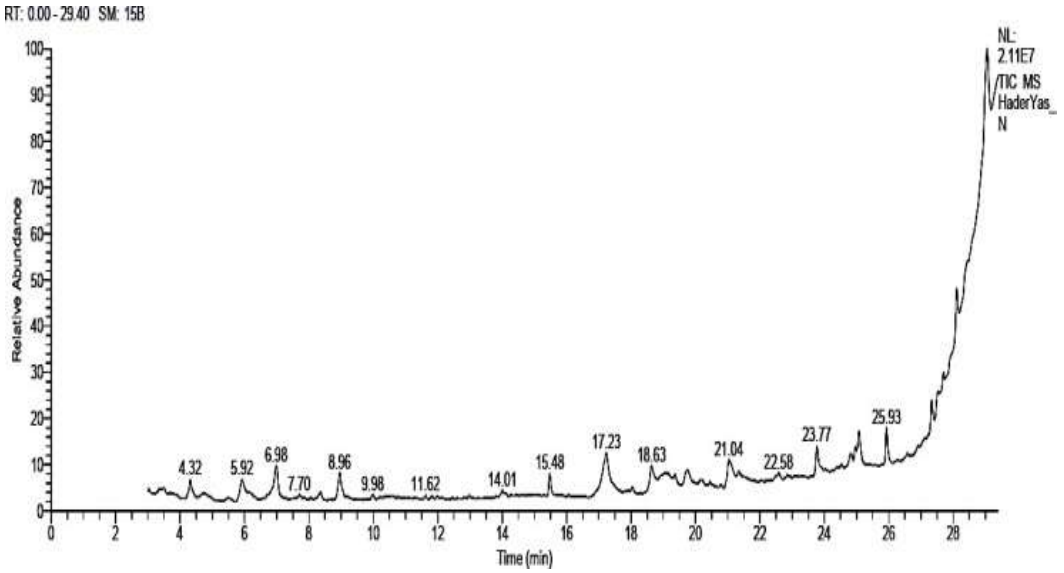

Fig. (1GC-MS Chromatogram of Pomegranate Peel Extract.

Table (1) Highest compounds recognized in Pomegranate peel extracts.

No	Name of Compound	Chemical formula	Retention Time
1	Geranyl vinyl ether	C15H26O2	1.19
2	Linalool	C12H20O	4.61
3	Benzoic acid	C7H6O2	5.92
4	Benzaldehyde	C10H12O	6.98
5	Oleic Acid	C18H34O2	8.37
6	Phenol, 2-methoxy-3-(2-propenyl)	C10H12O2	8.96
7	Eicosapentaenoic acid	C20H30O2	9.98
8	Acetic acid	C17H30O4	14.00
9	Oxirane undecanoic acid	C19H36O3	18.04
10	Hexadecanoic acid	C19H38O4	18.63
11	9-Octadecenamide	C18H35NO	19.02
12	9-Octadecenoic acid	C18H34O2	19.12
13	Oxirane undecanoic acid	C19H36O3	20.45

Minimum inhibitory Concentration (MICs) of PPE on E. coli isolate.

Table (2) MIC of PPE against E. coli bacteria tested using well diffusion method.

E.Coli isolate		
MIC (mg/L)	**Drug concentration Extract (mg/L)**	**Diameter of Inhibition Zones**
AEP	20	1.18 ±0.25[a]
	40	1.45±0.35[a]
	60	**2.65±0.21[a,b]**
	80	0.89±0.46[a]
	100	0.23±0.12[a]
MEP	20	0.1±0.12[a]
	40	0.5±0.17[a]
	60	0.3±0.15[a]
	80	0.5±0.20[a]
	100	**0.7±0.21[a]**

AEP. Aqueous Extraction of Pomegranate, **MEP**. Methanolic Extraction of Pomegranate.
Means (±SD) Within a column with different superscript letters (a,b) symbolize significant differences (P<0.05).

MICs of the PPE were measured to evaluate their impact on E.coli isolate as shown in Table (2). MICs for both aqueous and alcoholic extracts against E. coli were evaluated. The aqueous extract showed significant differences more than the alcoholic extract. The aqueous extract against E. coli was estimated to be 60 mg/L although the growth of this isolate was inhibited by 100 mg/L of aqueous extract. Our results showed antibacterial activity against the tested replicates of E. coli isolate, however, the aqueous extract have more effect.

4. Discussion

In this study, aqueous and methanolic PPE have antibacterial activities against E. coli strain. GC/MS chromatograph recognized and assessed the existence of the main compounds in pomegranate peel extracts. Chief constituents recognized in the extracts of the pomegranate peel extract are shown in Table (2). Thirteen compounds were found in the extract like Geranyl vinyl ether, Linalool, benzoic acid, Benzaldehyde, Oleic Acid, Phenol, 2-methoxy-3 (2-propenyl), and Eicosapentaenoic acid. In several studies, Most of these compounds were detected. Some of these compounds have been found to have impact on bacteria. MICs of pomegranate peel extraction against the test organisms were 1:16 for Streptococcus mutans (ATCC), S. mutans (CI), and S. sanguis. 1:128 for S. mitis and 1:64 for C. Albicans. Pomegranate extracts and Juglans regia have activity against H. Pylori, with a zone of inhibition of diameter 39 and 16 mm at 100ug disk [11].

Zones of inhibition and MICs values specified that the aqueous pomegranate peel extract was sophisticated than methanolic pomegranate peel extract by 60 mg/L. The MIC values detected the effect of pomegranate extracts against E. coli, but the aqueous pomegranate extracts have a higher effect than methanolic extract. This finding was consistent with the previous report. The antibacterial potential of aqueous pomegranate peel and pomegranate juice were detected on bacteria including S. aureus and the extract of pomegranate peel had the highest antibacterial activity [6].

Antimicrobial activity of many extracts from the different fragments of pomegranate shrub was previously premeditate, and numerous results were found by scientists [3]. In a study conducted by Nozohour et al. [16], the ethanolic extracts showed inhibitory effects on clinical isolates of P. aeruginosa and S. aureus clinical isolates. The pomegranate peel and seed extracts had minimum inhibitory concentrations of 12.5 and 25.0 mg|ml,respectively [16].

Similarly, Hussain et al. [9] has been identified that alcoholic extract of pomegranate peels have effect on S. aureus which may cause gingivitis. Same result was proved by Naziri et al who showed that the pomegranate peel extracts cause the zone of inhibition 15.3 - 25.7 mm and 16.0 - 25.3 mm, respectively, against S. aureus at 1 - 8 mg/disc [15].

At 500 mg|Kg aqueous-ethanolic fruit crust (50 percent) extracts suppressed inflammation by 82.14 percent (79 percent) against indomethacin at 10 mg|Kg. An aqueous extract of pomegranate peel inhibits neutrophil myeloperoxidase in vitro and reduces lung inflammation in mice [11]. The synergetic result of combining antibiotics with pomegranate extracts and resistant bacteria may lead to new selections for bacterial disease treatment.

5. Conclusions

This study proved that pomegranate peel extracts have antibacterial activity against tested *E.Coli isolate* , and the aqueous extract of pomegranate peel showed higher effect on tested *E.Coli* than methanolic extract.

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