
Antitumor and Antimicrobial activities of pomegranate (*Punica granatum*) dried powder peel in vitro

Asmaa M. Moghazy

Hormonal Evaluation Department, National Organization for Drug Control and Research. (NODCAR). Giza, Egypt. E-mail: asmaamoghazy@hotmail.com

Maie S. Khader

Medicinal Plant Department, National Organization for Drug Control and Research. (NODCAR). Giza, Egypt. E-mail:mselmym@yahoo.com

Neveen Saleh

Microbiology Department, National Organization for Drug Control and Research. (NODCAR). Giza, Egypt. E-mail:neveensaleh0@gmail.com

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ABSTRACT

Background: The pomegranate fruit possesses therapeutically important constituents that gained significant popularity in the treatment of many diseases. Therefore, this study was carried out to investigate the phytochemical compounds of aqueous extract of pomegranate peel (PPE) along with its antimicrobial and antitumor effect on Colorectal (Caco-2) and Colon (HCT-116) cancer cell lines.

Method: Aqueous extract of Pomegranate peel was analyzed by HPLC technique. Furthermore, antimicrobial activity was investigating against Gram-positive, Gram-negative and fungi. Likewise, antitumor activity was tested in vitro on the human cell line Colorectal (Caco-2), and Colon (HCT-116). The cytotoxicity was determined by using MTT assay. Cell cycle analysis, apoptosis, pre-apoptotic and apoptotic gene were analyzed by using Real-Time PCR. While catalase and hydrogen peroxide (H₂O₂) were determined by using ELIZA Kits. **Result:** According to the obtained data, PPE extract contain polyphenolic compounds among which protocatechuic acid, p-hydroxybenzoic acid, and rutin showing the most predominant ones. Besides, PPE has antibacterial activity against Gram-positive bacteria and anti-candida activity. Furthermore, PPE was cytotoxic to cancer cells in a concentration and cell type-dependent manner, where the Caco-2 cells were more sensitive to the PPE extract than HCT-116. Where IC₅₀ of Caco- and HCT-116 are 1434 and 2257 µg/ml respectively. Additionally, the anticancer activity was secure via the up/downregulation of the pro-apoptotic gene (Caspase-3 and p53), both genes expression was cell type-dependent as well. While anti-apoptotic gene Bcl-2 showed a significant decrease (P<0.05). Also, the apoptotic potential of PPE was confirmed via the arresting of cells in the G2/M phase and elevated apoptotic cell % in the pre-G1 phase. Data obtained also, increasing the level of H₂O₂ and decreasing catalase activity.

Conclusion: PPE has an anti-proliferative potential against Caco-2 and HCT-116 cancer cells through the induction of apoptosis. Besides, it has antimicrobial activity against Gram-positive bacteria.

Keywords: PPE, Caco-2, HCT-116, Caspase3, P53, Bcl2, cell cycle, Antimicrobial

Introduction

Pomegranate belongs to the family Punicaceae. It is categorized under medicinal fruits recommended by Prophet Muhammad (PBUH). According to Al-Qur'an, pomegranate grows in the gardens of paradise and it is mentioned three times in the Al-Qur'an as an example of God's good creation. Pomegranate (*Punica granatum L*) cultivate in most countries of the Middle East, Mediterranean Sea, USA, and South Africa (Fawole *et al.*, 2012). The pomegranate fruit possesses therapeutically important constituents, varying slightly in different parts of the fruit. Fruit of pomegranate consists of the outer peel which encloses inner seeds and arils.

The peel was used traditionally for treatment of ulcer and inflammation, and it has been showed antioxidant and bacterial activities (Rebogile *et al.*, 2016; Sreerkumar *et al.*, 2014). Several studies

Corresponding Author: Asmaa M. Moghazy, Hormonal Evaluation Department, National Organization for Drug Control and Research. (NODCAR). Giza, Egypt.
E-mail: asmaamoghazy@hotmail.com

have demonstrated that the pomegranate peel is a good source of bioactive compounds such as catechin, ellagitannins, epicatechin, rutin, and many others. Such bioactive compounds are responsible for many biological activities such as antimicrobial, antioxidant, antiinflammatory (Glazer *et al.*, 2012; Fawole *et al.*, 2015). Additionally, other studies have shown the therapeutic effect of pomegranate fruit, juice, and peel for treatment of lung cancer, esophagus, cardiovascular disorders and breast cancer. It has free radical scavenging activities which mostly related to its phytochemical components (Abdollahzadeh *et al.*, 2011; Qabaha, 2013). Besides, many studies have shown that pomegranate constituents can efficiently affect multiple signaling pathways involved in inflammation, cellular transformation, hyper-proliferation, angiogenesis, initiation of tumorigenesis, and eventually suppressing the final steps of tumorigenesis and metastasis (Khan *et al.*, 2007; Faria, and Calhau, 2011).

The aim of these studies was designed to inspect phytochemical constituents of aqueous extract of pomegranate peel, antimicrobial and antitumor activities of PPE on Caco-2 and HCT-116 cell lines.

Cell Death Pathways

There are two types of programmed cell death which decide the fate of cells of malignant neoplasms which are apoptosis and necrosis. They are distinguished by their morphological differences. Apoptosis, an active, gene-regulated form of cell death, is the major type of cell death that is characterized by specific morphological and biochemical changes of dying cells. The morphological changes include cell shrinkage, nuclear condensation and fragmentation, membrane blebbing and loss of adhesion to neighbors or extracellular matrix while biochemical changes include chromosomal DNA cleavage into inter nucleosome fragments (McIlwain *et al.*, 2013). On the other hand, necrosis is considered as accidental cell death, a random, uncontrolled process that caused profound effects on malignant cells. According to Ouyang *et al.* (2012), necrosis usually involves cell swelling, organelle dysfunction, and cell lysis. Necrosis also disrupts the integrity of the cells membrane which caused the release of intracellular materials, leading to an inflammatory response by immune cells. Apoptosis, a preferential way of elimination of damaged cells is regulated and controlled by a group of endoproteases known as caspases. Caspases play an important role in maintaining homeostasis by regulating cell death and inflammation. Caspases have been broadly categorized by their known functions in apoptosis [caspase-3, -6, -7, -8 and -9 in mammals] and in inflammation [caspase-1, -4, -5 and -12 in humans] (McIlwain *et al.*, 2013). Initially, caspases are produced as inactive monomeric procaspases which require dimerization and cleavage for activation. There are two types of caspases which are initiator [caspases-8 and -9] and executioner caspases [caspases-3, -6 and -7]. In apoptosis, executioner caspases are activated by initiator caspases. Various apoptotic pathways exist and most apoptotic programs fall into either extrinsic (death receptor-mediated) or intrinsic (mitochondria-mediated) pathway. The extrinsic apoptotic pathway is activated at the cell surface. The binding of pro-apoptotic ligands to the respective plasma membrane-localized receptors causes the monomeric pro-caspase-8 to be recruited to the death-inducing signaling complex [DISC] (McIlwain *et al.*, 2013) which then results in dimerization and activation. There are two outcomes resulted from the death receptor-mediated activation of caspase-8 which depends on the cell types. In type I cells, apoptosis is initiated directly by cleaving and activating the executioner caspases [caspase-3] whereas, in type II cells, caspase-8 must first activate the intrinsic apoptotic pathway to induce cell death.

Materials and Methods

Reagent and Media:

Fresh pomegranate fruits were collected from the local market at the Giza government, during September. Muller-Hinton agar (Difco, USA); Sabauod Dextrose Agar (SDA), Oxoid, USA; RPMI-1640 medium Trypsin 0.25%, fetal calf serum (GIBCO, USA), ethylene diamine tetra-acetic acid (EDTA), methanol and ethanol were purchased from Sigma Aldrich-USA.

Preparation of Pomegranate Peel Aqueous Extract (PPE):

Fresh pomegranate fruits were peeled manually and the collected peels were then rinsed with distilled water. Peels were further cut into small pieces and dried at 50-60°C in a hot air oven for

24hrs and ground to a coarse powder using a grinder. Dried pomegranate peel was used to make aqueous extracts. Powder (50 gm) of the dried peels were extracted with 250 ml boiled distilled water at room temperature for 1hrs. The PPE was concentrated by lyophilization using a freeze dryer (BUCHI Lyovapor L-200). The dried lyophilized powder was kept in a sealed glass container at -5°C in dark until use. The total yield of aqueous extract was 4%. Phytochemical constituents of aqueous extract of pomegranate peel (PPE) were carried out on an Agilent Technologies HPLC 1100 liquid chromatography according to Kim *et al.*, 2006.

Bacterial Strains:

Reference microorganisms used in this study were purchased from American Type Culture Collection (ATCC), including Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC14028, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027), Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633), yeast (*Candida albicans* ATCC 10231), and fungi *Aspergillus niger*, *Aspergillus flavus* were obtained from Microbiology Department at National Organization for Drug Control and Research. (NODCAR).

Antibiotic Susceptibility Testing:

Antibiotic susceptibility testing was performed using the Bauer-Kirby disc diffusion method by applying commercial antibiotic disc, Ciprofloxacin (5µg/disc). Breakpoint criteria were established according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2012).

Antimicrobial Assay of Pomegranate Peels Extract using the Agar-Well Method:

Pomegranate aqueous extract (PPE) was screened for their antimicrobial activity against different microorganisms using modified Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966) as follow: strains were inoculated in Mueller-Hinton broth (MHB) and Sabaurod Dextrose Agar (SDA) media then incubated at 35 °C and 25 °C for 24 and 48 hours for bacteria and fungi, respectively. Overnight cultures were diluted to 1/100 µl then adjusted to a concentration of 10⁸ CFU ml⁻¹ by making a suspension in 0.85% saline solution and match with the 0.5 McFarland turbidity standards (Hindler, and Jorgensen 1995), using a sterilized swab, aliquots from each tube were spread on Muller-Hinton agar medium, disks of Whatman filter paper soaked with aqueous extracts of 100 mg/ml were added and incubated at 35 °C and 25 °C for 24 and 48 hours for bacteria and fungi, respectively. Discs with water were used as a negative control and those with antibacterial and antifungal (ciprofloxacin and nystatin, respectively) used as a positive control. The experiment was tested in triplicates.

Cell Culture

Colon (Caco-2) and colorectal (HCT-116) cancer cell lines were provided by the tissue culture department (VACSERA-Egypt). Cells were cultured in RPMI 1640 medium and D-MEM supplemented with 10% fetal bovine serum (FBS) (GIBCO, USA) and 1% penicillin-streptomycin (Invitrogen, USA) and incubated (Jouan-France) at 37 °C in a humidified and 5% CO₂ atmosphere.

Cell Viability (MTT Assay)

Human Caco-2 and HCT-116 cancer cells were propagated in 75 cm² cell culture as previously mentioned according to Bussereau and Co-worker (Bussereau *et al.*, 1982), where confluent sheet were detached using 0.25% (w/v) trypsin solution and 0.05% (v/v) ethylene diamine tetra-acetic acid (EDTA) (GIBCO-USA) for 5 min. Cells were plated at a concentration of 2 x 10⁵ cell/ml in 96-well cell culture plates and incubated at 37°C for 24 hrs to achieve confluence. The growth medium was decanted and fresh medium containing 2-fold serially diluted PPE dispensed to cells (4 wells/dilution). Twenty-four hours later dead cells were washed out using phosphate buffered-saline (PBS) and 50 µl of MTT solution (0.5 mg/ml) were added to each well. After 4 hrs. In the incubation period, the supernatants were discarded, and the formazan precipitates were solubilized by the addition of 50 µl/well of dimethyl sulfoxide (DMSO). Plates were incubated in the dark for 30 min at 37°C and absorbance was determined at a wavelength of 570 nm using a microplate reader (ELX -800, BioTek-USA). The cell viability percentage was calculated using the following formula:

$$\text{Cell viability \%} = \frac{\text{Mean OD of test dilution}}{\text{OD of control well}} \times 100$$

Determination of PPE IC₅₀:

Cytotoxicity was conducted according to Van den Berghe *et al.* (Van den Berghe *et al.*, 1978), where growth medium was decanted from tissue culture Caco2 and HCT-116 cancer cell lines pre-cultured 96 microtiter plates. PPE sample was applied as a starting concentration of 10 mg/ml in a series of 2-fold serially diluted patterns in serum-free RPMI-1640 medium and D-MEM as 0.1 ml of each dilution/well. Negative non-treated cell control was considered. Plates were incubated at 37 °C (Jouan- France) and examined frequently for up to 3 days. Cells were checked for any toxic as partial or complete loss of monolayer, shrinkage, rounding, or cell granulation. The IC₅₀ values were determined using Master Plex 2010 Software.

Cell Cycle Analysis:

Caco-2 and HCT-116 were pre-cultured in 25 cm² surface area cell culture flasks were treated with the IC₅₀ of the tested product (PPE) in RPMI-1640 medium, for 24h. For cell cycle analysis, the cells were harvested and fixed gently with 70% (v/v) ethanol in PBS, maintained at a temperature of 4°C overnight, and re-suspended in PBS containing 40 µg/ml propidium iodide (PI), 0.1 mg/ml RNase and 0.1% (v/v) Triton X-100 in a dark room. After 30 min at 37°C, the cells were analyzed using a flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with an argon-ion laser at a wavelength of 488 nm. The cell cycle and sub-G1 group were determined and analyzed.

Real-Time PCR: mRNA Expression of Cell Apoptosis-Related Genes:

Total RNA was extracted from control and treated Caco-2 and HCT-116 cell lines using the Gene JET RNA Purification kit (Fermentus, UK) according to the manufacturer's protocol. The concentration and the integrity of RNA were assessed spectrophotometrically at 260/280nm ratio. First-strand cDNA was synthesized with 1µg of total RNA using a QuantiTect Reverse Transcription kit (Qiagen, Germany) according to the manufacturer's instructions. These samples were subsequently frozen at a temperature of -80°C until used for determination of the expression levels of Caspase-3, p53 and Bcl-2 genes using real-time PCR. Quantitative real-time PCR was performed on a Rotor-Gene Q cycler (Qiagen, Germany) using QuantiTect SYBR Green PCR kits (Qiagen, Germany) and forward with reverse primers for each gene. The nucleic acid sequences of the primers were as follows CASP-3-F 5'-TTC ATT ATT CAG GCC TGC CGA GG-3' and R 5'-TTC TGA CAG GCC ATG TCA TCCTCA-3' p53 F 5'-CCCCTCCTGGCCCCTGTATCTTC-3', R5'-GCAGCG CCTCA CAA CCTCCGTCAT-3'. Bcl-2 F 5'-CCTGTG GAT GAC TGA GTA CC-3' R 5'-GAGACA GCC AGG AGA AATCA-3' and β-actin F5'-GTGACATCCACACCCAGAGG-3' R 5'ACA GGA GTCAAAACTGCC-3'.

Real-time PCR mixture consisted of 12.5 µl 2x SYBR Green PCR Master Mix, 1 µL of each primer (10 pmol/µl), 2 µl cDNA and 8.5 µl Rnase-free water in a total volume of 25 µl. Amplification conditions and cycle counts were a temperature of 95°C for 15 min for the initial activation, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 60°C for the 30 s and elongation at 72°C for the 30s. Melting curves were performed after real-time PCR to demonstrate the specific cellular amplification of a single product of interest. A standard curve was performed to determine the amplification efficiency of the primers used, as well as relative fold changes in both of them.

Statistical Analysis:

All experiments were carried out in three independent tests. Data were expressed as the mean standard deviation (SD) and analyzed using a one-way analysis of variance (ANOVA). The results were considered statistically significant at $P < 0.05$.

Results

Identification and Quantification of Phenolic Compounds by HPLC Analysis:

The chromatograms illustrating the phenolic compounds in the PPE are shown in Fig (1), whereas, Table (1) gives the concentrations of individual phenolic compounds identified in PPE. A

total of 13 phenolic compounds were identified. Where, hydroxybenzoic acids such as protocatechuic acid, p-hydroxybenzoic, gallic acid, and vanillic acid, hydroxycinnamic acids such as chlorogenic, caffeic, syringic, and p-coumaric acid. Flavonoids such as rutin, Apigenin-7- glucoside, quercetin, and kaempferol were identified in PPE. The major phenolic acids are Protocatechuic (186.37 ug/ml), and p-hydroxybenzoic (155.08 µg/ml); While the major flavonoid is rutin (460 µg/ml).

Table 1: The Phenolic acids and flavonoids identified in pomegranate peel aqueous extract by HPLC.

Compound	Concentration (µg/ml)
Gallic	10.97
Protocatechuic	186.37
p-hydroxybenzoic	155.08
Catechin	4.83
Chlorogenic	6.88
Caffeic	5.33
Syringic	17.10
Vanillic	6.03
p-coumaric	6.76
Rutin	460.50
Apigenin-7-glucoside	5.36
Quercetin	2.24
Kaempferol	2.71

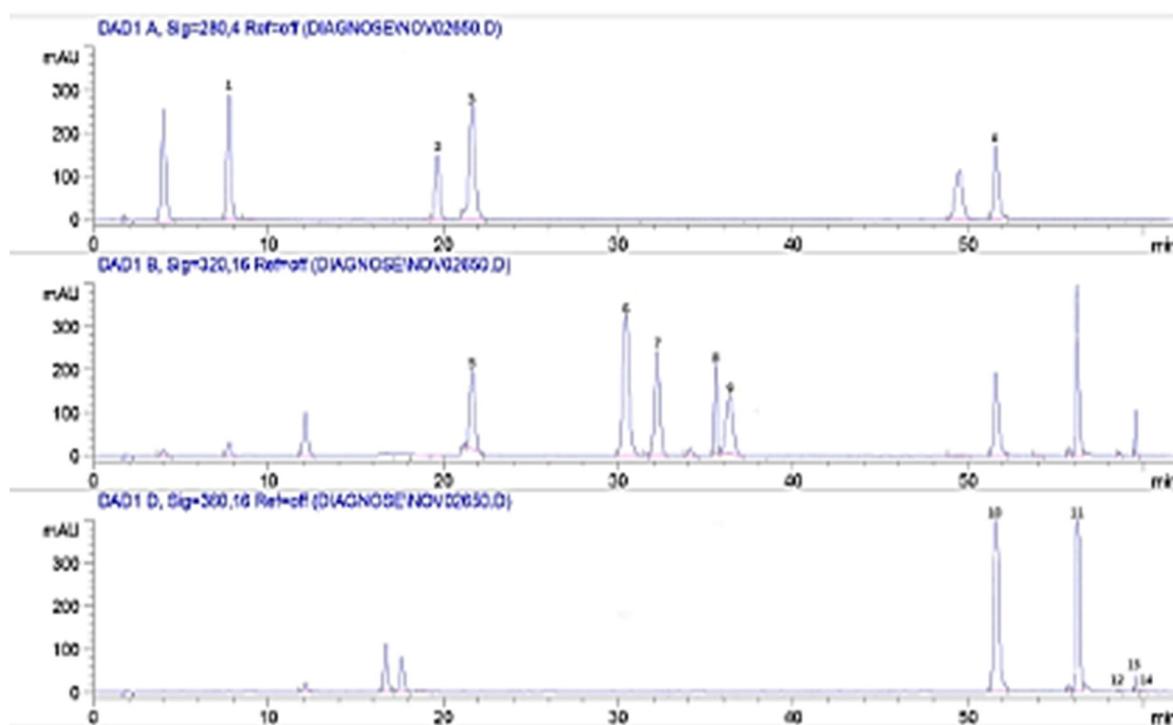


Fig. 1: Chromatogram of phenolic compounds in pomegranate aqueous extract cultivated in Egypt. Peaks:1 (Gallic acid), 2 (protocatechuic acid), 3 (catechin), 4(p-hydroxybenzoic acid), 5(vanillic acid), 6 (chlorogenic acid),7(caffeic acid), 8 (syringic acid), 9 (p-Coumaric acid), 10 (rutin),11(apigenin7-glucoside), 12(quercetin), 13 (kaempferol).

Aqueous extract of pomegranate (PPE) showed antibacterial activity against Gram-positive bacteria besides, anti-candida activity and no activity against Gram-negative bacteria was detected. The minimum inhibitory concentration of PPE was more than MIC's of standard antibiotics (Table 2). The antibacterial activity *in vitro* against the growth of the tested reference species of Gram-positive bacteria was shown as both concentration and species-dependent (Table 3). The tested extract had a mainly bacteriostatic effect (MBC/MIC ratio > 4) against *S. aureus* ATCC 25923 and *M. luteus*

ATCC 10240 and bactericidal effect against both *S. epidermidis* ATCC 12228 and *B. subtilis* ATCC 6633.

Table 2: Ciprofloxacin and nystatin were used as the standard drugs for bacterial and fungal strains

Microorganism	Ciprofloxacin MIC (mg/L)
<i>S. aureus</i> ATCC29213	0.24
<i>S. epidermidis</i> ATCC12228	0.49
<i>M. luteus</i> ATCC10240	0.98
<i>B. subtilis</i> ATCC6633	0.03
<i>K. pneumoniae</i> ATCC 13883	0.12
<i>E. coli</i> ATCC 25922	0.004
<i>S. Typhimurium</i> ATCC 14028	0.06
<i>P. aeruginosa</i> ATCC 9027	0.49
Microorganism	Nystatin MIC (mg/L)
<i>C. albicans</i> ATCC10231	0.48
<i>Aspragillus niger</i>	0.24
<i>Aspragillus flavus</i>	0.24

Table 3: Antimicrobial activity of PPE aqueous extract against test microorganisms and determination of MIC, MBC, and MFC.

Microorganism	Antimicrobial activity (mm)	MIC (mg/L)	MBC/MFC (mg/L)	Ratio MBC/MIC
Gram-positive bacteria				
<i>S. aureus</i> ATCC 25923	25	15.6	62.5	4
<i>M. luteus</i> ATCC 10240	22	7.8	31.3	4
<i>S. epidermidis</i> ATCC 12228	22.5	15.6	31.3	2
<i>B. subtilis</i> ATCC 6633	18.5	3.9	3.9	1
Gram-negative bacteria				
<i>S. Typhimurium</i> ATCC 14028	No inhibition	ND	ND	ND
<i>E. coli</i> ATCC 25922	No inhibition	ND	ND	ND
<i>K. pneumoniae</i> ATCC 13883	No inhibition	ND	ND	ND
<i>P. aeruginosa</i> ATCC 27853	No inhibition	ND	ND	ND
Yeasts and Fungi				
<i>C. albicans</i> ATCC 10231	16.0	>1000	>1000	ND
<i>Aspergillus niger</i>	No inhibition	ND	ND	ND
<i>Aspergillus flavus</i>	No inhibition	ND	ND	ND

Cytotoxicity of Pomegranate peel aqueous extract:

The cytotoxic effect of PPE was assessed by recording the various morphological changes of cells. The viability % of treated cells was cell type, extraction method, and concentration-dependent, it was found that Caco-2 was more sensitive to PPE than HCT-116 in an insignificant way ($P > 0.05$). Where, IC_{50} of Caco-2 is 1434 and IC_{50} of HCT-116 is 2257 $\mu\text{g/ml}$ [Fig.2 A, B, C].

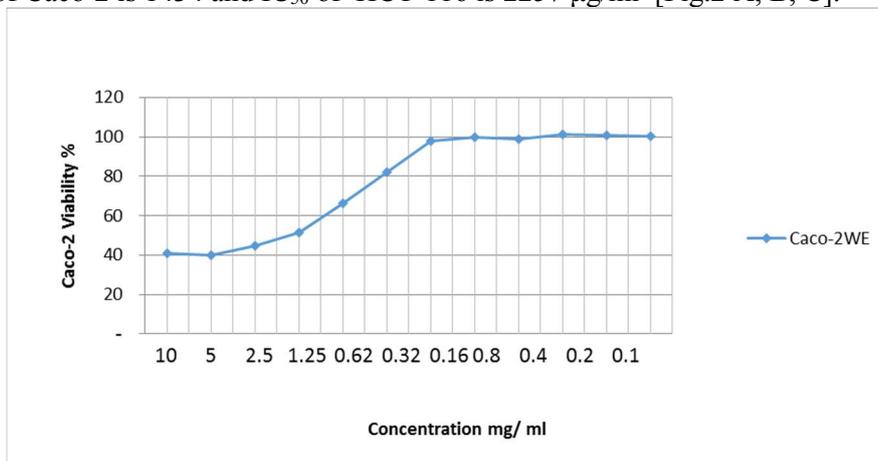


Fig. 2(A): Illustrate the correlation between the PPE concentration and the cell viability of Caco-2

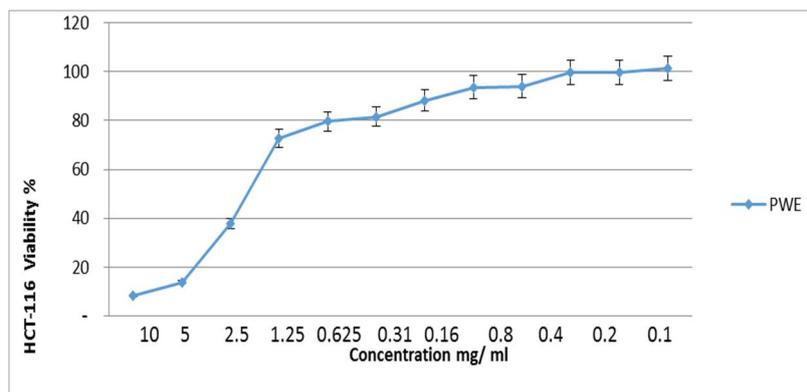


Fig. 2(B): Illustrate the correlation between the PPE concentration and the cell viability of HCT-116.

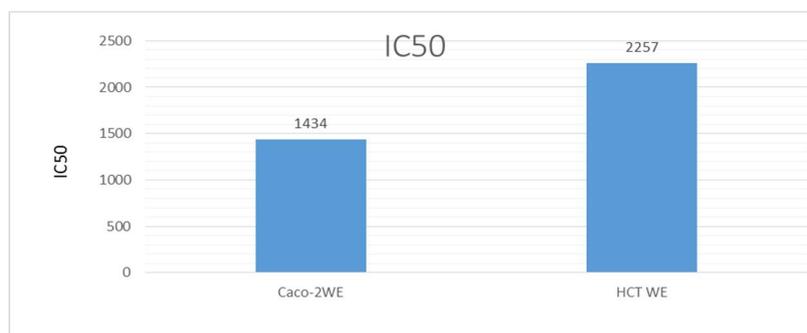


Fig. 2(C): Assessment of IC₅₀ values of PPE post-Caco-2 and HCT-116 cancer cell lines using MTT assay (ug/ml).

Cell Cycle and Apoptosis:

Concerning the effect of PPE extract on cell cycle profile, it was noticed that Caco-2 and HCT-116 cells in the G0-G1 phase is non-significant ($P > 0.05$) while, there was a significantly elevated cell accumulation during the Pre G1 and G2/M phase ($P < 0.05$) compared with cell control. Also, it was noticed that cell arrest was accompanied by apoptotic profile in the pre-G1 phase in a significant way in the case of both cell lines ($P < 0.05$) (Figure 3A & B). The apoptotic profile accompanied by cell arrest showed that early and late apoptosis were significantly elevated in Caco-2 than in the case of HCT-116 cells ($P < 0.05$) and to the values of cell control as well. Also, necrotic percentage showed the same profile (Figure 3 C). The anti-apoptotic activity of target extracts showed that pro-apoptotic gene (Caspase-3 and related p53) were significantly elevated post-treatment ($P < 0.05$) While, the anti-apoptotic gene (Bcl-2) was significantly declined ($P < 0.05$) compared with that in cell control as shown in (Figure 4). Finally, the biochemical changes accompanied by the apoptosis where H₂O₂ values significantly elevated ($P < 0.05$), and on the other hand, catalase enzyme activity was significantly decreased as compared with cell control values ($P < 0.05$), (Figure 5).

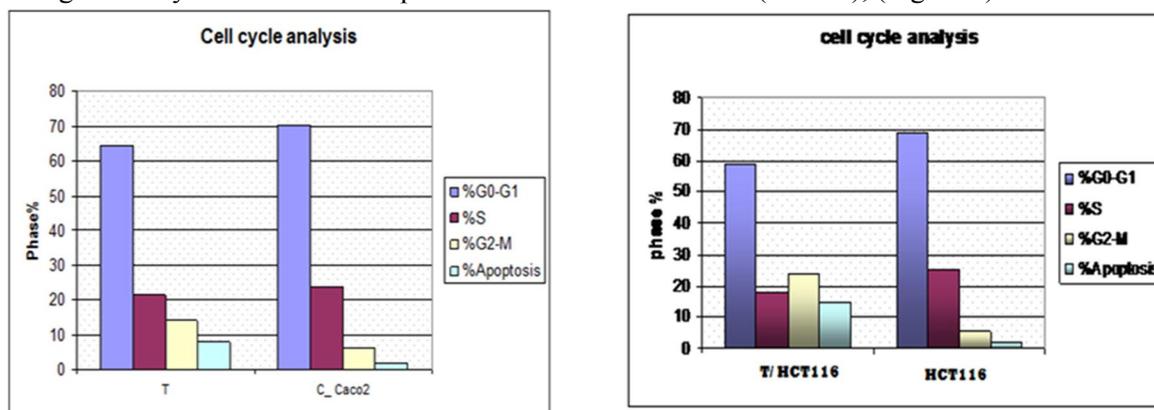


Fig. 3(A): Illustrate the cell cycle analysis and related % of arrested cells in different cell cycle phases post-treatment with PPE.

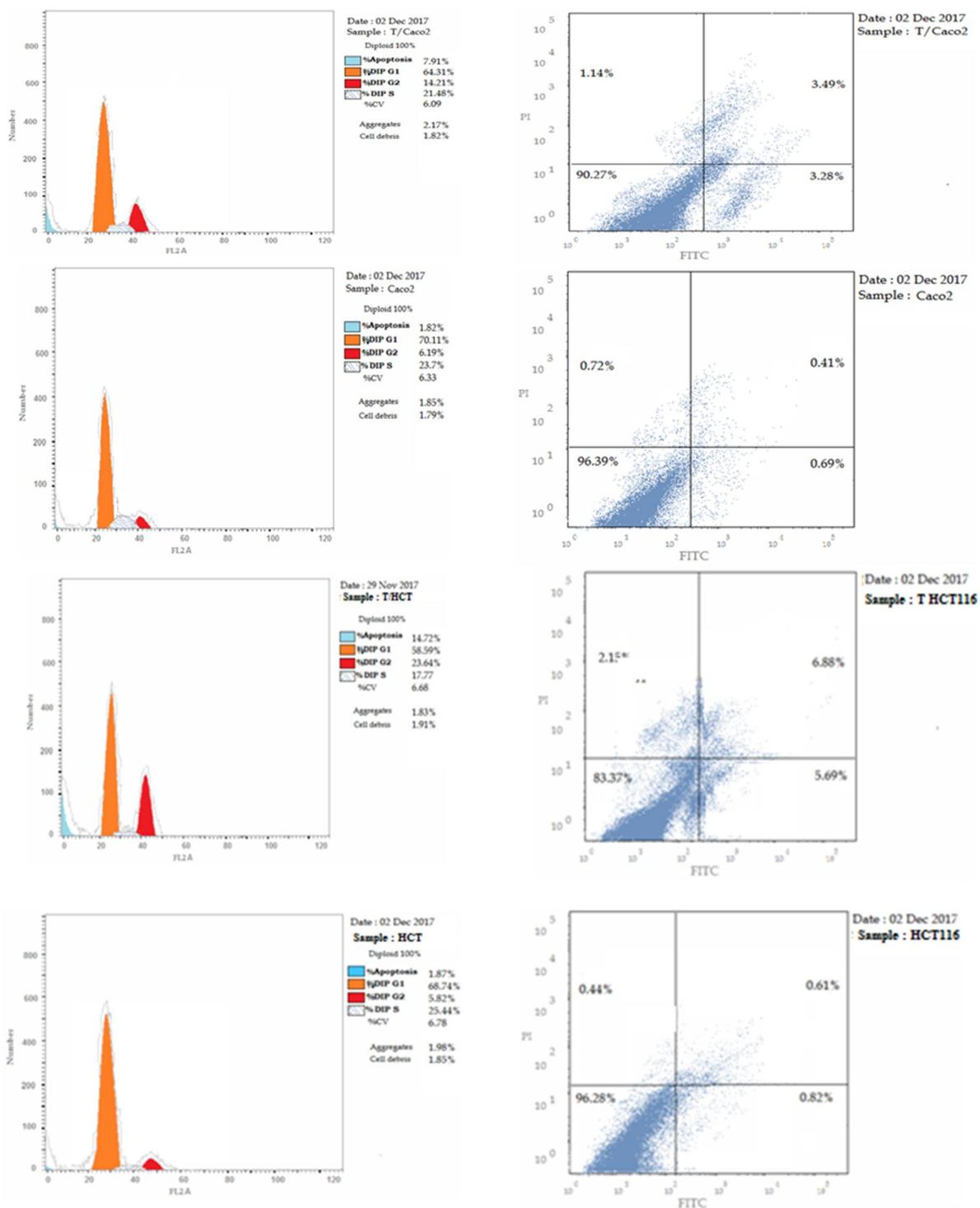


Fig. 3(B): Flow cytometric cell cycle analysis and related % of arrested cells in different cell cycle phases, as well as the apoptotic % post-treatment with PPE using PI and Annexin V stains

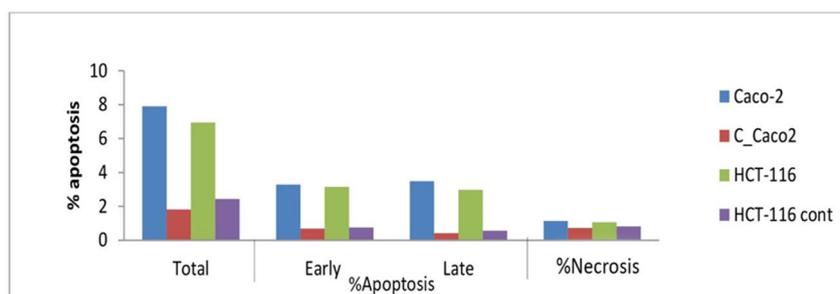


Fig. 3 (C): Apoptotic profile post- Caco-2 and HCT-116 treatment with PPE using Real-time PCR.

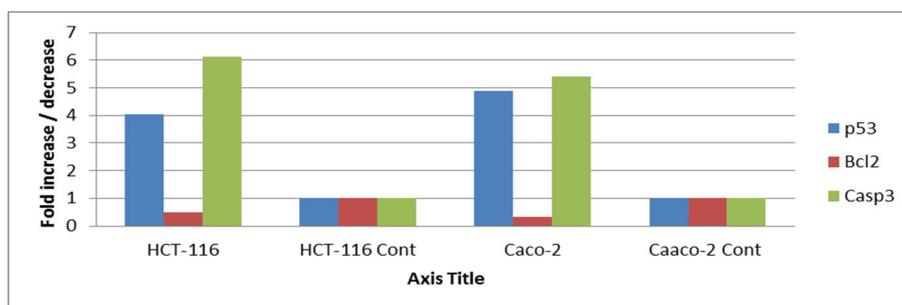


Fig. 4: Pro and anti-apoptotic gene profile post- Caco-2 and HCT-116 treatment with PPE using Real-time PCR.

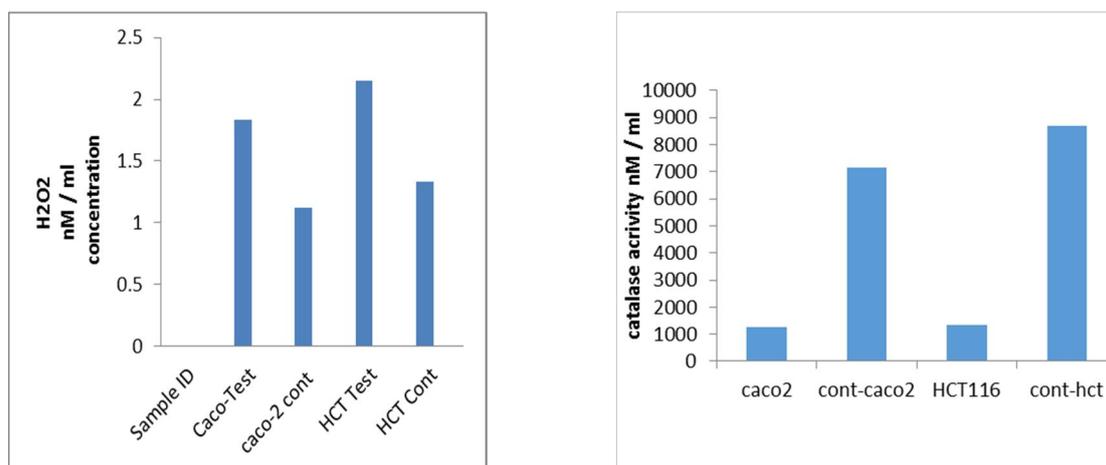


Fig. 5: Catalase and H₂O₂ activity post Caco-2 and HCT-116 treated with PPE using the biochemical assay.

Discussion

The pomegranate fruit possesses therapeutically important constituents that gained significant popularity in the treatment of many diseases. Therefore, the present study was designed to investigate the phytochemical constituent of aqueous extract of pomegranate peel (PPE), antimicrobial and anticancer potential of PPE in human colorectal cancer (Caco-2), and colon cancer (HCT-116) cell lines. Data in our investigation revealed that PPE was cytotoxic to cancer cells in a concentration and cell type-dependent manner, where the Caco-2 cells were more sensitive to the tested extract than HCT-116. The results regarding the anti-proliferative and cytotoxic potential matched the study recorded by El-Awady *et al.* (2015); Shalaby *et al.* (2019). In the current study, to establish the PPE as a good anticancer agent, its ability to arrest the cell cycle and induce apoptosis were evaluated. According to this study, PPE caused an accumulation during the Pre G1 and G2/M phase in treated Caco-2 and HCT-116 cells. Thus, PPE caused cell cycle arrest at Pre G1 and G2/M phase. This result agrees with a previous study done in colon cancer and breast cancer cells where PPE arrested cell cycle at pre G1 and G2/M phase, respectively (Khan *et al.*, 2007). The main purpose of cancer therapy is to target proliferating cells to induce cellular death pathways. The p53 protein is a transcription

factor, which can induce apoptosis by regulating the pro-apoptotic and anti-apoptotic genes. The ability of p53 to promote cell death could be directly linked to its tumor-suppressive function (Schmitt *et al.*, 2002). Bcl-2, an anti-apoptotic protein, is localized to the outer membrane of mitochondria, where it plays an important role in promoting cell survival and inhibiting the actions of pro-apoptotic proteins (Hardwick, and Soane, 2013). Bcl-2 promoter contains a p53-negative response element, raising the possibility that Bcl-2 may be a direct target of p53-mediated transcription repression (Liu, 2003). p53 may also directly impact Bcl-2 activity as part of a transcription-independent program of cell death. In this process, cytoplasmic p53 binds to pro-apoptotic Bcl-2-family proteins, leading to permeabilization of mitochondria and apoptosis (Engelbrecht *et al.*, 2007; Ather *et al.*, 2009). The results of the current study revealed a significant increase in p53 expression and a significant decline in Bcl-2 in both cell lines. Caspases play an important role in maintaining homeostasis by regulating cell death and inflammation. Caspases have been broadly categorized by their known functions in apoptosis [caspase-3, -6, -7, -8 and -9 in mammals] and in inflammation [caspase-1, -4, -5 and -12 in humans] (McIlwain *et al.*, 2013). The data obtained from the current work showed that a significant increase in the caspase -3 expression. Catalase, an antioxidant enzyme that brings about its cytoprotective action by suppressing or neutralizing the toxic hydrogen peroxide radicals produced (Sridhar *et al.*, 2011). The results of the current work showed a significant increase in H₂O₂ and a decrease in catalase activity. The study revealed that PPE is rich in phenolic acids such as gallic acid, Protocatechuic, p-hydroxybenzoic, chlorogenic acid, caffeic acid, and flavonoid like rutin where the antitumor and antibacterial activity may attribute to these compounds. This result agrees with Ross *et al.*, (2016) who reported that anticancer properties of phenolic acids, including scavenging free radicals, induction of enzymes involved in the metabolism of xenobiotics, regulation of gene expression, modulation of cellular signaling pathways including those involved in DNA damage repair, cell proliferation, and apoptosis. The result of the current study showed that the PPE has antibacterial activity against Gram Positive-bacteria with the MIC value less than 15.6 mg/l and *S. aureus* recorded as the most sensitive strain. PPE extract has bactericidal activity against all tested Gram Positive-bacteria. Our data was in contract with Al-Zoreky, (2009) who reported that the aqueous extract was inactive against *S. aureus* (2 strains), *B. subtilis*, while agree with the finding that PPE was inactive against Gram-negative and yeasts as *E. coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Yersinia enterocolitica*, *Candida utilis*, *Saccharomyces cerevisiae*, and *Aspergillus niger*. Different studies correlate the antimicrobial activity of herbal extracts to its contents of phenolic compounds, Pomegranate peel contains substantial amounts of polyphenols such as ellagic acid and gallic acids. Besides, other compounds as anthocyanins and flavonoid may act synergistically to exert their antimicrobial activity (Naz *et al.*, 2011)

Conclusion

Pomegranate peel extract has an anti-proliferative potential against Caco-2, and HCT-116 cancer cells through induction of apoptosis proved via the up-regulation of pro-apoptotic genes; Caspase-3. As well as downregulation of anti-apoptotic genes Bcl-2. However, apoptosis induction is both dose and time-dependent manner. Also, PPE has antimicrobial activity against Gram-positive bacteria and anti-candida activity.

Conflict of interest:

The authors have no conflicts of interest to declare

Abbreviation:

Pomegranate peel	PPE
Colorectal	Caco-2
Colon	HCT-116
hydrogen peroxide	H ₂ O ₂
Mueller-Hinton broth	MHB
Sabaurod Dextrose Agar	SDA
Dimethyl sulfoxide	DMSO
Ethylene diamine tetra-acetic acid	EDTA
Death-inducing signaling complex	DISC

phosphate buffered-saline	PBS
propidium iodide	PI
fetal bovine serum	FBS

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