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A Multi-Target Assessment of Antioxidant, Anticancer, and Antimicrobial Activities of Phenolic Compounds Fractionated from *Punica granatum* L. Peels Crude Extract

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ABSTRACT

Pomegranate peel is recognized as a rich source of phenolic compounds with significant biological activities. In the present study, phenolic constituents were isolated from pomegranate peel crude juice using solid-phase extraction (SPE), followed by fractionation through thin-layer chromatography (TLC). The chemical characterization of the obtained fractions was carried out using Fourier-transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrometry (GC/MS/MS). The analytical results confirmed the presence of several functional groups, including hydroxyl, carboxyl, carbonyl, and aromatic structures. Five major phenolic compounds were identified, namely gallic acid, ellagic acid, quercetin, catechin, and vanillin. The biological activities of these compounds were systematically evaluated. Ellagic acid demonstrated a pronounced antioxidant capacity, particularly in terms of metal chelation and reducing power, whereas vanillin exhibited strong free radical scavenging activity as determined by the DPPH assay. In addition, gallic acid and ellagic acid showed notable antibacterial effects against the tested bacterial strains. Regarding anticancer activity, catechin significantly reduced the viability of human breast cancer (MCF-7) and colon cancer (HCT-116) cell lines. Importantly, the safety profile of the studied phenolic compounds was assessed using normal human skin fibroblast (BJ1) cells, where no significant cytotoxic effects were observed at the tested concentrations. These findings support the potential application of pomegranate peel-derived phenolics as safe and effective natural agents with antioxidant, antimicrobial, and anticancer properties

Keywords: phenolic compounds, antioxidant activity, antibacterial activity, cytotoxicity.

Introduction

Plants produce a vast range of vital substances referred to as phytochemicals which can be used as a source of drugs for curing diverse ailments. These phytochemicals are not only safe for human consumption but also for a friendly environment.

Pomegranate (*Punica granatum* L.) is considered one of the Punicaceae family and ancient edible fruits vastly cultured in Far-East countries and extend to the Middle-East and European continent through to the Mediterranean zone (Konsoula *et al*., 2016). In recent years, pomegranate has attracted significant scientific interest due to its rich phytochemical profile and diverse pharmacological properties. Different parts of the fruit, particularly the peel and juice, have been reported to exhibit promising therapeutic effects against a wide range of chronic disorders, including cancer, gastrointestinal diseases, and metabolic syndromes (Fahmy *et al*., 2020 and Sharma *et al*., 2022).

Moreover, pomegranate botanical parts can treat some chronic ailments such as breast, colon, prostate, skin cancers, and stomach ulcers because of their anticancer features (Singh *et al*., 2023; Derakhshan *et al*., 2018)

Among the major bioactive constituents of pomegranate are polyphenolic compounds, which are well known for their strong antioxidant capacity. These compounds act as free radical scavengers and

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metal ion chelators, thereby reducing oxidative stress and preventing lipid peroxidation. Accumulating evidence suggests that polyphenols contribute significantly to the prevention of chronic diseases such as cancer, diabetes mellitus, and cardiovascular disorders through multiple biological mechanisms (Li *et al*., 2023; Ain *et al.,* 2023).

In addition to their antioxidant potential, polyphenols also exhibit antimicrobial properties against a broad spectrum of foodborne pathogens and spoilage microorganisms, enhancing their importance in both medical and food preservation applications (Mo *et al*., 2022).

Polyphenol compounds are scaving free radicals and metal chelators. They can exhibit various physiological activities such as antioxidants and inhibit lipid peroxidation. Cancer, diabetes, cardiovascular disease, and certain chronic diseases could be prevented and treated by phenolic compounds (Del Rio *et al*., 2013). Also, they can inhibit many spoilage bacteria and foodborne pathogenic (Zambrano *et al*., 2019). Subsequently, the present study was focused on investigating the biological activities of some fractionated polyphenols from pomegranate crude juice.

2. Experimental technique and materials

2.1. Collection of plant sample

In October 2019, pomegranate ripe fruits were picked from the Faculty of Agric., Cairo Univ., Giza, Egypt (Research Farm). Samples were picked by hand from different trees and authenticated by the specialist in Horticulture Department, Dr. Abdalatif, A. M. (Associate Prof.).

2.2. Pomegranate peels crude juice preparation

Pomegranate peels (4 kg) were manually separated, cleaned, and removed seeds. A hydraulic laboratory press instrument was used to produce peels' juice mechanically (Carver Model C S/N 37000-156; Fred S. Carver NC, USA). The force was 10 tons/ 2 inch (capacity 1 kg). The resultant crude juice (300ml) had been put up and kept in brown bottles at -5 ºC until use.

2.3. Solid-phase extraction (SPE) of phenols

Pomegranate peels crude juice was firstly filtered by column (Spe-JT Baker Inc, Phillipsburg, N.J., USA) then extracted by solid-phase extraction (SPE) technique using Extra-Sep C18-cartridges 0.2 g and manifold JT Baker vacuum chamber (JT Baker Inc, Phillipsburg, N.J., USA) and oil-less pump used with vacuum = -20 kilopascal (kpa) for the extraction. The cartridges were conditioned with 1 ml of methanol then pomegranate peels crude juice (10 ml, diluted 1:10 v/v) was loaded into the cartridges and passed slowly through the extraction device using the vacuum pressure at a flow rate of 29 drops/min. and eluted with 1 ml ethanol (Rodriguez *et al*., 2000).

2.4. Fractionation of phenolic compounds by thin-layer chromatography

The thin-layer chromatographic experiment was done with TLC silica gel 60 F_{254} coated on glass plates (20 × 20 cm) (Ragazzi *et al*., 1973). An aliquot (10 µl) of the extracted polyphenols by SPE of pomegranate peels crude juice was applied to the chromatographic plates. All planar chromatograms were developed in the TLC twin trough chamber with stainless steel lid (Switzerland, CAMAG, Muttenz) which was previously saturated with mobile phase vapor (30 min). The solvent system consisted of chloroform- ethyl acetate-methanol (5:4:1, v/v/v). Mobile phase was ethyl acetate: Formic acid : chloroform: (5:4:1, v/v/v). The chromatograms were developed 17 cm high and then dried at room temperature for 1 h. After drying, each chromatogram was visualized under a UV hand lamp (Specrtoline, ENF-260C/FE, Spectronics Co., Westbury, New York, USA) at λ= 254 and 365 nm then photographed using a phone camera (iPhone 7 Plus, 12 MP, California, USA) and the R_f was calculated and presented in Table 1.

Table 1: TLC profile of the phenolic compounds of pomegranate peels crude juice.

Solvent system	No. of spots	R_f value
Chloroform: ethyl acetate: methanol (5:4:1, v/v/v)	1	0.2
	2	0.28
Chloroform: ethyl acetate: formic acid (5:4:1, v/v/v)	3	0.68
	4	0.73
	5	0.78

2.5. Identification of the fractionated polyphenols from pomegranate crude juice

2.5.1. FTIR analysis (Fourier-Transform Infrared) Spectroscopy

The FTIR analysis was used to identify functional groups and active compounds of polyphenolic compounds in pomegranate crude juice that was fractionated by TLC. The FTIR analysis was done on the liquid samples by using Nicolet 380 FT-IR Spectrometer (Thermo Scientific, China) in the region of (400-4000) cm^{-1}.

2.5.2. GC-MS/MS assay

The analysis of the fractionated phenolic compounds of pomegranate peels crude juice was using GC/MS/MS. An aliquot of pomegranate leave and peels crude juices (0.2 ml) was diluted with 2 ml of ethanol and injected into the GC/ MS/ MS. The sample was analyzed at the Agriculture Research Centre, Regional Centre for Food and Feed (Organic Pollutants Laboratory). The carrier gas was Helium (linear velocity of 1ml/min). The oven temperature program was 55 ^{o}C for 3 min and raised to 280 ^{o}C with a rate of 11 ^{o}C/min. The detector and injector temp. were 220 ^{o}C and 220 ^{o}C respectively. The volume injected was 1 µl. Selected ion monitoring (Scan) mode was applied using m/z at start mass 35 and end mass 600. The MS operating parameters condition were: ionization potential 70 eV, interface temperature 280 ^{o}C.

Components were recognized by comparing their retention time and mass spectra with authentic compounds and by computer matching with NIST and WILEY library.

2.6. Determination of antioxidant activity

2.6.1. DPPH assay (2, 2-Diphenyl-1-picryl-hydroxyl)

The scavenging potency of 2, 2-diphenyl-1-picryl-hydroxyl (DPPH) radical of the polyphenolic compounds of pomegranate crude juice was determined (Cano-Lamadrid *et al*., 2018). The different concentrations of polyphenols were mixed with DPPH (1 ml, 0.004 % w/v). After shaking, the mixture was stood for 30 min (dark place at room temperature). The remaining amount of DPPH was measured by the absorbance at 517 nm. Butylated hydroxyl toluene (BHT) was used as standard. The calculation of DPPH radicals scavenging ability was according to the next equation:

$$\text{Inhibition \%} = \frac{\text{A control - A test}}{\text{A control}} \times 100$$

Where;

A_{test} = Absorbance of fractionated phenolic compounds of pomegranate peels crude juice.

$A_{control}$ = Absorbance of control reaction.

Results were expressed as IC_{50} (half-maximal inhibitory concentration) by comparing with the standard.

2.6.2. Assay of Reducing power

The reducing powers of the fractionated phenolic compounds of pomegranate peel crude juice was described (Ayoub *et al*., 2019). Phosphate buffer (2.5 ml,0.2 M, pH 5.6) was added to an aliquot of the phenolic compounds (1 ml) and potassium ferricyanide (2.5 ml, 10 g/L), incubation at 50ºC for twenty min. Trichloro acetic acid (2.5ml, 10 %) was added to the mixture and centrifuged at 1000 xg (10 min). Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and then 0.5 ml $FeCl_3$ (1g/L). UV-Vis spectrophotometer (Shimadzu, UVmini-1240, Japan) recorded the absorbance at 700 nm. Phosphate buffer was the blank solution. Ascorbic acid was a standard.

The antioxidant activity of the phenolic compounds was expressed as IC_{50} and compared with the standard.

2.6.3. Determination of Metal chelating activity

The chelating activities of the fractionated phenolic compounds of pomegranate peel crude juice were determined (Oche *et al*., 2019). An aliquot of the phenolic compound solution (40 µl) was mixed with Tris HCl buffer (168 µl,0.1 M, pH 7.4) and saline (218 µl,0.9 % NaCl w/v). Ferrous sulfate solution (150 µl, 500 µM) was added to the mixture and left to stand for 5 min at room temperature. Finally, 13 µl of 1, 10 phenanthroline (0.25 % w/v) was added. The UV-Vis spectrophotometer (Shimadzu, UVmini-

1240, Japan) was recording absorbance at 510 nm. EDTA (Ethylene di-amine-tetra acetic acid) was utilized as a standard. The chelating ability was calculated as % chelation using the following equation:

$$\text{Chelation \%} = \frac{\text{A control - A sample}}{\text{A control}} \text{ x } 100$$

Where ;
A_{sample} = Absorbance of phenolic compounds.
$A_{control}$ = Absorbance of control reaction.
The ability of chelation was displayed as IC_{50}, in addition, to comparing with the standard.

2.7. Antibacterial activity of the fractionated phenolic compounds of pomegranate peels crude juice.

2.7.1. Bacterial strains

The antibacterial activity of the phenolic compounds fractionated from pomegranate peels crude juice against several pathogenic microorganisms like Gram-positive and Gram-negative food contaminant/pathogenic bacteria. The Gram-positive bacteria were *Bacillus cereus* RCMB 027 and *Staphylococcus aureus* ATCC 25923 (1) and the Gram-negative bacteria were *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Bacteria were maintained on Mueller-Hinton agar and sub-cultured on their respective media (37 °C for 24 h). All the strains screened for antibacterial activity were obtained from AL-Azhar University, Regional Center for Mycology and Biotechnology unit (RCMB), and the American Type Culture Collection (ATCC), Cairo, Egypt.

2.7.2. Antibacterial activity

The antibacterial activity of the phenolic compounds fractionated from pomegranate peels crude juice was implemented by the agar well diffusion method (Hindler *et al*.,1994). The inoculant suspension was established from colonies grown overnight on an agar plate and inoculated in Mueller-Hinton broth agar.

The inoculated medium was spilled into a sterilized petri dish (9 cm). It permitted to become solid. Each 6 mm, is cut through agar by a sterilized borer. After that agar was removed leaving hollow wells filled with 100 µl of phenolic fractions then left for 1 h for adequate diffusion of the crude juices. After incubation for 24 h at 37 ºC, the inhibition zone was measured around each well. The values were compared with the positive control (Gentamycin) for antibacterial susceptibility. Experiments were done in triplicate. The mean values of inhibition zones were calculated ± standard error.

2.8. Assessment of Minimum Inhibitory Concentration (MIC)

MIC of the phenolic compounds fractionated from pomegranate peels crude juice against *Bacillus cereus* RCMB 027 (1), *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922 were assessed according to agar well diffusion method as previously mentioned. An aliquot (100 µl) of different concentrations (up to 100 µg/ml) of the phenolic compounds fractionated from pomegranate peels crude juice were placed in sterile Petri dishes' wells which were inoculated with the tested bacteria (incubated at 37 ºC for 24 h). MIC was estimated as the latter dilution prevents the growth of the tested bacteria. The lower MIC represents the greater antimicrobial effect.

2.9. Anticancer activity of the fractionated phenolic compounds of pomegranate peels crude juice

2.9.1. Cell lines sources and culture

Cell culture Human transformed cell lines, from the colon (HCT-116) and breast (MCF-7) were obtained from the American Type Culture Collection (ATCC). Cells were preserved in Dulbecco's Modified Eagle Medium (DMEM) complemented with 10 % fetal bovine serum and 1 % penicillin G potassium and streptomycin in humidified atmosphere and 5 % CO_2 at 37 °C with a complete medium in a 25 cm^3 cell culture flask.

2.9.2. Determination of anticancer activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method

The anticancer activity of phenolic compounds fractionated from pomegranate peels crude juice was achieved (Mosmann, 1983). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl-tetrazolium bromide) method.

Cells were cultured in CO_2 incubators (5 % CO_2 at 37 °C) in a DMEM medium. After 90 % confluency, media was removed and the cells were trypsinized by 3 ml (0.25 %) trypsin–EDTA solution and incubated for 2 min then were seeded in 96 wells plates (20×10^3 cells/well) then incubated for 24 h at 37 °C. Additionally, the cells were treated with each tested crude juice (100 µl) at different concentrations and incubated for 48 h, then 40 µl of MTT solution had added to each well. After incubation for another 4 h, the medium was eliminated and dimethyl sulfoxide (DMSO, 150 µl) was added. A microplate reader was used to record the cell viability at 490 nm. Cell viability was calculated by the next equation:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of the cells treated with the samples}}{\text{The absorbance of the untreated cells}} \text{ x } 100$$

Control (medium only, 100 % viability) and blank (no cells, 0 % viability) groups were also carried out. Data were displayed as means ± standard error. All the experiments were fulfilled in triplicates.

2.9.3. *In vitro* cytotoxicity effects on normal cells

Cytotoxicity effects of phenolic compounds fractionated from pomegranate peel crude juice were tested against normal human skin fibroblast cell line (BJ1) by the MTT (3 - (4,5-dimethylthiazol-2-yl)-2,5- diphenyl- tetrazolium bromide) assay (Vijayakumar *et al*., 2018). The steps are mentioned in detail in the determination of anticancer activity.

Cell culture normal human cell line, from skin fibroblast (BJ1) normal cell line, were obtained from the American Type Culture Collection (ATCC). Cells were in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum and 1 % penicillin G potassium and streptomycin in humidified atmosphere and 5 % CO_2 at 37 °C with a complete medium in a 25 cm^3 cell culture flask.

2.10. Results Statistical analysis

ASSISTAT program, Version 7.7 beta (2014) was used for statistical analysis.
The difference between treatments was calculated by the least significant difference (L.S.D) test and Analysis of variance (ANOVA). All analyses were accomplished in triplicates and data was reported as ± standard error (SE). The confidence limits were based on ($P< 0.01$).

3. Results and Discussion

The presence of natural active compounds in plant extracts affects chemical structures, biological characteristics, and mechanisms of action. The prospects of plant extracts for co-therapy (*in vitro* and *in vivo*) of diverse global spread diseases were studied by several pharmacological researchers and supported the traditional medicine in cases like diabetes mellitus, cancer, cardiovascular diseases, and parasitic infections.

Pomegranate (*Punica granatum* L.) belongs to the *Punicaceae* family and is one of the ancient edible fruits vastly cultured in Far-East countries and extending to the Middle-East and European continent through to the Mediterranean zone (Konsoula *et al*., 2016). Moreover, pomegranates' different parts can treat some chronic diseases such as breast, colon, prostate, skin cancers, and stomach ulcers because of their anticancer features (Fahmy *et al*., 2020; Derakhshan *et al*., 2018).

Polyphenols are one of the most phytochemicals (phenolic acids, tannins, flavonoids, and anthocyanins) and are liable for antioxidant activities and free radical scavenging. Accordingly, the objective of the present study was to investigate the biological role of phenolic compounds fractionated from pomegranate peels crude juice.

3.1. Identification of the fractionated polyphenols from pomegranate crude juice

3.1.1. Fourier-Transform Infrared Spectroscopy (FTIR) analysis

Identification of functional groups of active components was performed by FTIR spectrum which is present in phenolic compounds fractionated from pomegranate feels crude juice. The functional groups were identified based on the peak value obtained in the spectrograph and they were illustrated in figs 1-5. The graphs of the fractionated phenolic compounds revealed the presence of various functional groups such as OH, COOH, C=C, C-C, CHO, and C=O.

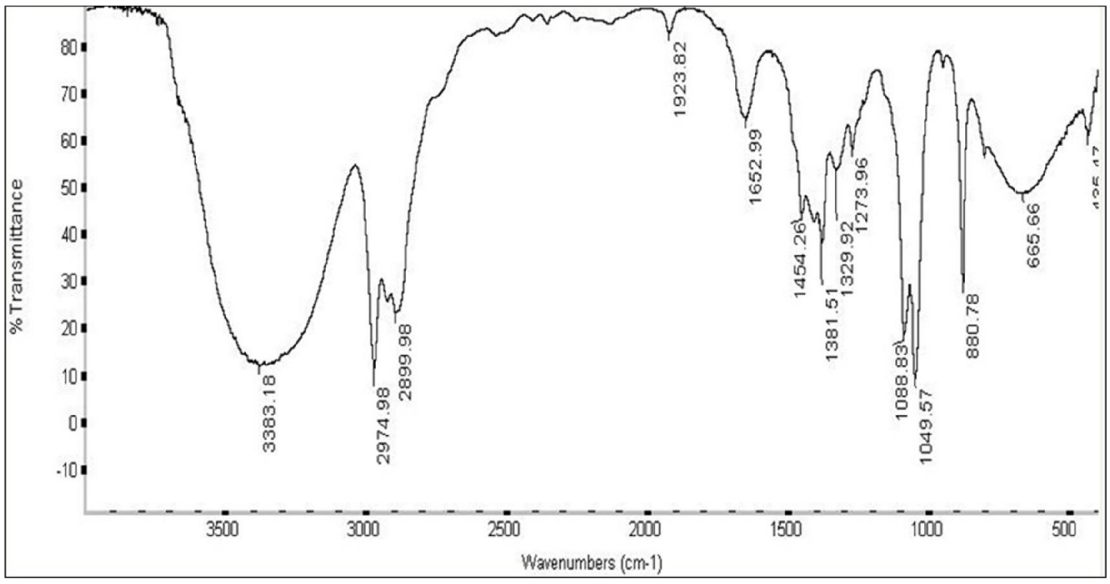

Fig. 1: FTIR spectrum of various functional groups of the phenolic compound P1isolated from pomegranate peels crude juice

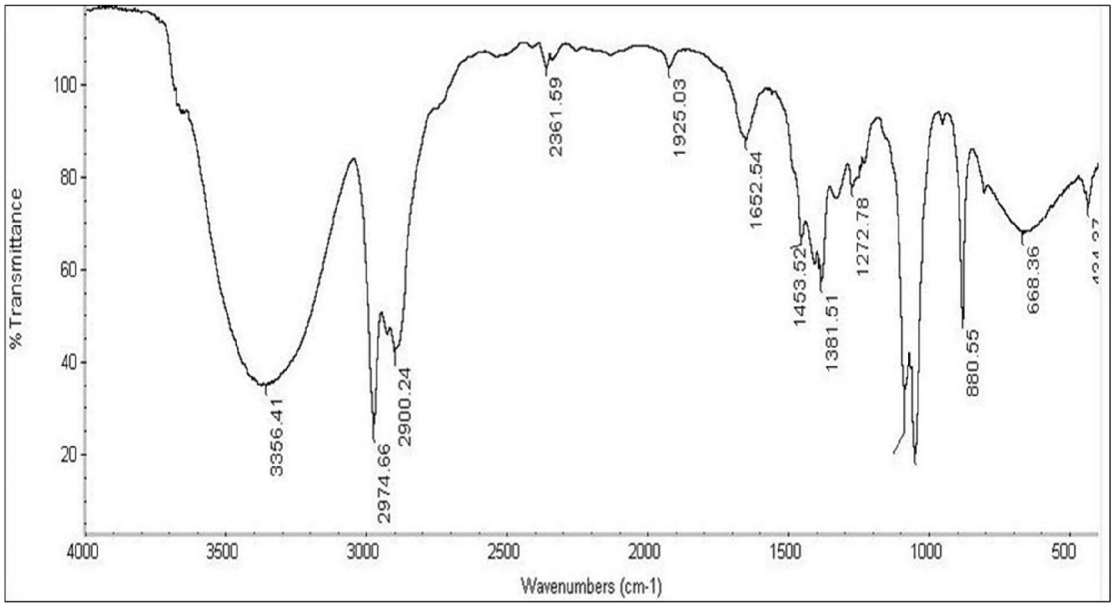

Fig. 2: FTIR spectrum of various functional groups of the phenolic compound P_2 isolated from pomegranate peels crude juice

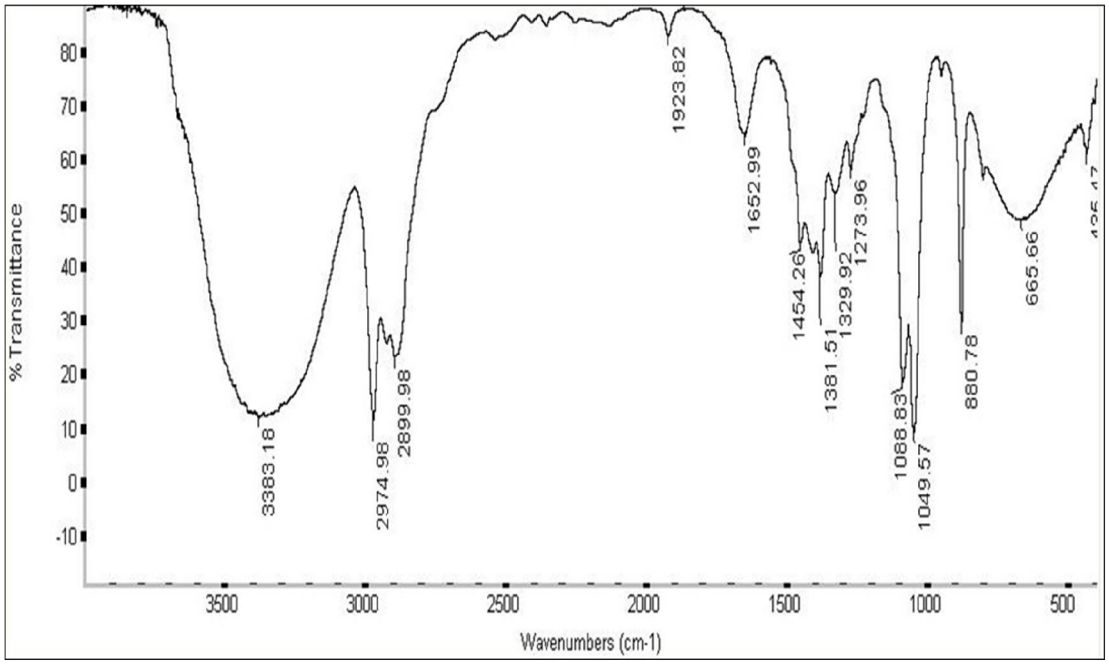

Fig. 3: FTIR spectrum of various functional groups of the phenolic compound P_1isolated from pomegranate peels crude juice

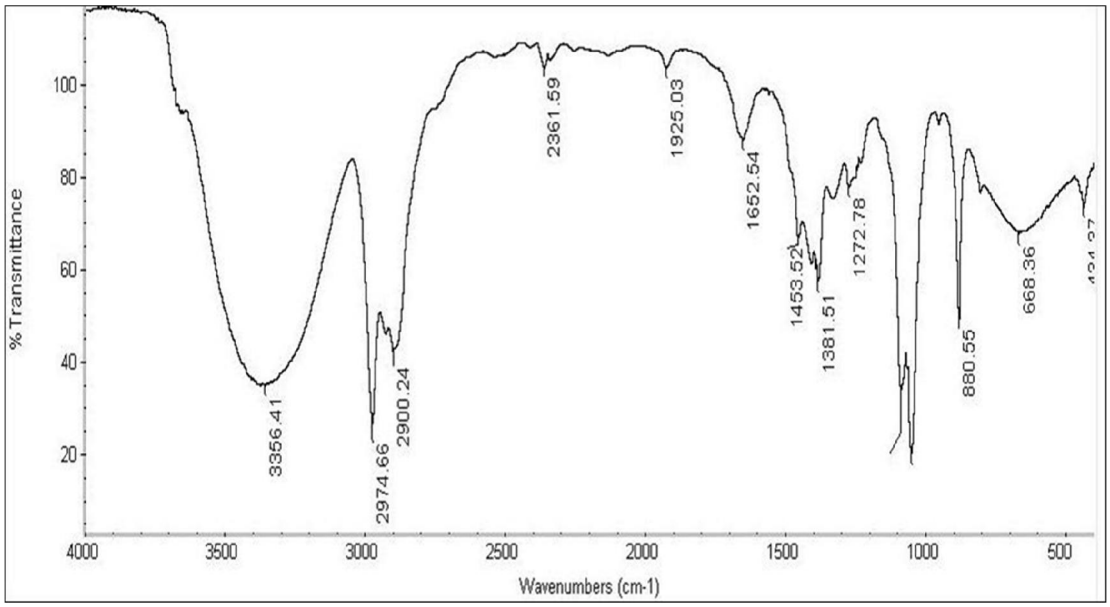

Fig. 4: FTIR spectrum of various functional groups of the phenolic compound P_4 isolated from pomegranate peels crude juice

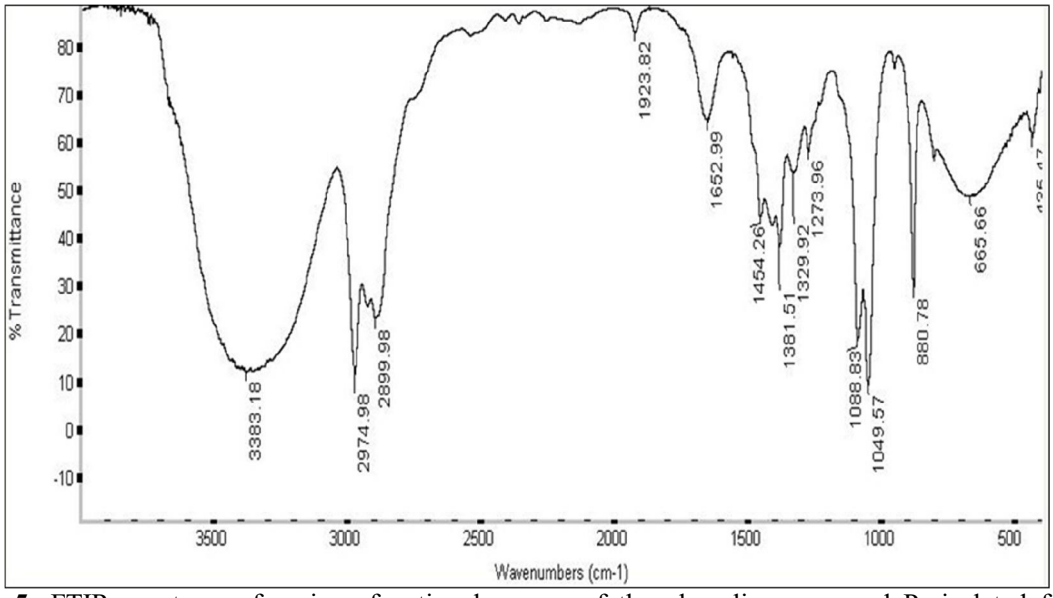

Fig 5: FTIR spectrum of various functional groups of the phenolic compound P_5 isolated from pomegranate peels crude juice

3.2. GC-MS/MS assay

3.2.1. Antioxidant activity of fractionated phenolic compounds

Antioxidants protect the body from the injurious by the action of free radicals generated as byproducts of normal metabolism. A vital role played by Antioxidants in preventing pathogenic processes associated with cardiovascular disease, cancer, respiratory disorder, macular degeneration, and cataracts, and are able to enhance the immune system (Nakilcioğlu and Hışıl, 2013).

Plants are rich in Natural antioxidants like flavonoids, tannins, phenolics, terpenoids, curcuminoids, coumarins, xanthones, and lignans are found in different plant products (Farag *et al*., 2003; Jeong *et al*., 2004). The authors are renowned to shield components of food that are capable of oxidizing easily from oxidation. This effect differs extensively relying on the increasing conditions, extraction process, and a multitude sides of the chemical structure of the active constituents, i.e., the amount, particle size, molecular weight, position of hydroxyl groups, the concentration of solvent, time of contact, temperature, and mass-solvent ratio, between others factors (Soto-Garcia *et al*., 2016).

Phenolic compounds possess free radical repression, metal inhibition, peroxide degradation, or oxygen suppression in biological systems besides blocking oxidative disease (Babbar *et al*., 2005). Thus, the recent work was intended to assess the antioxidant activity of fractionated phenolic compounds of pomegranate peels crude juice.

No individual method is adequate for evaluating the antioxidant capacity of foods, since diverse methods were able to yield quite varied findings (Huang *et al*., 2005). Several methods, based on various mechanisms must be applied. Therefore, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), reducing power assays, and metal chelating activity were applied.

Considering data in Fig.6, and table 2 indicate the maximum antioxidant activity by DPPH assay was recorded in P5 (vanillin) followed by P1 (gallic acid), P3 (quercetin), and P2 (ellagic acid) while it was the lowest in P4 (catechin). Comparable results gained from the reducing power assay, ellagic acid (P2) exhibits a better reducing power activity. A minor activity was observed for quercetin (P3). ellagic acid (P2) exhibited the strong potential to act as a metal chelator while gallic acid (P1) show a minor capacity for metal ion chelation.

Gallic acid, ellagi-tannins, and ellagic acid, are the most dependable for the antioxidant activity of pomegranate peels. Furthermore the ellagic acid was the most phenolic which plays a significantly important role in antioxidant activity (Al-Rawahi *et al*., 2014; Amjad and Shafighi, 2012). This acid can react with free radicals due to its ability to chelate with metal cations, a potent oxidant against lipid peroxidation in mitochondrion and microsome.

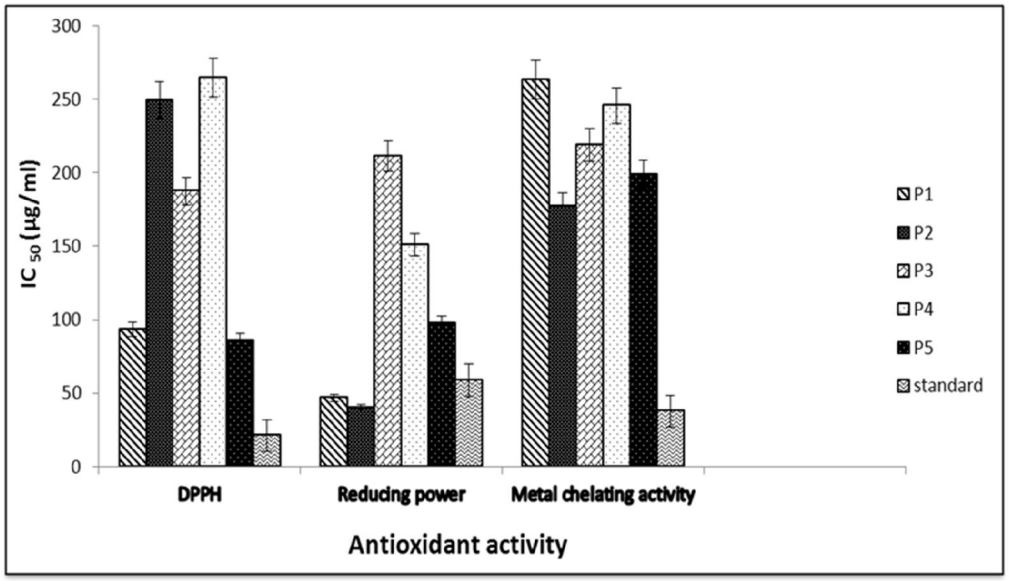

Fig. 6: The antioxidant activity of phenolic fractions isolated from pomegranate crude juice. P1, P2, P3, P4, and P5 refer to gallic acid, ellagic acid, quercetin, catechin, and vanillin, respectively.

Table 2: Retention times and area % of polyphenolic compounds fractionated from pomegranate peels crude juice obtained by GC-MS/MS.

Sample no.	RT (min)	Compound	Molecular weight (g/mol)	Molecular formula
P1	14.35	**Gallic acid**	170.12	$C_7H_6O_5$
P2	7.91	**Ellagic acid**	302.197	$C_{14}H_6O_8$
P3	10.74	**Quercetin**	302.236	$C_{15}H_{10}O_7$
P4	8.12	**Catechin**	290.26	$C_{15}H_{14}O_6$
P5	12.37	**Vanillin**	152.15	$C_8H_8O_3$

3.2.2. Antibacterial activity of the fractionated polyphenols from pomegranate crude juice

Bioactive compounds can be obtained from different kinds of plant materials. These natural substances have gained attention in food research as possible growth inhibitors of foodborne pathogenic and spoilage bacteria (Tako *et al*., 2020).

The phenolic compounds can express their antimicrobial activity through different modes of action, e.g., by inhibition of biofilm formation, reduction of host ligand adhesion and neutralization of bacterial toxins, reducing the fluidity of the membrane, inhibiting the synthesis of nucleic acids and the cell wall or energy metabolism (Quideau *et al*., 2011 and Górniak *et al*., 2019).

The antioxidant properties are correlated to the number of hydroxyl groups in phenolic compounds. In addition, changes in the position of the hydroxyl group could play an important role in antimicrobial activity and the interactions with cell membrane structures (Takó *et al*., 2020).

Preliminary screening of the antibacterial activity of fractionated polyphenols from pomegranate crude juice was estimated using a good diffusion method against Gram-positive bacteria (e.g., *S. aureus* and *B. cereus*) and Gram-negative bacteria (e.g., *P. aeruginosa* and *E. coli*) by presence or absence of inhibition zone diameters. The larger the inhibition zone is the more susceptible bacteria to a particular plant's crude juice.

Table 3 revealed that P1 (gallic acid) and P2 (ellagic acid) exhibited significant inhibitory activity against all bacterial strains under study when compared to other phenolic compounds under study. It was observed that the maximum inhibition zone of P1 was against *P. aeruginosa* (13.03 ± 0.20mm) while the minimum inhibition zone was against *B. cereus*(8.9 ± 0.21mm). In the case of P2, the maximum inhibition zone was observed against *S. aureus* (16.9 ± 0.26 mm) while the minimum inhibition zone was against *B. cereus*(11± 0.23 mm) also. P3 (quercetin) exhibited antibacterial activity against *B. cereus* and *P. aeruginosa*only with inhibition zones 8.13 ± 0.09 mm and 9.07 ± 0.12 mm,

respectively, while P5 (vanillin) had bactericidal activity against only *P. aeruginosa* and *E. coli*. On the other hand, P4 (catechin) did not show any inhibition zone at all.

3.3. Minimum inhibitory concentration (MIC)

MIC was only assessed for P2 (ellagic acid) due to the highest efficacy that it presented against all the tested bacteria in comparison with the other phenolic compounds understudy in the aforementioned preliminary antibacterial activity.

The MIC of ellagic acid against Gram-negative bacteria (e.g., *P. aeruginosa* and *E. coli*) and Gram-positive bacteria (e.g., *S. aureus and B. cereus*) are presented in Fig 7. The data illustrate that the higher MIC (6.25µg/ml) was against *B. cereus* while the lowest MIC was against *S. aureus* (25µg/ml).

Table 3: Antibacterial activity of phenolic fractions isolated from pomegranate crude juice.

Tested bacteria	Zone of inhibition (mm)*					
	P1	P2	P3	P4	P5	Control (Gentamycin)
Gram-positive bacteria						
***Staphylococcus aureus* ATCC 25923**	12± 0.58	16.9± 0.26	NA	NA	NA	24 ± 0.231
2- *Bacillus cereus* RCMB 027 (1)	8.9± 0.21	11± 0.23	8.13 ± 0.09	NA	NA	25 ± 0.289
Gram-negative bacteria						
***Escherichia coli* ATCC 25922**	9.13± 0.09	11.27 ± 0.15	NA	NA	9.13 ± 0.08	30 ± 0.17
***Pseudomonas aeruginosa* ATCC 27853**	13.03 ± 0.20	15 ± 0.29	9.07 ± 0.12	NA	10.07 ±0.07	27 ± 0.231

***** The obtained results for a zone of inhibition (mm) represented the mean of triplicate determinations. Values are means of three replicates of each parameter ± standard error.P1, P2, P3, P4, and P5 refer to gallic acid, ellagic acid, quercetin, catechin, and vanillin, respectively.
NA refers to no activity.

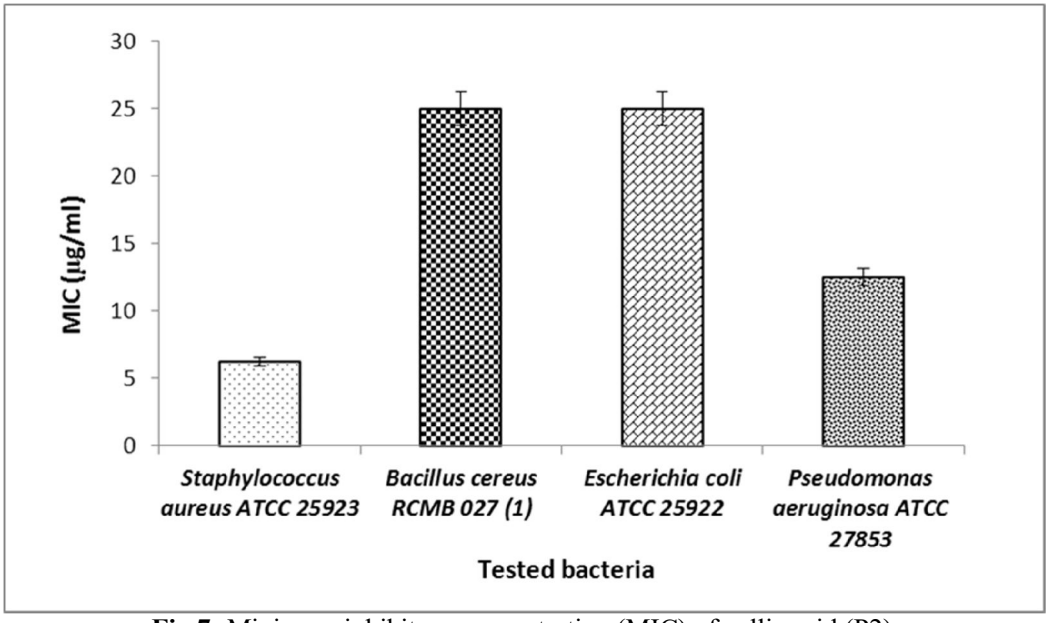

Fig.7: Minimum inhibitory concentration (MIC) of gallic acid (P2).

3.4. Anticancer activity of fractionated polyphenols from pomegranate crude juice

Cancer is vastly recognized for the time being as the disease of the century due to the numeral of rising cases worldwide. In addition, enormous studies consecrated to this domain and its rising rate of success (Hassanpour and Dehghani, 20172017).

Breast cancer is the second very prevalent cancer worldwide and the most common malignancy that takes place in women. It has markedly higher happening than any cancer else between disease in women (Del Pup and Peccatori, 2018). Also, breast cancer has spreading attributes and a high proportion of metastasis, which leads to a high disease and death rate (Acevedo-Diaz *et al*., 2019; Parvin *et al*., 2019).

Colorectal cancer represents about 10 % of whole yearly diagnosed cancers and deaths related to cancer throughout the world and deem as the second most very popular cancer diagnosed in women and the third most in men (Bray *et al*., 2018). It is the world's fourth most fatal cancer with nearly 900,000 deaths yearly (Dekker *et al*., 2019).

Phenols have a great history of offering benefits in preventing and curing chronic diseases such as cancer (Zhou *et al*., 2016). These components possess anticancer properties via several varied mechanisms of action, such as the inhibition of tumor cell growth, the induction of apoptosis, DNA damage, topoisomerases I and II inhibition, the stimulation of apoptosis, and others (Lichota *et al*., 2018).

Many *in vitro* and *in vivo* researches had indicated that the combining of naturalistic polyphenols with chemotherapeutics can enhance anticancer efficiency, lowering the bad effects of chemotherapy and getting over the chemo- or radio-resistivity of cancer cells (Fantini *et al*., 2015). Subsequently, the present study focused on the anticancer activity of pomegranate peels crude juices on breast adenocarcinoma (MCF-7) and human colon cancer (HCT-116) cell lines.

Considering the data in Figs. 8 and 9, P4 (catechin) treatment decreased the viability of MCF-7 breast adenocarcinoma cells and human colon cancer cells (HCT-116).

Numerous researchers noticed that Gallic acid prevents MCF-7 breast cancer cells. proliferation (IC_{50} = 80.5 µM), and stimulated apoptosis through the intrinsic and extrinsic pathways Ellagic acid exhibited anti-proliferation, and pro-apoptotic influences on colon cancer cell lines in a concentration-dependent way (Yousef *et al*., 2016).

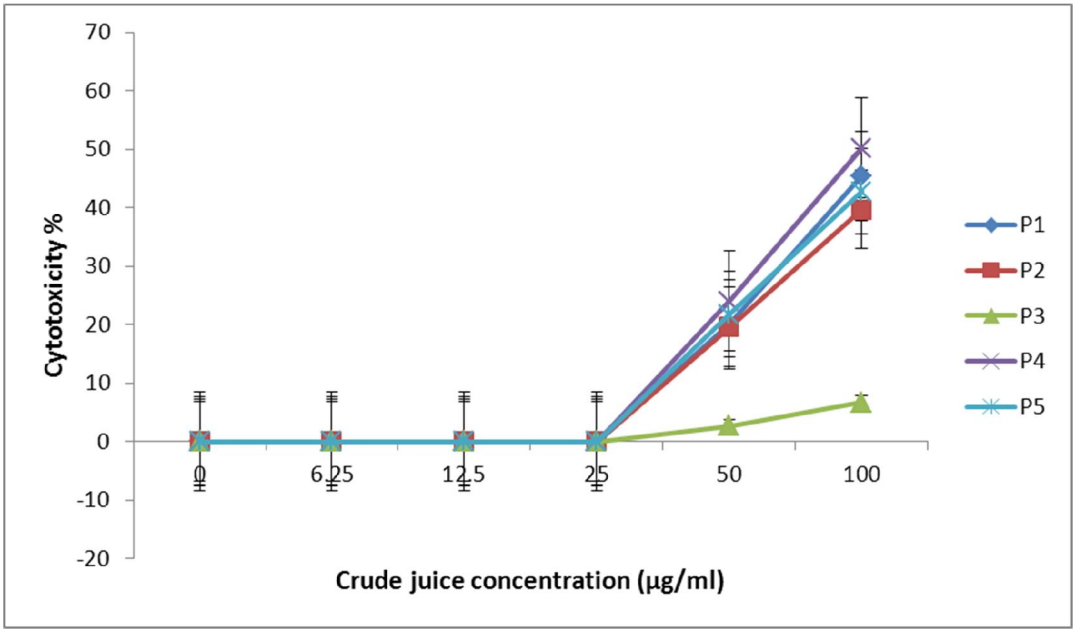

Fig. 8: Cytotoxicity effects of different concentrations phenolic fractions isolated from pomegranate crude juice on human colon cancer cell line (HCT-116).

P1, P2, P3, P4, and P5 refer to gallic acid, ellagic acid, quercetin, catechin, and vanillin, respectively.Values are means ± standard error.

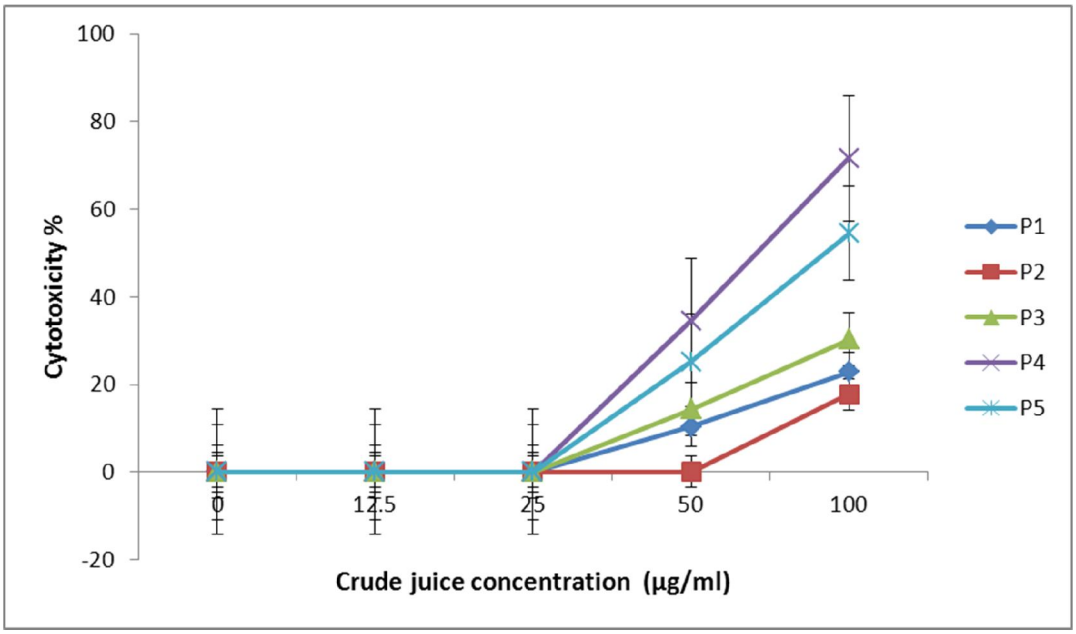

Fig. 9: Cytotoxicity effects of different concentrations of phenolic fractions isolated from pomegranate crude juice human breast adenocarcinoma cell line (MCF-7).
MCF-7 refers to Michigan Cancer Foundation-7 (the institute in Detroit where the cell line was established in (1973). P1, P2, P3, P4, and P5 refer to gallic acid, ellagic acid, quercetin, catechin, and vanillin, respectively. Values are means of three replicates of each parameter ± standard error.

3.5. Cytotoxicity effects of the fractionated polyphenols from pomegranate crude juice

The cytotoxic effects of the fractionated polyphenols from pomegranate crude juice at different concentrations were estimated on skin fibroblast (BJ1) normal cell line of the aforementioned phenolic compounds as presented in Fig. 10. The data revealed no cytotoxic effects on the viability of the skin fibroblast (BJ1) normal cell line at the concentrations used in the present study.

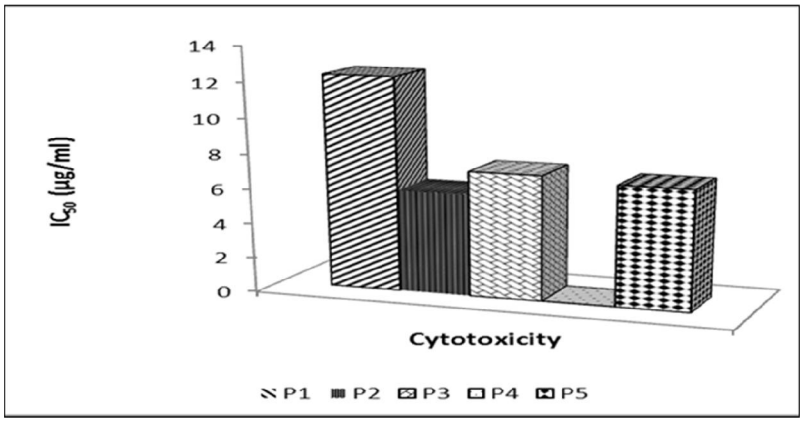

Fig. 10: Cytotoxicity effects of phenolic fractions isolated from pomegranate crude juice were tested against skin fibroblast (BJ1) normal cell line.
P1, P2, P3, P4, and P5 refer to gallic acid, ellagic acid, quercetin, catechin, and vanillin, respectively.

4. Conclusion

Briefly, this study highlights the benefit of non-edible part of pomegranate "pomegranate peel" which act as safety and effectiveness promising natural drugs. Our current study achieved that pomegranate peel extract able to treat some chronic diseases such as breast cancer, prostate cancer, skin cancer and stomach cancer. The fractionated phenols from pomegranate peels indicate no cytotoxicity effect. The survival of human colon cancer cells (HCT-116) and MCF-7 breast adenocarcinoma cells

was decreased by catechin. The results indicated that pomegranate peels has antioxidant, antibacterial and anticancer potentials.

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Conflict of interest

No conflict of interest was affirmed by the authors.

Ethical statement

The research does not contain any studies with human participants, animal studies, biosecurity, or clinical trials that require ethical approval.

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