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Phytochemical screening and antioxidant activity of pomegranate peels and leaves of peach

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Abstract

Pomegranate (*Punica granatum* L.) is a nutrient-dense food rich in beneficial phytochemicals. The aim of this study is to investigate the antioxidant activity, total phenolic, and flavonoids content and compared them with the antioxidant activity of Peach leaves. The phytochemical screening was assessed by using different extracts: ethanol, butanol, and ethyl acetate. The total phenolic content was determined spectrophotometrically by Folin-Ciocalteu's method and the total flavonoid content were also determined by using the aluminum chloride complex formation assay. Results showed that the amount of total phenolic and flavonoid content in Pomegranate was higher than those in peach's leaves. They ranged from 0.352 to 1.35µg/ml and varied from 0.223 to 0.701µg/ml respectively. Also, the extracts of Pomegranate possessed stronger antioxidant activity than extracts of leaves of peach in radical scavenging activity by DPPH' where the DPPH' radical scavenging activity ranged from 18.5% to 45% in ethyl acetate extract of Pomegranate.

Keywords: Phytochemistry, pomegranate, leaves of peach, polyphenols, folin-ciocalteu and DPPH

1. Introduction

Free radicals are produced naturally under aerobic conditions and are part of normal physiological processes, but excess free radicals can damage all cellular macromolecules including proteins, carbohydrates, lipids, and nucleic acids. The free radicals initiate reactions such as DNA oxidation which can ultimately cause genetic material mutations and possibly cancer [1]. One type of endogenous damage is that arising from oxygen-free radicals (OFR) reduction intermediates, this disorder generates the oxidative stress that affects not only the bases but also the deoxyribosyl backbone of DNA through redox imbalance. OFR are also known to attack other cellular elements, such as lipids, leaving reactive species behind that in turn can couple to DNA bases [2].

Current therapeutic work is aimed at finding naturally occurring antioxidants, particularly those of plant origin. Many species of plants have recorded possessing potential biomolecules to become drug source [3]. Plants are capable of synthesizing compounds with complex structures that are known as secondary metabolites, such as terpenoids, flavonoids, alkaloids, quinones, polyphenols, such metabolites that are called natural products are used in the fields with medicinal chemistry and pharmacognosy and are already recognized as having potential activities to cure various forms of disease. Such plants with curative constituents for number of listed diseases are termed as Medicinal plants [4].

Pomegranate peels contain a number of different phytochemical components include alkaloids "caffeine", anthocyanidins "cyanidin", ellagic acid, flavonoids "catechin, Epicatechin, and luteolin", terpenes and terpenoids " punicaone, lupeol, betulin " these constituents of peels have (lipophilic terpenes such aspunicaone, as well as hydrophilic derivatives of flavonoids like catechin) that could be effective antioxidant to scavenge free radicals in both, lipophilic parts as well as aqueous fluids of the body [5, 6].

In general, the reviewed studies point out the use of pomegranate and its constituents as dietary supplements or as adjuvants in vascular diseases treatment, such as hypertension, coronary artery disease, and peripheral artery disease. Also, use as adjuvant therapy for the treatment of several forms of oncological diseases, particularly Alzheimer's disease in the prostate cancer [5].

Single components (e.g., punicalin, punicalagin, ellagic acid, and gallic acid) isolated from pomegranate fruit suppressed the development of advanced glycation end products (AGEs, known to contribute to a number of diseases including diabetic complications and arteriosclerosis) from bovine serum albumin and sugar *in vitro* antiglycation assay [7].

2. Materials and methods

2.1 Plant materials

Peels of pomegranate were harvested in September, 2016 from pomegranate trees while leaves of peach were harvested in July, 2016 from peaches tree. The samples of Peels and leaves were taken from different trees of commercial Elbyda province (Eastern of Libya 36-39°C). Samples were collected, sun-dried and powdered. The powder of Peel and leaves was extracted by hot continuous extraction, Soxhletion process was used for the extraction of the plant material. Peels and leaves 10 g were extracted by 150 ml of the following solvents: ethanol, butanol and ethyl acetate. The extracts were filtered through Whatman No.1 filter paper for removing of particles. Then each extract has been used to prepare 500 µg/ml for assays.

2.2 Chemicals

1,1-Diphenylpicrylhydrazyl (DPPH) was obtained from Sigma chemicals, ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid, sodium carbonate, and pyrogallol, were obtained from ALPHA Chemical Co.

2.3 Determination of total phenolic content (TPC)

TPC were estimated by the Folin-Ciocalteu method reported in^[8]. From each extract (100,200,300,400,500 µg/ml) was diluted by 2ml of distilled water followed by "600 µl" of Folin-Ciocalteu reagent. The tubes were allowed to stand for 5 min. before 2 ml of 20% Na₂CO₃ were added. All tubes were incubated for 1 minute in a boiling water bath after cooling the blue colour was formed. The reading of the absorbance was made at 765 nm using a double beam uv-vis spectrophotometer. Quantification was done with respect to stander calibration curve of pyrogallol the results were expressed as pyrogallol "µg/ml".

2.4 Determination of total flavonoids content (TFC)

The amount of TFC in the extracts was measured spectrophotometrically following the method^[9]. This method was based on the formation of a complex flavonoid aluminum, having the maximum absorbance at 415 nm. (100,200,300,400,500 µg/ml) of extracts mixed with 0.1 ml of 10% aluminum chloride and 0.1 ml of 1 M potassium acetate followed by 2.8 ml of distilled water. The tubes kept at room temperature for 30 min. the absorbance of the complex was measured at 415 nm using a double beam uv-vis spectrophotometer. Quercetin solution in methanol at concentrations (100 to 500 µg/ml) was used to make a calibration curve.

2.5 Reducing power assay (RPA)

Substances, which have reduction potential, react with potassium ferricyanide (Fe³⁺) to form Potassium ferrocyanide (Fe²⁺), which then react with ferric chloride to a form ferric ferrous

complex that has an absorption maximum at 700nm. This experiment was carried out asdescribed by Baba SA, *et al.*^[10]. Extract solution of (100,200,300,400,500 µg/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide then mixture was incubated in a water bath at 50 °C for 20 minutes. 2.5 ml of trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 minutes. Finally 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 1 ml FeCl₃. The absorbance of prussian blue colour was measured at 700 nm. Quantification was done with respect to the standard calibration curve of ascorbic acid the results were expressed as ascorbic acid (µg/ml).

2.6 DPPH free radical scavenging activity (RSA)

The scavenging activity on DPPH[•] radical of different extracts was determined following the method reported by Park HR *et al.*^[11]. Stock solution of DPPH[•] (0.2 mM) in methanol was prepared. The DPPH scavenging activity of extracts was estimated by mixing (100, 200,300, 400, 500 µg/ml) of extracts with 2 ml of 0.2 mM DPPH in methanol. The mixture was kept in darkness at room temperature for 30 minutes. The absorbance of the sample was measured at 517 nm by UV-visible spectrophotometer. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula:-

$$\% \text{DPPH "RSA"} = [\text{Abs. of Control} - \text{Abs. of Sample} / \text{Abs. of Control}] \times 100$$

3. Results and discussions

3.1 Total phenolic content

It has been recognized that phytochemicals that occur in food and natural health items play an important role in the prevention and promotion of diseases. Bioactivities in herbal and natural products constitute an abundance of chemical compounds, among which phenolic substances often play a primary or a synergistic function. Phenolic compounds are known to act as antioxidants not only because they donate hydrogen or electrons but also they are stable radical intermediates, that prevent oxidation of various food ingredients^[12]. Table 1 shows the phenolic content of different extracts of punica and leaves of peach in this study. The total phenolic content was determined by using the Folin-Ciocalteu reagent where the blue color formed according to reduction of Molybdenum "Mo" where Mo(VI) reducing to Mo(V) by accepting an electron from reducer (i.e. antioxidant)^[13].

Color intensity increases with the increasing concentration of punica and leaves of peach which are considered to be an indicator to increase the total phenolic content and finally reflect its antioxidant activity. Were the punica has more phenolic content than the leaves of peach and the ethyl acetate extract was extract in both plants contain phenolic content. The results expressed according to pyrogallol as a standard phenolic compound.

Table 1: Total phenolic content of Punica and leaves of peach.

Concentration (µg/ml)	Absorbance of extracts						
	Pyrogallol	Ethanol		Butanol		Ethyl acetate	
		Punica	Leaves of peach	Punica	Leaves of peach	Punica	Leaves of peach
100	0.438 ± 0.024	0.271 ± 0.052	0.222 ± 0.022	0.293 ± 0.023	0.253 ± 0.073	0.352 ± 0.046	0.302 ± 0.026
200	0.725 ± 0.037	0.523 ± 0.042	0.543 ± 0.072	0.532 ± 0.032	0.502 ± 0.022	0.612 ± 0.062	0.572 ± 0.032
300	1.070 ± 0.021	0.802 ± 0.046	0.752 ± 0.026	0.812 ± 0.031	0.772 ± 0.051	0.843 ± 0.052	0.821 ± 0.051
400	1.307 ± 0.019	1.05 ± 0.052	0.924 ± 0.032	1.12 ± 0.051	1.02 ± 0.091	1.19 ± 0.034	1.15 ± 0.065
500	1.564 ± 0.027	1.21 ± 0.044	1.15 ± 0.084	1.28 ± 0.047	1.20 ± 0.027	1.35 ± 0.066	1.35 ± 0.029

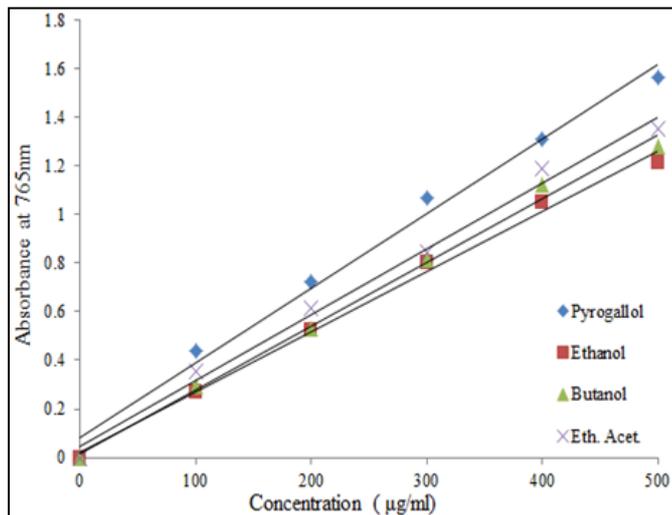


Fig 1: Total phenolic content of peels

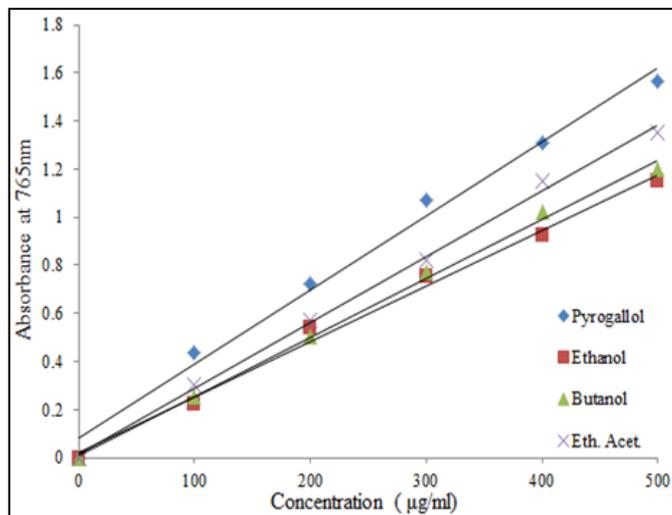


Fig 2: Total phenolic content of leaves

3.2 Total flavonoid content

Polyphenols are the principal compounds of plants with high levels of antioxidant activity. Such behavior may be attributed to their capacity to absorb and neutralize free radicals and to quench them. Their capacity as free radical scavenger could also be due to their redox properties, the existence of conjugated ring structures and the carboxylic group that have been documented to inhibit lipid peroxidation [14].

The procedure used for the quantification of flavonoids is based on the reaction between the flavonoids and aluminum chloride forming a yellow colored complex which can be measured by spectrophotometer at a wavelength of 415 nm [15]. The colorimetric aluminum chloride procedure was used to evaluate the flavonoids. The calibration curve was prepared at concentrations of 100 to 500 µg/ml with quercetin solution in methanol. The spectrophotometric study based on the complex formation of aluminum chloride which is one of the

most commonly applied analytical procedures for determining the content of flavonoids.

Aluminum chloride forms labile acid complexes with ortho-dihydroxyl groups in the flavonoid rings A or B. The C₅ hydroxyl group and the ortho dihydroxyl groups in the B ring formed a complex with strong absorption at 415 nm [16].

Based on the absorbance values of the different concentrations, and compared to the standard solution of quercetin results obtained in this study, the level of flavonoid in Punica and leaves of peach was shown in Table 2 to be relatively lower than that of phenolic content. The compounds of high molecular weight might be as important as the monomer flavanols such as catechin, which have demonstrated high antioxidant potential in phenolic compounds furthermore the antioxidant activity of a sample could have a synergic effect among several compositions, rather than a single compound.

Table 2: Total Flavonoid content of Punica and leaves of peach.

Concentration (µg/ml)	Extracts						
	Quercetin	Ethanol		Butanol		Ethyl acetate	
		Punica	Leaves of Peach	Punica	Leaves of Peach	Punica	Leaves of Peach
100	0.307 ± 0.074	0.181 ± 0.065	0.103 ± 0.075	0.215 ± 0.083	0.126 ± 0.027	0.223 ± 0.022	0.164 ± 0.056
200	0.612 ± 0.027	0.302 ± 0.087	0.188 ± 0.094	0.334 ± 0.053	0.208 ± 0.031	0.382 ± 0.074	0.252 ± 0.032
300	0.954 ± 0.077	0.382 ± 0.033	0.237 ± 0.022	0.427 ± 0.023	0.338 ± 0.097	0.512 ± 0.083	0.375 ± 0.072
400	1.203 ± 0.082	0.501 ± 0.071	0.319 ± 0.083	0.553 ± 0.095	0.456 ± 0.053	0.621 ± 0.053	0.497 ± 0.035
500	1.511 ± 0.033	0.619 ± 0.083	0.439 ± 0.024	0.672 ± 0.056	0.574 ± 0.092	0.701 ± 0.026	0.627 ± 0.021

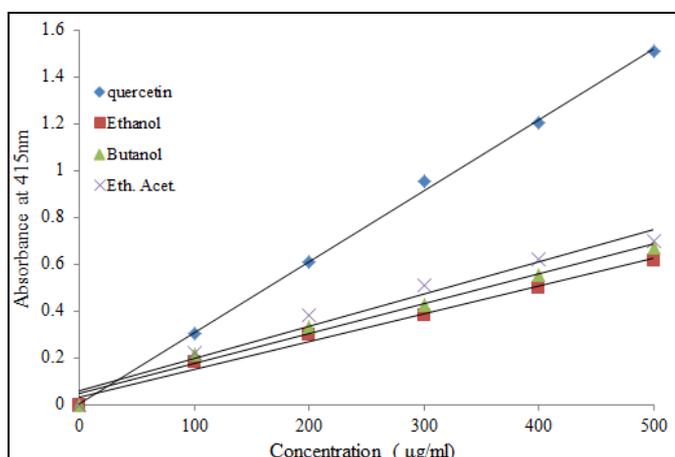


Fig 3: Total flavonoid content of peels

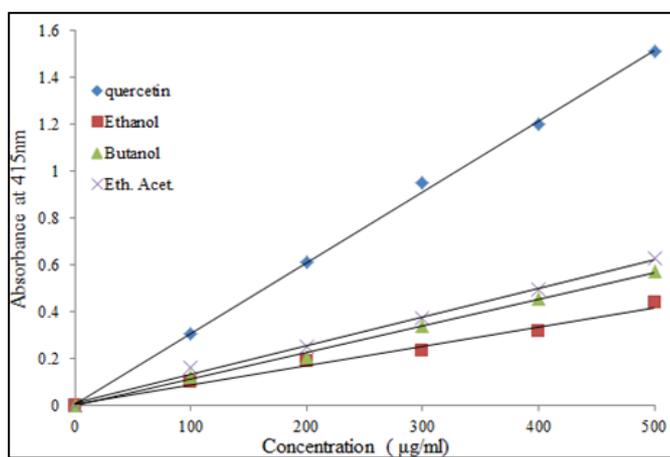


Fig 4: Total flavonoid content of leaves

3.3 Reducing power assay

Antioxidant activity is consistent with the reduction force of bioactive compounds. Therefore, it is necessary to determine the reduction power of phenolic constituents in order to clarify the relationship between their antioxidant effects and their reduction power [17]. Table 3 shows the reduction capacity of ferric chloride in peel of punica and leaves peach extract.

The reductive potential was determined by changing ferric Fe⁺³ oxidation states to ferrous Fe⁺². Where the test solution changes to prussian blue. The intensity of the color increase with increasing of the amount extracts was measured at 700nm by uv-vis-spectrophotometer [18]. The result obtained showed that the extracts had a concentration-dependent antioxidant activity which reflects its reduction capability, the results expressed according to ascorbic acid as a standard.

Table 3: Reducing power assay of Punica and leaves of peach.

Concentration (µg/ml)	Extracts						
	Vitamin C	Ethanol		Butanol		Ethyl acetate	
		Punica	Leaves of Peach	Punica	Leaves of Peach	Punica	Leaves of Peach
100	0.468 ± 0.044	0.291 ± 0.025	0.242 ± 0.015	0.298 ± 0.033	0.281 ± 0.067	0.373 ± 0.044	0.312 ± 0.033
200	0.712 ± 0.067	0.477 ± 0.044	0.452 ± 0.074	0.505 ± 0.062	0.487 ± 0.071	0.618 ± 0.029	0.593 ± 0.092
300	0.972 ± 0.027	0.693 ± 0.082	0.601 ± 0.072	0.701 ± 0.043	0.692 ± 0.077	0.735 ± 0.059	0.771 ± 0.042
400	1.22 ± 0.062	0.841 ± 0.033	0.802 ± 0.023	0.871 ± 0.066	0.853 ± 0.083	0.948 ± 0.028	0.983 ± 0.055
500	1.51 ± 0.073	1.08 ± 0.062	1.05 ± 0.084	1.12 ± 0.045	1.09 ± 0.062	1.21 ± 0.055	1.26 ± 0.061

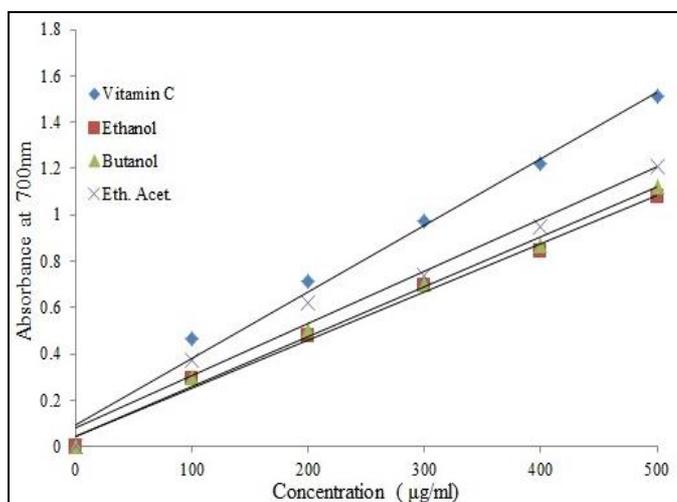


Fig 5: Reducing power of peels

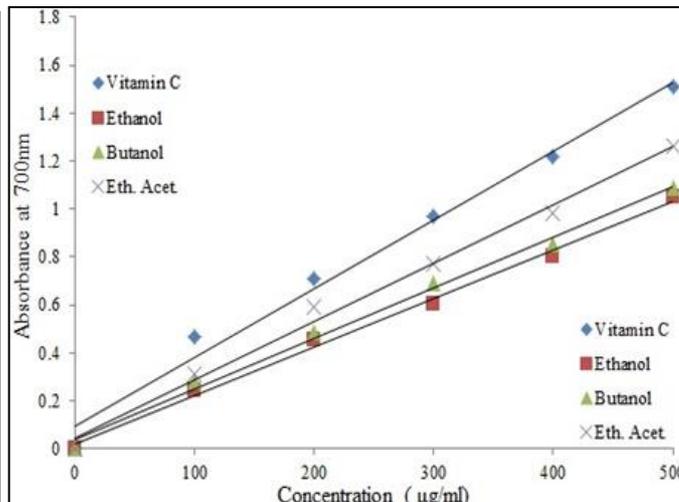


Fig 6: Reducing power of leaves

3.4. DPPH radical scavenging

Reactivity of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) is a popular method of screening the free radical scavenging capacity of compounds or the antioxidant activity of plant extracts and has been widely used as a free radical for the assessment of reduction substances [19]. Table 4 indicates the percentage of inhibition of DPPH radical scavenging by using the extracts of Punica and leaves of peach. DPPH is a free radical, stable at room temperature, which produces a purple solution in methanol, where the absorbance measured at 517nm. DPPH's purple color altered to the yellow methanolic solution that causes a decrease in DPPH absorption when reacting with the plant extracts. The lower absorbance at

517nm indicates a greater activity of radical scavenging and finally, its antioxidant activity may provide us strong facts about its radicals scavenging. The percentage of radical scavenging activity calculate from the equation below.

$$[\% \text{ RSA} = [\text{Abs. of Control} - \text{Abs. of Sample} / \text{Abs. of Control}] \times 100]$$

According to the above equation, the percentage of DPPH radical scavenging operation of Punica ranged from 18.5 percent at 100 µg / ml to 45 percent at 500 µg / ml in Punica's ethyl acetate extract, its radical scavenging ranged from 14 percent at 100 µg / ml to 44 percent at 500 µg / ml in Punica as well as in peach leaf extracts.

Table 4: Percent of DPPH radical inhibition by of Punica and leaves of peach

Concentration (µg/ml)	Extracts						
	Vitamin C	Ethanol		Butanol		Ethyl acetate	
		Punica	Leaves of Peach	Punica	Leaves of Peach	Punica	Leaves of Peach
100	27%	12.6%	11%	13.4%	12%	15.5%	14%
200	40%	20%	17.5%	21.3%	19.5%	26.4%	20.4%
300	61%	28.6%	26.4%	30%	28%	36%	30%
400	75%	35%	32%	36%	33%	45%	35.7%
500	87%	43%	41.5%	43%	42.4%	53%	44%

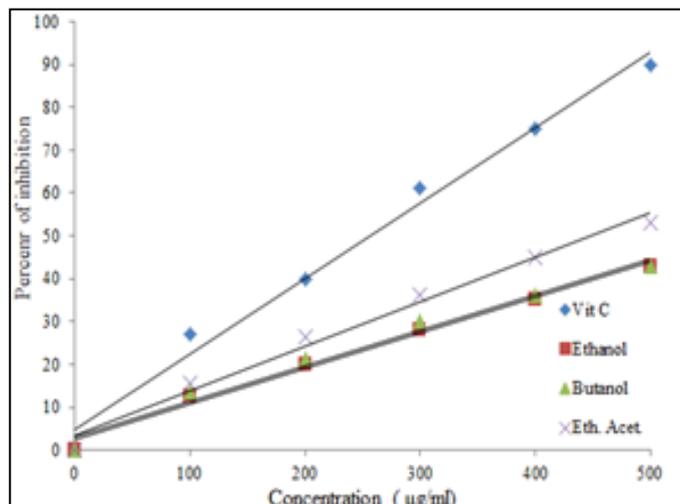


Fig 7: DPPH scavenging activity of peels

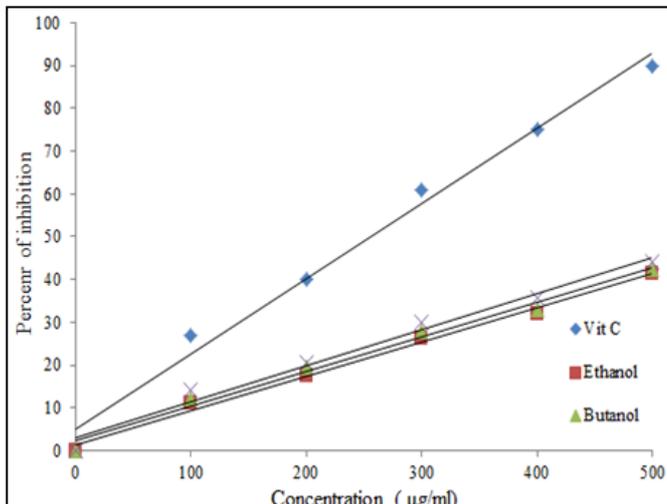


Fig 8: DPPH scavenging activity of leaves

4. Conclusion

In conclusion, the presence of phenols, polyphenols, and flavonoid compounds has appeared in the phytochemical screening of various pomegranate extracts and leaves of peach; these compounds have biological and pharmacological activities. So that might be employed these compounds for the development of traditional medicines which may be created a new way to treat many incurable diseases.

5. Conflict of interests

There are no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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