

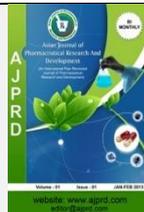
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Research Article

Antimicrobial and Antioxidant Activity of Pomegranate Peel Extracts Obtained by Sequential Extraction Method

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ABSTRACT

Punica granatum L. (Pomegranate) is a plant belongs to Lythraceae family. The objective of this study was to evaluate the antimicrobial activity of petroleum ether, chloroform, acetone, ethyl acetate and ethanol extracts from the peel fruit of *Punica granatum* against standard microorganism. This plant has been used as a traditional treatment for several diseases such as microbial infections. Extracts were evaluated for their effectiveness against four bacterial strains including both Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria as well as fungal species (*Candida albicans* and *Aspergillus niger*) using disc diffusion method. The chloroform, petroleum ether and ethyl acetate extracts showed higher antibacterial activity than the acetone and ethanol extracts with inhibition zone ranged between (7-16 mm). The petroleum ether and chloroform extracts showed the higher antifungal activity compared with the other extracts, the diameter of inhibition zone ranged between (11-16 mm). The antioxidant potential of peel extracts was determined on the basis of their scavenging activity of the stable 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) free radical and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS). All extracts showed high antioxidant activity. The highest result of antioxidant activity by DPPH scavenging assay was in acetone extract (89.40%), but high result by ABTS in ethanol extract (72.07%).

KEYWORDS: *Punica granatum* L. peel, antibacterial activity, antifungal, antioxidant activity, sequential extraction

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INTRODUCTION

Plants are known as natural source of many bioactive compounds, which provide desirable health benefits beyond basic nutrition¹. Medicinal plants are a good source for preparation of modern pharmaceuticals and novel therapeutic agents as alternative medicines over traditional drug regimens^{2, 3}. Due to their minor effects and low cost, the medicinal natural products are very frequently used in developing countries such as Sudan to treat various infectious human diseases⁴.

The pharmacological activities of plants are attributed to presence of polyphenols⁵. The phenolic compounds are defined as secondary metabolites which are nonnutritive and not involved in the plant's growth and reproduction⁶. The phenolic compounds from medicinal herbs include

phenolic acids, flavonoids, tannins. Various bioactivities of phenolic compounds are responsible for their therapeutic properties (e.g., antioxidant, anticarcinogenic, or antimutagenic, antimicrobial, antifungal and anti-inflammatory)^{7, 8}.

Punica granatum L. commonly called pomegranate and belongs to the family *Punicaceae* is an important fruit of tropical and subtropical regions⁹. The pomegranate's fruit is one of the oldest edible fruits that has achieved great interest due to its increased medicinal benefits in the last years¹⁰. All parts of pomegranate's fruit are excellent sources of bioactive compounds and have been used in folk medicine for treating a variety of illness¹¹. Pomegranate peel are the most abundant part in the fruit with bioactive compounds and have stronger biological activities than the juice¹².

Pomegranate fruits peels are inedible part and they are byproduct of juice processing industry¹³. A various bioactive compounds with antioxidant activity have been identified in pomegranate peel such as organic and phenolic acid, flavonoids, sterols, triterpenoids, alkaloids and tannins^{6,14}. The pomegranate peel has been reported to have antibacterial, anti-inflammatory, anti-mutagenic, antiviral and antifungal activity^{15,16}. Pomegranate peel are known as a popular folk medicine throughout the world due to their strong astringency^{17,18}. The therapeutic properties of pomegranate peel include healing wounds, curing diarrhea, antimalarial, anti-parasitic agent, blood tonic, remedy for diabetes, cardioprotective, dental conditions, treatment male infertility and anti-hypertensive^{6,12,19}.

Herbal drugs could be used to formulate new antimicrobial drugs to overcome the problem of resistant of available synthetic antibiotics²⁰. Studies reported that, pomegranate peel extracts have antimicrobial activity against some human pathogen so they might be used as alternative to antibiotics in microbial therapies²¹.

The extraction methods of plants and type of extracting solvents have a great effect on the bioactive properties of the extracts due to difference in polarity of active compounds²². The sequential extraction technique is a common method for the extraction of active components from natural sources as the sequential extraction procedure involves different solvents of various polarities that can provide optimum effect of extraction and better activity than direct solvent extraction²³. Several studies revealed that phenolic content differ with extractor solvent polarities, hence current study was aimed to evaluate the effect sequential extraction using five solvents with different polarities on the antioxidant, antimicrobial and antifungal activities of peel of pomegranate.

MATERIALS AND METHODS

Chemicals standards and reagents

Ascorbic acid (98.7%) was purchase from Supelco (Bellefonte, USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) were from Sigma Chemical Company (St. Louis, MO, USA). Potassium persulfate (98%) and dimethyl sulphoxide were obtained from SDFCL (Mumbai, India). Petroleum ether (60-80 °C, 95%) was from CDH (New Delhi, India). Chloroform (99.5%) was from Romil (Essex, UK). Ethyl acetate (99.5%) was purchase from LOBA Chemi (Mumbai, India). Acetone (99%) was from LabTech Chemicals (India). Ethanol (99.8%) was obtained from Duksan (Sinwonro, Korea).

Pomegranate peel sample

Ripe fruits were collected local markets available in Khartoum. The pomegranates were peeled manually, the peels were air dried at room temperature (about 30 °C) for approximately 5 days. When dried, the material was ground to a coarse powder using a pestle and mortar and stored in a clean container ready for analysis until extraction.

Instrumentation

The instrument used for determination of antioxidant activity was a Ripe fruits were collected from local markets available in Khartoum. SPECROstar Nano from BMG LabTech (Ortenberg, Germany).

Extraction of plant materials

Sequential extraction method was employed to extract the plant powder using different organic solvents from low polar to high polar namely petroleum ether, chloroform, acetone, ethyl acetate and ethanol. An aliquot of 60 grams of powder was soaked in 200 mL of petroleum ether in closed conical flask and kept on an electric shaker for three days. The supernatant was filtered using Whatman filter paper No.1 filter paper. This procedure was repeated twice to ensure the complete separation of all constituents. All filtrates have been combined in one flask. Petroleum ether was evaporated under reduced pressure by using rotatory evaporator and dried extract was stored in room temperature until used. The remaining sample was allowed to air dry and used for chloroform, acetone, ethyl acetate and ethanol extraction, respectively similar to the procedure carried out for petroleum ether.

Test strains and culture media

Standard strains of microorganism were used in this study and were obtained from Medicinal and Aromatic Institute of Research, National Research Center, Khartoum. The bacterial species used were the Gram-negative bacteria; *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and the Gram-positive bacteria; *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923). Fungal species were *Candida albicans* (ATCC 7596) and *Aspergillus niger* (ATCC 9763). Bacteria were grown in Mueller Hinton Agar and fungi were grown in Sabouraud Dextrose Agar. The concentration of bacterial suspensions were adjusted to 10⁸ cells mL⁻¹, and that of fungal suspensions to 10⁷ cells mL⁻¹.

Preparation of culture media

Preparation of Mueller Hinton agar

Thirty eight gram of the powder of Mueller Hinton agar was weighed, dissolved in 1.0 L of distilled water and allowed to soak for 10 minutes. The medium was placed in water bath to dissolve, swirled to mix and sterilized by autoclaving for 15 min at 121°C, cooled to 47 °C and mixed well then poured into sterile Petri dishes.

Preparation Sabouraud Dextrose agar

Sixty two gram of the powdered of Sabouraud dextrose agar, was weighed, dispersed in 1.0 L of water and allowed to soak for 10 min, swirled to mix then sterilized by autoclaving for 15 minutes at 121 °C, cooled to 47 °C, mixed well then poured in to sterile Petri dishes.

Assay for antibacterial activity

Disc diffusion method

In vitro antibacterial activity of different crude extracts obtained by sequential extraction was evaluated using disc diffusion method²⁴. Crude extracts solutions at concentration level of 100 mg mL⁻¹ were prepared in 5% dimethyl sulfoxide (DMSO). The test microorganisms were seeded into respective medium by spread plate method. After solidification, filter paper discs with a diameter of 6.0 mm were impregnated with 10 µL of crude extracts followed by drying off. Antibacterial discs were dispensed onto the surface of the inoculated agar plates and Petri plates were incubated for 24 h at 37 °C. Diameters of clear zone of inhibition produced around the discs were measured and recorded.

Bioassay for antifungal activity

The same method described for antibacterial activity assay has been adopted. To test antifungal activity, Sabouraud Dextrose Agar was used. The inoculated medium was incubated at 25 °C for two days for the *Candida albicans* and three days for *Aspergillus niger*.

Determination of antioxidant activity

DPPH radical scavenging assay

The scavenging activity on DPPH radical of pomegranate peel was determined according to the method of Shimada *et al.*²⁵ with some modification. Briefly, 10 µL of sample extracts (5.0 mg mL⁻¹ in DMSO) were transferred to a 96-wells plate and then 300 µL of DPPH solution in ethanol (0.5 mM) has been added. The mixtures were allowed to react for half an hour at 37 °C. After incubation, decrease in absorbance was measured at 517 nm using multi plate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. Ascorbic acid (2.0 mg mL⁻¹) was used as standard and all tests and analyses were run in triplicates. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A₀ is the absorbance of the control and A₁ is the absorbance of the sample.

ABTS radical scavenging activity

ABTS radical-scavenging activity of the pomegranate peel extracts were determined according to Re *et al.*²⁶. The ABTS⁺ cation radical was produced by the reaction between 5.0 mL of 14 mM ABTS solution and 5.0 mL of 4.9 mM potassium persulfate solution, stored in the dark at room temperature for 16 h. Before use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. Aliquots of 10 µL pomegranate peel extracts (5.0 mg mL⁻¹ in DMSO) have been homogenized with 300 µL of ABTS⁺ cation solution and allowed to react for 6.0 min then their absorbance were recorded at 734 nm. Ascorbic acid (2.0 mg mL⁻¹) was used as standard and all tests and analyses were run in triplicates. The inhibition percentage of ABTS⁺ radical was calculated using the following formula:

$$\text{ABTS}^+ \text{ scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A₀ is the absorbance of the control, and A₁ is the absorbance of the sample.

Statistical analysis

Antimicrobial activity experiment was performed in triplicates and data were presented as means ± standard deviation (SD). Statistical analysis for all the assays results were done using Microsoft Excel program (2010).

RESULTS AND DISCUSSION

Antibacterial activity

Antibiotics are effective remedy in the inhibition of bacteria growth. On the other side, antibiotics resistance against microorganism is one of the major problems in the use of antibiotics against microorganism²⁷. Medicinal plants and their extracts have extensively studied by researchers in order to produce potential drugs of medicinal properties with reduced toxicity. Therefore, this study investigated the potential antibacterial activity of pomegranate peel extracts.

The antibacterial activity of the petroleum ether, chloroform, acetone, ethyl acetate and ethanol extracts from peel of *Punica granatum* was determined against the Gram-positive *B. subtilis* and *S. aureus* and the Gram-negative *E. coli* and *P. aeruginosa*. Different extracts showed variable activity against the tested bacteria as shown in Table 1 and Figure 1. For *B. subtilis*, the inhibition zones values of five different extracts were between 7.0 mm and 13 mm. The maximum and lowest inhibitory effects were found in acetone and chloroform extracts, respectively. For *S. aureus*, the inhibition zones values ranged from 13 mm to 15 mm. The high effect was noted for petroleum ether extract while the other extracts showed same inhibition zone (13 mm) against *S. aureus*. In the case of *E. coli*, the inhibition zones values were between 11 mm and 16 mm. The chloroform extract presented greater inhibitory activity against *E. coli* while petroleum ether, acetone and ethanol extracts showed similar and lowest activity. All extracts have inhibitory effects on *P. aeruginosa* with inhibition zones ranged from 7.0 mm to 13 mm. The largest diameter of growth inhibition zone was achieved by petroleum ether extract and the lowest one observed in the chloroform extract.

Table 1: Antibacterial activity of peel of *Punica granatum* extracts

Extract 20 mg mL ⁻¹	MDIZ (Mean diameter of growth inhibition zone , mm)			
	Bacteria strains			
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Petroleum Ether	10±0.0	15±0.7	11±0.0	13±1.4
Chloroform	7±0.7	13±0.7	16±0.7	7±0.0
Acetone	13±0.7	13±0.0	11±0.7	10±0.0
Ethyl acetate	11±0.7	13±0.0	14±0.7	11±0.0
Ethanol	12±1.4	13±1.4	11±0.0	9±0.0

Interpretation of results: MDIZ (mm) : < 9 mm Inactive ; 9-12 mm Partially active ; 13-18mm Active ; >18 mm: Very active .

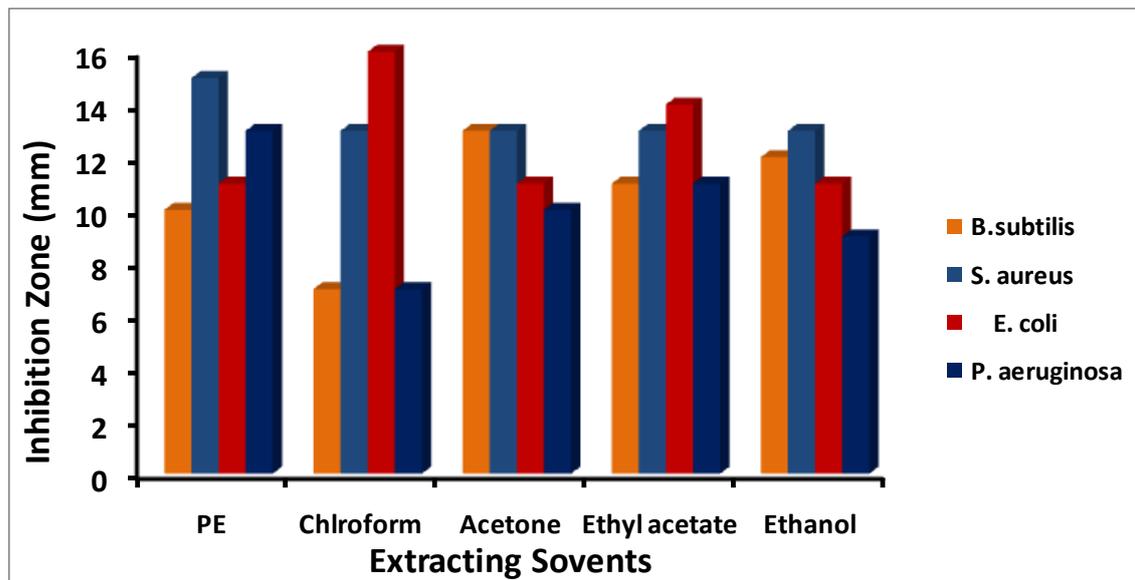


Figure 1: Effect of pomegranate peel extracts using different extracting solvents on bacterial strains

Generally, for the five different extracts the chloroform, petroleum ether and ethyl acetate extracts showed higher antibacterial activity than the acetone and ethanol extracts. The chloroform extract showed the highest activity against *E. coli* with inhibition zone of 16 mm while, petroleum ether extract displayed best inhibition against *S. aureus* (15 mm). The ethyl acetate extract showed the highest activity against *E. coli* (14 mm). Acetone and ethanol extracts exhibited good antibacterial activity against *B. subtilis* and *S. aureus*, respectively, with inhibition zone of 13 mm for both. Previous study based on pomegranate peel reported that peel has antibacterial effect²⁸. Our results of antibacterial activity are agree with that obtained by Ismail *et al.*²⁹, who reported that extracts of *Punica granatum* peel showed good antibacterial activity against *S. aureus*, *P. aeruginosa*, *E. coli* and *B. subtilis*. It was reported that, the extracts of pomegranate peel obtained by sequential extraction using ethyl acetate, acetone, methanol, and aqueous, exhibited inhibitory effect against *S. aureus*, *P. aeruginosa*, *E. coli* and *B. subtilis* strains. In this earlier study, the acetone extract showed the highest activity against the test strains while the ethyl acetate extract showed the lowest one³⁰. It was observed that the effect of ethanolic pomegranate peel extract has a stronger effect on Gram-positive bacteria than Gram-negative bacteria. This finding is consistent with previous report³¹.

Also the acetone extract has shown greater activity against the Gram-positive bacteria than Gram-negative bacteria whereas the other extracts have no definite trend against Gram-positive and Gram-negative bacteria that studied here. The observed antibacterial activity of pomegranate fruit peel extracts may be due to the presence of metabolic

toxins or broad spectrum antimicrobial compounds that act against both Gram-positive and Gram-negative bacteria.

Antifungal activity

In this study the antifungal effects of pomegranate peel different extracts were evaluated on two fungi (*A. niger* and *C. albicans*) using the disc diffusion method and the results were shown in Table 2. All extracts exhibited high. All extracts exhibited high antifungal activity with inhibition zones ranged from (11-15 mm) and (13-16 mm) against *A. niger* and *C. albicans*, respectively. Petroleum ether extract showed the highest antifungal activity against *C. albicans* while, the acetone extracts showed the lowest effect.

The chloroform extract exerted the greater antifungal effect whereas the ethanol extract exhibited the lowest activity against *A. niger*. In our knowledge, the inhibitory effects of pomegranate peel extracted with sequential extraction, have been rarely studied against *A. niger* and *C. albicans* strains. Most of the published researches on antifungal activity of pomegranate peel were used alcoholic extracts. There is no data found in the literature about the application of sequential extraction on pomegranate peel and then studied its antifungal activity against *A. niger* fungi. Therefore, our results for antifungal effect of pomegranate peel different extracts against *C. albicans* strain have been compared with those reported by Aravindaraj *et al.*³² as shown in Table 2. In the current study, relatively higher antifungal activities against *C. albicans* strain have been obtained in comparison to that obtained by Aravindaraj *et al.*³². These finding may be due to, the plants used in this study and the previous study have varied chemical composition which it depends on the cultivar, growing region³³.

Table 2: Antifungal activity of peel of *Punica granatum* extracts

Extract 20 mg mL ⁻¹	MDIZ (Mean diameter of growth inhibition zone, mm)		
	Fungi strain		
	<i>C. albicans</i> (Current study)	<i>A. niger</i>	<i>C. albicans</i> (Previous study ³²)
Petroleum Ether	16±0.7	14±0.7	10
Chloroform	14±0.0	15±0.7	10
Acetone	13±0.0	13±0.0	14
Ethyl acetate	14±0.7	12±0.0	12
Ethanol	14±0.0	11±0.0	12

Interpretation of results: MDIZ (mm) : < 9 mm Inactive ; 9-12 mm Partially active ; 13-18 mm Active ; >18 mm: Very active .

Antioxidant activity

In this study, the antioxidant activities of pomegranate peel extracts obtained by sequential extraction using petroleum ether, chloroform, acetone, ethyl acetate and ethanol as extracting solvents were evaluated using DPPH and ABTS assays.

DPPH Radical Scavenging Activity

This method is based on the ability of antioxidant to reduce the purple-colored stable free radical of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) into yellow-colored 2, 2-diphenyl-1-picrylhydrazine molecule. The DPPH free radical is decolorized by accepting an electron that donated by an antioxidant compound. The scavenging potentials of the sample extract are proportional to degree of DPPH discoloration. The quality of the antioxidants in the extracts was determined by the IC_{50} values, which the effective concentration of the sample required to inhibit 50% of the initial DPPH free radical. The IC_{50} value is inversely proportional to the antioxidant activity⁵. In the present study, all the extracts of pomegranate peel were able to decolorize DPPH. The values of DPPH free radical scavenging activities of pomegranate peel different extracts

are calculated as percentage and IC_{50} values as presented in Table 3 and Figure 2. The percentage of radical scavenging activity and IC_{50} of petroleum ether, chloroform, acetone, ethyl acetate and ethanol extracts varied from 77.79 % to 89.40% and 0.106 mg mL⁻¹ to 0.24 mg mL⁻¹, respectively. These results are relatively good compared with percentage DPPH radical scavenging activity of ascorbic acid (93.50 %) as antioxidant standard.

It was observed the highest DPPH scavenging activity in acetone extract and the lowest in chloroform extract. Similarly, Karthikeyan & Vidya³⁴ reported that the acetone extract of pomegranate peel shows maximum DPPH scavenging activity when compared to aqueous, ethanol and hexane extracts. In current study, the extracts obtained using polar solvents (acetone ethanol and ethyl acetate) show higher scavenging activity than those obtained using less polar solvents (chloroform and petroleum ether). This may be attributed to high content of polar phenolic compounds in pomegranate fruit peel, which are responsible for the antioxidant activity¹⁸. These compounds are usually extracted with polar solvents such as aqueous acetone and methanol³⁵. It can be stated that pomegranate peel extracts were good DPPH free radical scavengers.

Table 3: Antioxidant activity of peel of Punica granatum extracts by DPPH assay

Extract	DPPH %	IC_{50} (mg mL ⁻¹)
Petroleum Ether	79.3 ±0.11	0.2454
Chloroform	77.97 ±0.4	0.2223
Acetone	89.40 ±0.07	0.1063
Ethyl acetate	86.36 ±0.01	0.1994
Ethanol	85.90 ±0.06	0.1876
Ascorbic acid	93.5 ±0.00	-

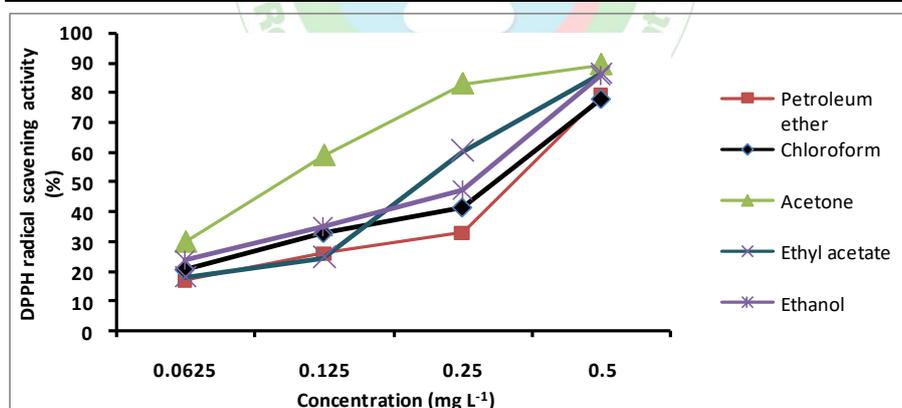


Figure 2: IC_{50} of plant extracts by DPPH assay

ABTS⁺ radical scavenging activity

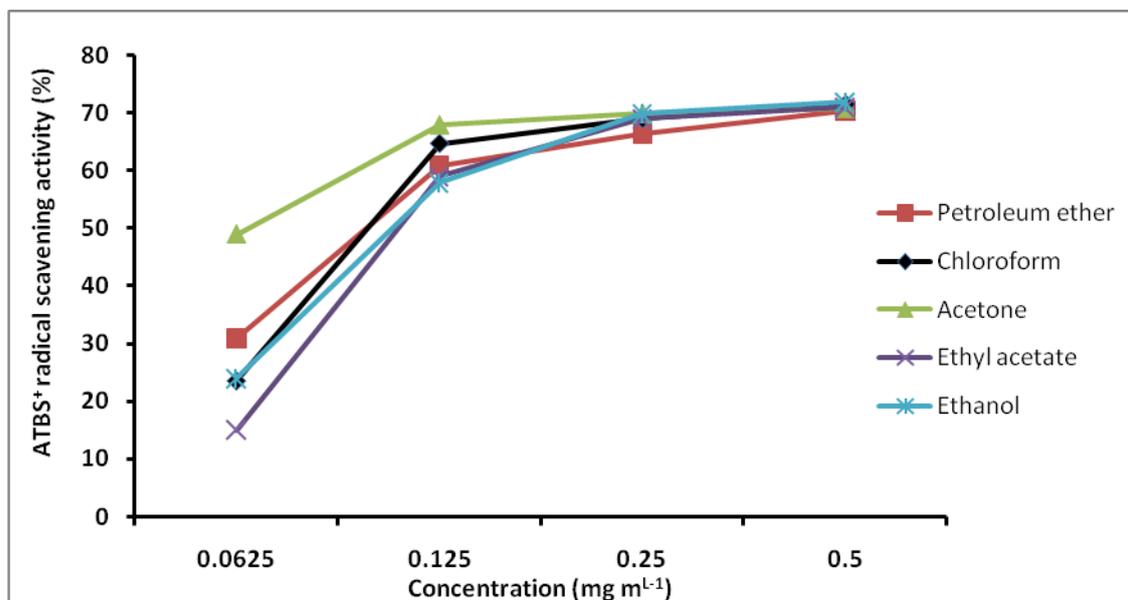
Due to different chemical properties of antioxidant compounds and their different response manner to radical or oxidant sources, there is no universal method for the determination of antioxidant capacity. In this work, ABTS⁺ assay has been employed to measure the antioxidant activity beside to DPPH assay.

ABTS⁺ assay measures the relative capacity of the antioxidants in sample to inhibit and decolorization the radical ABTS⁺ compared with a reference antioxidant standard³⁶. The ABTS⁺ radical scavenging capacities of pomegranate peel extracts are evaluated as percentage and IC_{50} values as shown in Table 4 and Figure 3. The

percentage ABTS⁺ radical scavenging activity and IC_{50} values of pomegranate extracts varied between 72.07% to 70.53% and 0.061 to 0.159, respectively. The highest radical scavenging activity was observed in ethanol extract and the lowest in petroleum ether extract. The obtained results of ABTS⁺ radical scavenging activity of different pomegranate peel extracts are very close. It was observed that, the reducing power values determined using DPPH assay were higher than those obtained by using ABTS⁺ radical scavenging assay for all solvents extracts. This finding, likely because of differences in reaction ability of the free radicals in the assays and reaction rates of the antioxidants under actual analytical conditions³⁷.

Table 4: Antioxidant activity of peel of Punica granatum extracts by ABTS assay

Extract	ABTS %	IC ₅₀ (mg mL ⁻¹)
Petroleum Ether	70.53 ±0.01	0.128
Chloroform	71.77 ±0.02	0.138
Acetone	70.91 ±0.01	0.061
Ethyl acetate	71.72 ±0.00	0.159
Ethanol	72.07± 0.02	0.141
Ascorbic acid	95.05 ±0.00	-

**Figure 3:** IC₅₀ of plant extracts by ABTS⁺ method

CONCLUSION

In the current study different pomegranate peel extracts obtained by sequential extraction using petroleum ether, chloroform, acetone, ethyl acetate and ethanol were screened for their antibacterial, antifungal and antioxidant activities. All different extracts of pomegranate peel show significant antibacterial, antifungal and antioxidant activities. The results reveal that, different types of solvents had a great effect on the antibacterial, antifungal and antioxidant properties of obtained extracts. The results of the present work indicated that the extracts obtained from less polar solvents (chloroform and petroleum ether) have higher antibacterial and antifungal activity compare to those obtained from high polar solvents against all tested organism except *B. subtilis* bacteria which is more inhibited

by acetone extract. In this study, the antioxidant activities determined using DPPH assay are higher in the extracts of relatively polar solvents (acetone, ethyl acetate and ethanol) than those of non-polar solvents (chloroform and petroleum ether). The results indicate that, the total antioxidant capacity determined using DPPH assay were higher than those obtained by using ABTS⁺ radical scavenging assay for all solvents extracts. Additional research is recommended to identify and isolate of bioactive pure compounds from peel extracts to find out which responsible for their antibacterial, antifungal and antioxidant property to be used in pharmaceutical industry.

Conflicts of Interest

The authors declare no conflicts of interest.

REFERENCES

1. Cartea ME, Francisco M, Soengas P, Velasco P. Phenolic compounds in Brassica vegetables. *Molecules*. 2011; 16(1):251-80.
2. Paul D, Biswas K, Sinha SN. Biological activities of Adenium obesum (Forssk.) Roem. & Schult.: a concise review. *Malaya Journal of Biosciences*. 2015; 2(4):214-21.
3. Sangeetha J, Vijayalakshmi K. Physicochemical Evaluation and Chromatographic Fingerprint Profile of the Rind of Ganesh Variety of Punica granatum Linn. *IJPBS* 2012.3:329-37.
4. Karar MGE, Kuhnert N. Herbal drugs from Sudan: Traditional uses and phytoconstituents. *Pharmacognosy reviews*. 2017; 11(22):83.
5. Ali SI, El-Baz FK, El-Emary GA, Khan EA, Mohamed AA. HPLC-analysis of polyphenolic compounds and free radical scavenging activity of pomegranate fruit (*Punica granatum L.*). *Int J Pharm Clin Res*. 2014; 6(4):348-55.
6. Al-Rawahi AS, Edwards G, Al-Sibani M, Al-Thani G, Al-Harrasi AS, Rahman MS. Phenolic constituents of pomegranate peels (*Punica granatum L.*) cultivated in Oman. *European Journal of Medicinal Plants*. 2014:315-31.
7. Barros ZMP, Salgado JM, Melo PS, de Oliveira Biazotto F. Enrichment of commercially-prepared juice with pomegranate (*Punica granatum L.*) peel extract as a source of antioxidants. *Journal of Food Research*. 2014; 3(6):179.
8. Dadwal V, Bhatt Sh., Sonkhla K., Joshi R. and Gupta M. Quantification of free and bound phenolics in Bio-Waste pomegranate peel and formulation of punicalagin rich rice extruded snacks. *International Journal of Food and Nutritional Science*. 2017; 4(2):98-104.

9. Farag R, Abdelatif M, Emam S, Tawfeek L. Phytochemical screening and polyphenol constituents of pomegranate peels and leave juices. *Agr Soil Sci* 2014; 1(6): 86. 2014;93.
10. Hmid I, Elothmani D, Hanine H, Oukabli A, Mehinagic E. Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (*Punica granatum* L.) cultivars grown in Morocco. *Arabian Journal of Chemistry*. 2017; 10:S2675-S84.
11. Wu S, Tian L. Diverse phytochemicals and bioactivities in the ancient fruit and modern functional food pomegranate (*Punica granatum*). *Molecules*. 2017; 22(10):1606.
12. Alexandre EM, Silva S, Santos SA, Silvestre AJ, Duarte MF, Saraiva JA, et al. Antimicrobial activity of pomegranate peel extracts performed by high pressure and enzymatic assisted extraction. *Food research international*. 2019; 115:167-76.
13. Rowayshed G, Salama A, Abul-Fadl M, Akila-Hamza S, Mohamed EA. Nutritional and chemical evaluation for pomegranate (*Punica granatum* L.) fruit peel and seeds powders by products. *Middle East Journal of Applied Sciences*. 2013; 3(4):169-79.
14. Entessar H, Al-Mosawe A-SI, Al-Saadi I. The Extraction and purification of gallic acid from the pomegranate rind. *Al-Mustanisyah J Sci*. 2012; 23:184-92.
15. Bhandari PR. Pomegranate (*Punica granatum* L.). Ancient seeds for modern cure? Review of potential therapeutic applications. *International Journal of Nutrition, Pharmacology, Neurological Diseases*. 2012;2(3):171.
16. Singh R, Chidambara Murthy K, Jayaprakasha G. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of agricultural and food chemistry*. 2002; 50(1):81-6.
17. Kaneria MJ, Bapodara MB, Chanda SV. Effect of extraction techniques and solvents on antioxidant activity of pomegranate (*Punica granatum* L.) leaf and stem. *Food Analytical Methods*. 2012; 5(3):396-404.
18. Panichayupakarananta P, Issuriya A, Sirikatitham A, Wang W. Antioxidant assay-guided purification and LC determination of ellagic acid in pomegranate peel. *Journal of chromatographic science*. 2010; 48(6):456-9.
19. Middha SK, Usha T, Pande V. A review on antihyperglycemic and antihepatoprotective activity of eco-friendly *Punica granatum* peel waste. *Evidence-Based Complementary and Alternative Medicine*. 2013; 2013.
20. Yehia HM, Elkhadragey MF, Moneim AEA. Antimicrobial activity of pomegranate rind peel extracts. *African Journal of Microbiology Research*. 2011; 4(22):3664-8.
21. Ferrazzano GF, Scioscia E, Sateriale D, Pastore G, Colicchio R, Pagliuca C, et al. In vitro antibacterial activity of pomegranate juice and peel extracts on cariogenic bacteria. *BioMed research international*. 2017.
22. Santos MC, Gonçalves ÉC. Effect of different extracting solvents on antioxidant activity and phenolic compounds of a fruit and vegetable residue flour. *Scientia Agropecuaria*. 2016; 7(1):7-14.
23. Uthayarasa K, Pathmanathan K, Jeyadevan JP, Jeeyaseelan E. Antibacterial activity and qualitative phytochemical analysis of medicinal plant extracts obtained by sequential extraction method. *IJIB*. 2010; 10(2):76-81.
24. Kil HY, Seong ES, Ghimire BK, Chung I-M, Kwon SS, Goh EJ, et al. Antioxidant and antimicrobial activities of crude sorghum extract. *Food Chemistry*. 2009; 115(4):1234-9.
25. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of agricultural and food chemistry*. 1992; 40(6):945-8.
26. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*. 1999; 26(9-10):1231-7.
27. Rahmani AH, Alsahli MA, Almatroodi SA. Active constituents of pomegranates (*Punica granatum*) as potential candidates in the management of health through modulation of biological activities. *Pharmacognosy Journal*. 2017; 9(5).
28. Devatkal SK, Jaiswal P, Jha SN, Bharadwaj R, Viswas K. Antibacterial activity of aqueous extract of pomegranate peel against *Pseudomonas stutzeri* isolated from poultry meat. *Journal of food science and technology*. 2013; 50(3):555-60.
29. Ismail T, Akhtar S, Sestili P, Riaz M, Ismail A, Labbe RG. Antioxidant, antimicrobial and urease inhibitory activities of phenolics-rich pomegranate peel hydro-alcoholic extracts. *Journal of Food Biochemistry*. 2016; 40(4):550-8.
30. Negi P, Jayaprakasha G. Antioxidant and antibacterial activities of *Punica granatum* peel extracts. *Journal of food science*. 2003; 68(4):1473-7.
31. Naziri Z, Rajaian H, Firouzi R. Antibacterial effects of Iranian native sour and sweet pomegranate (*Punica granatum*) peel extracts against various pathogenic bacteria. 2012.
32. Aravindraj S, Preethi M, Sivapathasundharam B. Antimicrobial Effects of *Punica granatum* Extracts on *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Enterococcus faecalis* and *Candida albicans*. *Int J Curr Microbiol App Sci*. 2017; 6(9):2762-74.
33. Bassiri-Jahromi S, Doostkam A. Comparative evaluation of bioactive compounds of various cultivars of pomegranate (*Punica granatum*) in different world regions. *Food*. 2018; 4:41-55.
34. Karthikeyan G, Vidya A. Phytochemical analysis, antioxidant and antibacterial activity of pomegranate peel. *Research Journal of Life Science, Bioinformatics, Pharmaceutical and Chemical Science*. 2019; 5(1) Page No.218
35. Wissam Z, Ghada B, Wassim A, Warid K. Effective extraction of polyphenols and proanthocyanidins from pomegranate's peel. *Int J Pharm Pharm Sci*. 2012; 4(Suppl 3):675-82.
36. Lucas-Abellán C, Mercader-Ros M, Zafrilla M, Gabaldón J, Núñez-Delgado E. Comparative study of different methods to measure antioxidant activity of resveratrol in the presence of cyclodextrins. *Food and Chemical Toxicology*. 2011; 49(6):1255-60.
37. Jalal H, Pal MA, Hamdani H, Rovida M, Khan NN. Antioxidant activity of pomegranate peel and seed powder extracts. *J Pharmacogn Phytochem*. 2018; 7(5):992-7.