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Phytochemical Screening and Biological Activity (Antioxidant and Antibacterial) of *Punica granatum* Fruit peel extract Cultivated in Sa'dah Governorate-Yemen

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Abstract

Punica granatum (Pomegranate) is a valuable source of secondary plant metabolism compounds (SMs) that are widely used in the pharmaceutical, cosmetics, and food industries. The phytochemical screening illustrates that the Sa'dah *P. granatum* cultivar peel ethanol extract containsvarious beneficial components, including Flavonoids, Alkaloids, Steroids, Phenols, and Tannins. Moreover, the Sa'dah *P. granatum* cultivar peel ethanol extract (1.3 μg/ml) exhibited significant antioxidant activity compared to low concentrations of ascorbic acid (0.4, 0.6, 0.8, and 1 μg/ml). Furthermore, the Sa'dah *P. granatum* cultivar peel ethanol extract displays potent antibacterial activities against selected pathogenic bacteria (*Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*) compared to two nominated antibiotics (Ampicillin and Gentamicin). This remarkable performance can be attributed to the presence of phenols and flavonoid compounds, which exhibit high antioxidant activity.

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Sa'dah *Punica granatum* cultivar
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1. INTRODUCTION

The food industry requires natural materials that are eco-friendly and preferred by consumers because these natural materials possess unique properties that enable them to treat a variety of diseases, the most significant of which are anti-cancer and antipathogenic bacteria [1]. Plants are valuable sources of secondary plant metabolism compounds (SMs), including flavonoids, terpenoids, phenols, steroids, tannins, and aromatic, which are widely utilized in the pharmaceutical, cosmetics, and food industries [2]. Secondary plant metabolites are not essential for the plant's growth and development but are considered defensive compounds that interact with the environment for adaptation [3]. Additionally, few studies mentioned that environmental factors can impact metabolites, growth, and productivity in plants [4]. *Punica granatum* L. is commonly known as the Pomegranate shrub and belongs to the Punicaceae family; it is a small cultivated shrub native to Persia that grows up to 3 meters in height [5]; its cultivation has expanded in a large area of the world, including China, South Africa, India, the United States, as well as in Yemen due to its sweet edible fruits, moreover, its fruit peels are important, as an anticancer and resistance to pathogenic microbes [6]. Pomegranates (*P. granatum*) are considered one of the main economic crops in Yemen. The country produces 50,309 tons of pomegranates per year, with about 3,021 hectares of Yemeni agricultural land dedicated to pomegranate cultivation. Approximately 38.2% of Pomegranate Production in Yemen comes from the Sa'dah governorate, which produces 19,214 tons of Pomegranates per year from 1,618 hectares [7]. Numerous studies have demonstrated the

various applications of Pomegranate in medicine and the food industry [8, 9]. Moreover, some studies mentioned that the Pomegranate peel and juice contain several flavonoid components, particularly anthocyanins, which are responsible for the fruit's color and also its medicinal effects [10, 11]. Additionally, several studies have shown that extracts from different parts of the Pomegranate, including the peel, are rich in antioxidants and exhibit antimicrobial activities [1, 12]. Furthermore, it was mentioned that Pomegranate peel has long been used as a treatment for various diseases, such as stomach ulcers and bacterial infections, possibly due to the presence of beneficial compounds, particularly antioxidants [13]. Based on the former, numerous studies have evaluated the antioxidant and antimicrobial properties of various Pomegranate cultivars. However, no studies have investigated the antioxidant and antibacterial activity of the Sa'dah Pomegranate (Sa'dah *P. granatum* cultivar) that grows in the Sa'dah governorate, Yemen. Therefore, this work aims to evaluate the phytochemical, antioxidant, and antibacterial activities of the ethanolic extract of Sa'dah Pomegranate cultivars peel.

2. MATERIALS AND METHODS

2.1. Plant Material

Fruits of the Sa'dah *P. granatum* cultivars were collected from *P. granatum* plantation in Sa'dah (Figure 1) during the period January- February 2021 and identified by Dr. Hassan Ibrahim, head of the herbarium of the Biological Sciences Department, Faculty of Science, Sana'a University, and preserved by the herbarium number (152) at the Biological science Department herbarium, Faculty of Science, Sana'a University, after it has been dried (Sun-dry), pressed and mounted on a herbarium sheet with few leafy branches.

Figure 1. Sa'dah *P. granatum* cultivar.

2.2. Chemicals

All chemicals were purchased from Sigma, Aldrich (USA), and Fluka (Germany).

2.3. Preparation of *P. granatum* fruit peel extract

The peel of *P. granatum* fruits was removed, let to be air-dried in a shaded place (Figure 2), and then blended into a powder by using a blender. About 100g of the peel powder was extracted twice using the maceration method and 1000 ml ethanol (96%) with a shaker for 24 hours before it was filtered using Whatman filter paper. The crude ethanolic extract was dried (ethanol was evaporated) using a water bath at 50℃; then the crude ethanolic extract was weighed and maintained in a sealed container at 4℃ for future studies [14]. For Phytochemical analysis, the dry crude ethanolic extracts were dissolved in the same solvent[15].

Figure 2. Sa'dah *P. granatum* cultivar dried fruit peels

2.4. Phytochemical screening

2.4.1. Determination of Flavonoids

In a test tube, 3 ml of the filtered peel crude extract was mixed with 4 ml of 1% aluminum chloride in methanol. The presence of flavonols, flavones, and chalcones was shown by the formation of a yellow color [16].

2.4.2. Determination of Alkaloids

One ml of the filtered peel crude extract and 1 ml of Dragondroff's reagent were mixed in a test tube. A reddish-brown precipitate was interpreted as proof that alkaloids were present [14, 16].

2.4.3. Determination of Steroids

The presence of steroids was detected by the color changing from violet to blue or green when 0.5 ml of the filtered crude fruit peel extract was mixed with 2 ml of acetic anhydride and 2 ml of sulphuric acid [16].

2.4.4. Determination of Phenolic Compounds and Tannins

2 ml of distilled water and a few drops of 10% aqueous ferric chloride solution was added to 1 ml of filtered crude fruit peel extract. The development of a black hue suggested the existence of tannins and phenolic compounds [14].

2.4.5. Determination of Saponins

A test tube containing 0.2 g of filtered crude fruit peel extract was filled with 5 ml of distilled water and heated to boiling. If a creamy mass of tiny bubbles formed, the extract contained saponins [14].

2.4.6. Determination of Resins

One ml of filtered crude fruit peel extract was treated with a few drops of acetic anhydride solution, then 1 ml of concentrated H_2SO_4 was added to the mixture. The presence of resins is indicated by the orange-to-yellow coloring [17].

2.5. Determination of Antioxidant capacity by using Ferric-bipyridine assay

This procedure is grounded on the establishment of the ferrous form of the Fe^{3+}-Bipyridine complex through reduction at pH4, and the antioxidant activity is measured by observing the change in absorption taken at 535 nm [18]. Different concentrations of ascorbic acid (0.4 0.6, 0.8, 1, 2, and 4μg/ml) and one concentration of *P. granatum* fruit peels ethanolic extract (1.3) were reacted with 1.0 ml 0.01M $FeCl_3$ solution, 1.0 ml bipyridine and 2.0ml 0.3M acetate buffer (Acetate buffer, 3g of Sodium acetate trihydrate and 15ml of glacial acetic acid) to adjust the pH to 4. A standard curve of ascorbic concentration was drawn at A535nm to obtain the equivalent value of ascorbic acid (g/L) corresponding to the sample in absorbance by comparing the absorbance of the sample with the absorbance values of the different ascorbic acid concentrations [15]. The results of antioxidant capacity were shown as (mg ascorbic acid equivalents g/L the extract).

2.6. Determination of Antibacterial Activities

2.6.1. Tested Pathogens

The antibacterial activity of *P. granatum* fruit peels ethanolic extract was Investigated three human pathogenic bacteria: one positive–gram bacteria *(Staphylococcus aureus)* and two negative-gram bacteria (*Escherichia coli*, and *Pseudomonas aeruginosa*) which were obtained from Aldbahani Laboratories, Sana'a, Yemen.

2.6.2. Antibacterial activity

The antibacterial activity of *P. granatum*fruit peels ethanolic extract was assessed using the agar well-diffusion method on Mueller Hinton Agar (MHA) medium. Two petri dishes containing MHA were prepared for each bacterial pathogen. One dish was used for testing, including three concentrations of the extract, and the other dish served as a positive control. Each tested petri dish was inoculated with 106 CFU/ml of the test bacteria, which were spread on the surface of the medium using a sterile swab [14]. Four wells of 5 mm diameter were made in the agar medium using a cork borer–one in the center for the negative control (DMSO) and three at the corners for the different concentrations of the extract. To assess the antibacterial activity, three concentrations (5, 2.5, and 1.25 mg/ml) of *P. granatum* fruit peels ethanolic extract were prepared using Dimethyl sulfoxide (DMSO) as a solvent [19, 20]. As a positive control, the antibiotics Ampicillin (5 mg) and Gentamicin (5 mg) were utilized. The inhibitory zone diameter was measured in millimeters for each concentration and antibiotic (positive control) after the Petri dishes were incubated for 24 hours at 37℃ [15].

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

The preliminary phytochemical qualitative tests illustrate that the ethanol extract of Sa'dah *P. granatum* cultivars fruit peels contains five active pharmacological components: Flavonoids, Alkaloids, Steroids, Phenols, and Tannins. These results are consistent with the findings of Sajjad et al. [21], Karthikeyan & Vidya [22], and Singh et al. [23], where they note the presence of these components in the ethanol extract of *P. granatum* fruit peels. However, Saponins and Resin were not detected in the ethanol of Sa'dah *P. granatum* cultivars fruit peels extract, which is in agreement with the findings of Sajjad et al. [21] where they recorded the absence of Saponins in the ethanol extract of *P. granatum* fruit peels.

3.2. Determination of Antioxidant capacity by using Ferric-bipyridine assay

According to Table 1 and Figure 3, the Sa'dah *P. granatum* cultivar fruit peels ethanolic extract (1.3μg/ml) demonstrated a higher total antioxidant activity compared to lower concentrations of ascorbic acid (0.4, 0.6, 0.8, and 1μg/ml). This is attributed to its higher ability to re-

Table 1. **Antioxidant activity of Sa'dah *P. granatum* cultivar fruit peels ethanolic extract**

Ascorbic acid (μg/ml)	Absorbance (535 nm)	Sa'dah *P. granatum* cultivar peel ethanol extract (μg /ml)	Absorbance (535 nm)
4	0.421	1.3	0.133
2	0.212		
1	0.100		
0.8	0.078		
0.6	0.059		
0.4	0.039		

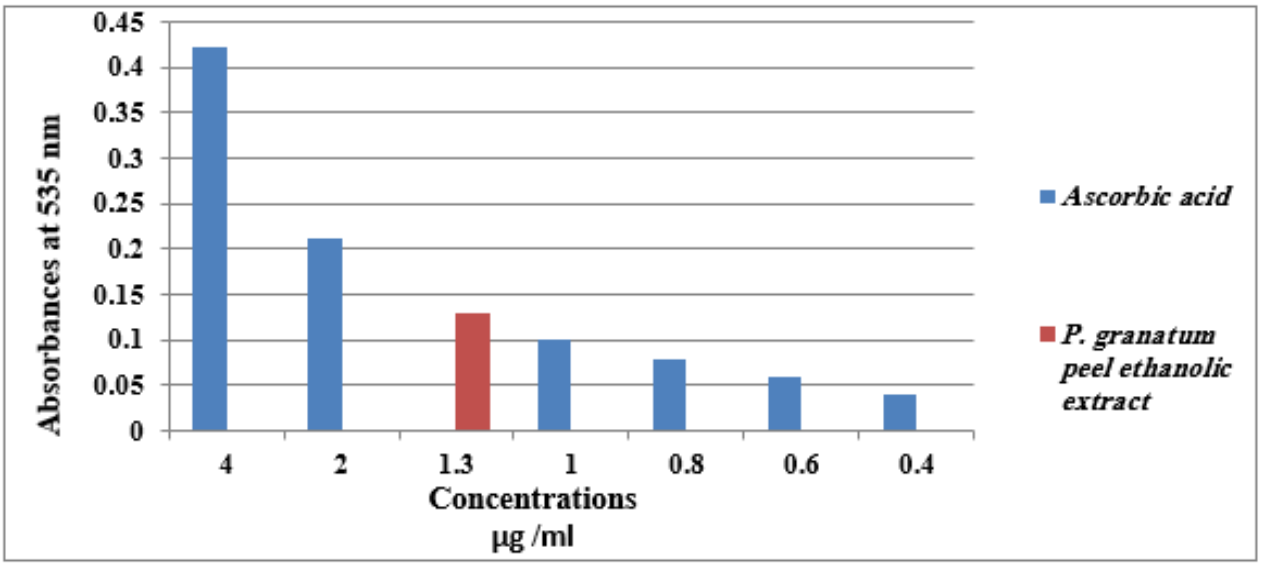

Figure 3. Antioxidant activity of Sa'dah P. granatum cultivar fruit peels ethanolic extract

duce the ferrous form of the Fe^{3+}-Bipyridine complex, as indicated by the absorbance readings at 535 nm (1.290) when compared with the lower concentrations of ascorbic acid (0.039, 0.059, 0.078, and 1, respectively). Furthermore, the results displayed in Table 1 and Figure 3 correlate with the conclusions of Hanafy et al. [19], Abu-Niaaj et al.[20], and Abdelrahman et al. [24], where they reported that *P. granatum* peel extracts (methanolic and ethanolic extracts) exhibit a high antioxidant capacity (ability to donate hydrogen and scavenge free radicals) which is may be due to its high total phenolic (TPCs) and flavonoid (TFs) content, which are influenced by a number of variables, such as the cultivation region, extraction solvent, and technique.

3.3. Determination of Antibacterial Activities

The Sa'dah *P. granatum* cultivar fruit peels ethanolic extract, as shown in Table2 and Figure4, exhibits antimicrobial activity against the selected gram-positive pathogens where it inhibited the growth of *S. aurous*; 15, 22, and 25 mm at concentrations of 1.25, 2. 5 and 5 mg/ml respectively. Moreover, at concentrations of 1.25, 2. 5, and 5 mg/ml, the Sa'dah *P. granatum* cultivar peel ethanol extract showed antibacterial action against *E. coli* (with an inhibition zone of 15, 18, and 22 mm correspondingly). Furthermore, Table 2 and Figure 4 illustrate that Sa'dah *P. granatum* cultivar fruit peels ethanolic extract at concentrations 1.25, 2.5 and 5 mg/ml displays antibacterial activity against *P. aeruginosa* (with an inhibition zone of 13, 17, and 20 mm sequentially). In addition, the Sa'dah *P. granatum* cultivar fruit peels ethanolic extract at a concentration of 5 mg/ml (Table 2 and Figure 4), exhibits a strong antibacterial effect against *S. aureus* and *E. coli*, with inhibition zones of approximately 25 mm and 22 mm, respectively. This effect is higher compared to the antibacterial activity of the selected antibiotics; Ampicillin 5mg (with inhibition zones of 13 mm and 8 mm, respectively) and Gentamycin 5mg (with inhibition zones of 20 mm and 16 mm, respectively). Furthermore, the two selected antibiotics showed no activity against

Table 2. Antioxidant activity of Sa'dah *P. granatum* cultivar fruit peels ethanolic extract

Tested microorganism (Bacterial Pathogens)	Type of Bacteria	Extraction concentration mg/ml			- Control (DMSO)	+ Control (Antibiotics)	
		5	2.5	1.25		AMP 5mg	GEN 5mg
		Inhibition zone diameter (mm)				Inhibition zone diameter (mm)	
S. aurous	G +ve	25	22	15	-	13	20
E. coli	G -ve	22	18	15	-	8	16
P. aeruginosa	G -ve	20	17	13	-	-	-

G +ve: Positive-gram bacteria, G –ve: Negative-gram bacteria, AMP: Ampicillin antibiotic, GEN: Gentamycin antibiotic.

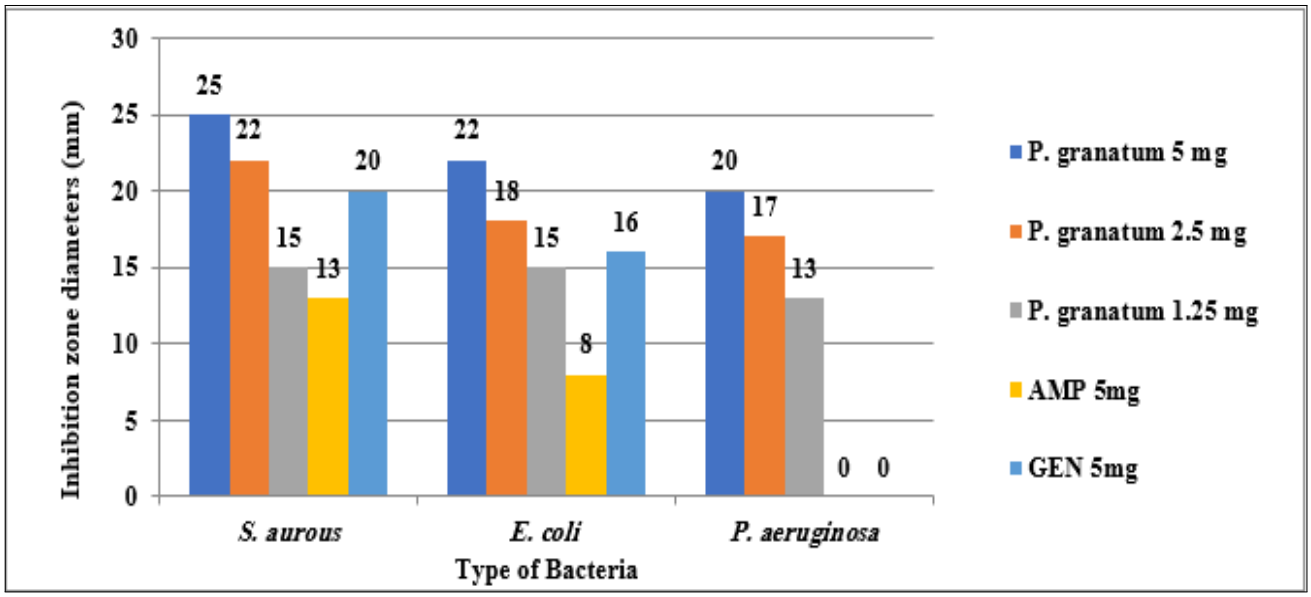

Figure 4. Inhibition zone diameters (mm) of Sa'dah *P. granatum* cultivar fruit peel ethanol and selected antibiotics extract against tested pathogenic bacteria

the growth of *P. aeruginosa* (Table 2 and Figure 4), whereas the three concentrations of the ethanolic extract of Sa'dah *P. granatum* cultivar fruit peels (1.25, 2.5, and 5 mg/ml) demonstrated an inhibitory effect on the growth of *P. aeruginosa*, with inhibition zones of 13 mm, 17 mm, and 20 mm, respectively. The results in Table 2 and Figure 4 are in line with the findings of Abu-Niaaj et al. [20], where they reported that the lowest concentration of *P. granatum* methanolic extract that inhibits the growth of pathogenic bacteria (*Salmonella typhimurium* and *E. coli*) was 5mg/ml, while the lowest concentration of *P. granatum* methanolic extracts that inhibition the growth of *P. aeruginosa* was 2.5 mg/m.

Moreover, Abdelrahman et al. [24] reported that the acidified ethanol extract of *P. granatum* fruit peels with a concentration of 50 μg/ml gives an inhibition zone of about 8.13, 8.44, 8.12, 8.43, 7.49, 7.77, 8.66, 8.38 mm agents the growth of *S. aureus*, *Streptococcus pyogenes*, *Listeria monocytogenes*, *L. ivanovii*, *Klebsiella oxytoca*, *S. typhimurium*, *P. aeruginosa* and*E. coli correspondingly*. In addition, previous studies [19, 20, 24] have indicated that fruit peels of *P. granatum* contain a high amount of phenolic and flavonoid compounds. These compounds have the ability to scavenge free radicals and interact with reactive oxygen species (ROS). This interaction can lead to oxidative stress and damage to microbial cells [25].

4. CONCLUSION

The Phytochemical exploration shows that the ethanol extract of Sa'dah *P. granatum* cultivar fruit peels contains various pharmacological components, including Flavonoids, Alkaloids, Steroids, Phenols, and Tannins. Furthermore, the Sa'dah *P. granatum* cultivar fruit peels ethanol extract (1.3μg/ml) exhibited a significant antioxi-

dant activity compared to low concentrations of Ascorbic acid (0.4, 0.6, 0.8, and 1 μg/ml). Additionally, the Sa'dah *P. granatum* cultivar fruit peels ethanol extract displays high antibacterial activities against selected pathogenic bacteria compared to two nominated antibiotics (Ampicillin and Gentamycin). This could be attributed to the presence of phenols and flavonoid compounds which have high antioxidant activity.

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