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PHYTOCHEMICAL PROFILING OF WILD POMEGRANATE (*PUNICA GRANATUM* L.): UNVEILING ANTIOXIDANT POTENTIAL AND ANTIBACTERIAL ACTIVITY

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Abstract

Wild Pomegranates *Punica granatum* L., is a medicinal plant, native to Northern Pakistan and is known for its richer phytochemicals profile and bioactive properties. In this study, we used HPLC to analyze the phytochemical composition and assess the antioxidant and antibacterial activities of different parts of the plants. Interestingly, the seed extract exhibited the highest antioxidant activity, with IC50 values of 795 µg/mL for DPPH and 975 µg/mL for ABTS. Among the tested parts, rind extract showed the highest bacterial growth inhibition against *Xanthomonas oryzae* and the effect may be attributed to its high phenolic contents. Similarly, HPLC characterization of methanolic extract of different parts of wild pomegranate identified various phytochemicals, including malic acid, vitamin C, chlorogenic acid, quercetin, ellagic acid, epigallocatechin gallate and pyrogallol. However, specifically, the rind contained five phytochemicals including, malic acid, vitamin C, quercetin, chlorogenic acid and pyrogallol. The root contained six phytochemicals: malic acid, vitamin C, epigallocatechin gallate, quercetin, chlorogenic acid, and ellagic acid. Similarly, the seed contained six phytochemicals: malic acid, vitamin C, epigallocatechin gallate, quercetin, chlorogenic acid and ellagic acid. It was concluded that wild pomegranate is a rich source of beneficial phytochemicals, with the seed extract displaying potent antioxidant properties and the rind extract showing strong antibacterial activity. Further *In vivo* and toxicological studies are necessary to validate these findings.

Key words: Wild pomegranate, Antioxidants, HPLC, Phytochemicals, Antibacterial activity, DPPH, ABTS, Phenolic compounds.

Introduction

In the past, people gathered plants and animals for diet, well-being and shelter. Many people in underdeveloped states rely heavily on animal and plant resources for their daily needs and livelihood. Due to their high demand as potential medicines, wild plant species are increasingly being overused, leading to the depletion of natural resources (Bhukta, 2020; Islam *et al*., 2021; Rahman *et al*., 2023). This has drawn the attention of ecologists, as some species face extinction soon if their misuse is not reined in. Because of their significant phytochemical contents, therapeutic plants are regarded as the foundation of the pharma industry in the modern world (Maxted & Brehm, 2021). It is a general exercise worldwide, especially in the modern world, to practice plants or plant extracts such as decoctions, drinks, or solutions for home-based therapies to treat several common diseases. They have significant developments in human medicine, the emergence and blowout of transmittable diseases caused by fungi, bacteria and viruses, remain a serious risk to common people's health (Tiwari *et al*., 2021; Begum *et al*., 2023). Plants are an excellent source of natural antioxidants and health-promoting compounds. Phytochemicals, the bioactive compounds found in plants, possess diverse medicinal qualities, such as antibacterial, antifungal, antidiabetic, antioxidant, and anticancer properties (Ullah *et al*., 2021; Abbas *et al*., 2024). These natural compounds are often more effective and have fewer adverse effects than those associated to their synthetic counterparts (Maliński *et al*., 2021).

Wild pomegranates, (*Punica granatum* L.) is the members of the Punicaceae family. The pomegranate tree usually reaches a height of 12 to 16 feet. It is grown in the USA, China, Japan, Afghanistan, and Pakistan (Holland *et al*., 2009). The fleshy seeds of sour pomegranates are dried and marketed as "Anar dana," which is used in the condiments. Fruits are the most common raw materials used in the wine industry due to their ease of fermentation. Pomegranate is rich in carbohydrates, minerals like calcium, iron, and Sulphur, as well as vitamin C (Dar *et al*., 2012). Pomegranate fruits contain medicinal and therapeutic potential. Sweet variants are laxative, whilst sour varieties are beneficial against stomach irritation and heartburn. Bark is also employed in the tanning industry (Verma *et al*., 2010). Pomegranate trees' capacity to flourish in varied Agro-climatic sites around the world has enabled their distribution throughout. the world as well its adaptability in all kinds of environments and they show high genetic diversity (Zeinalabedini *et al*., 2012). Pomegranates have a long history of domestication in the Mediterranean region, where they were prized for their therapeutic qualities and were seen as an integral part of nutrition. Pomegranate agriculture moved from Iran to the Indian peninsula in the first century (CE). It began to thrive in Indonesia in 1416, and Spanish sailors

brought it to the New World. The Greeks dispersed pomegranate horticulture throughout Europe. The majority of the plant's components are abundant in phytochemicals, which further enhances their health-promoting qualities (Noda *et al*., 2002). The rind and arils of cultivated and wild pomegranates are high in ellagic acids, flavonoids, anthocyanins, and hydrolysable tannins (Sudhakar *et al*., 2015). In the current study, different phytochemicals were characterized from various parts of wild pomegranate. This was achieved using high-performance liquid chromatography (HPLC). Additionally, the antioxidant potential of the extracts was evaluated. The antibacterial activity of these extracts against *Xanthomonas oryzae* bacteria was also examined.

Material and Methods

Site description and sampling: During the 2017-18 session, fresh parts of the wild pomegranate, including roots, and leaves, seeds and rind were collected from different localities i.e., Dir, Sheringal, Kalkot, Barawal, Larham and Wari of Tehsil in the Upper Dir District of Khyber Pakhtunkhwa, Pakistan. The samples were taken to the Department of Botany, University of Hazara, for onward research and analysis.

Extraction: For extraction, Nazir *et al*., (2018) procedure was followed. The samples including, the seed, root, leaves, rind and stem bark were allowed to air dry at room temperature in the shade. After being allowed to dry, the plant materials were ground with a grinder into fine powder and kept in storage at 4°C. A total of 500 g of powder (for each part) was macerated in 80% methanol, filtration was done after each fourth day with a three times solvent replacement. Using a rotary evaporator, the filtered extract was dried at 40°C. To facilitate additional phytochemical analysis, the dried extracts were ultimately stored at 4°C.

Qualitative screening of phytochemical

Screening of phenols: For the estimation of phenols, one-milligram extract was mixed in two milliliters of distilled water along with ten percent ferric chloride. The blue or green color indicated the presence of phenol (Soloman *et al*., 2013).

Screening of flavonoids: For estimation of flavonoids 1 mg of extract was mixed with 1 mL of Sodium hydroxide. Presence of yellow indicated flavonoids (Aziz, 2015).

Quantitative screening of phytochemicals

Characterization of total phenolic content: The total phenolic content (TPC) of the plant extract was analyzed by using spectrophotometric methods. Phenolic compounds are familiar for their antioxidant potential. The antioxidant activity has a direct relationship with phenolic compounds and flavonoid content. Therefore, the phenolic content of the methanolic extract of each fragment of wild pomegranate was assessed by using the method established by (Singleton *et al*., 1999). A 100 µL of the prepared extract solution (1000 µg/mL) was shifted to a 50 mL volumetric flask and diluted to five hundred microliters with the help of distilled water. The mixture was mixed with 100 µL of Folin-Ciocalteu reagent after it had been left for six minutes. One milliliter of a sodium carbonate solution containing seven percent was then added. After giving the mixture a good shake, it was left to react at room temperature for two hours. After that, the mixture was incubated at the same temperature for an additional hour. Using gallic acid as the standard, the absorbance of each sample was measured using a spectrophotometer set to 760 nm in wavelength. Milligrams of gallic acid equivalents (GAE) were used to measure the total phenolic content per gram of the dry extract fraction. Three measurements of each sample were taken to guarantee accuracy and precision.

Characterization of total flavonoids content: The total flavonoid content was determined using the method described by (Kim *et al*., 2003). Firstly, a 100 µg/mL prepared solution was transferred to a 50 mL volumetric flask and diluted to 500 µL using distilled water. After adding 100 µL of 5% sodium nitrite, the mixture was left to react for six minutes. The mixture was then allowed to react for five minutes after 150 µL of 10% aluminum chloride was added. Subsequently, two hours were spent stirring and letting the mixture sit at room temperature before adding 200 µL of 1 M sodium hydroxide. In this assay, quercetin was used as the standard. One gram of dry extract or fraction was used to calculate the milligrams of quercetin equivalents (QE) that comprised the total flavonoid content. A spectrophotometer was used to measure the mixture's absorbance at 510 nm, and the quercetin calibration curve was used to calculate the flavonoid content. To ensure the accuracy and reproducibility of the findings, the experiment was carried out with three replications.

Preparation of extract for high-performance liquid chromatography (HPLC) characterization: HPLC characterization was performed using the technique described by (Zeb, 2015). To prepare extracts for HPLC study. 1g of the ground material was mixed with a 1:1 water and methanol solution (20 mL; v/v). The mixture was heated at 70°C in a water bath for 1 hour and after heating, the mixture was centrifugated for 10 minutes at four thousand rotations per minute. After centrifugation, 2 mL of the supernatant was filtered into HPLC vials with the help of Whatman filter papers.

The Agilent-1260 Infinity HPLC system was employed to measure the amount of phenolic substances. The apparatus comprised of an auto-sampler, quaternary pump, degasser, and ultraviolet array detector. On an Agilent-Zorbax-Eclipse column (XDB-C18), separation was carried out. Two solvents, B and C, made up the gradient system. In solvent B, deionized water, methanol, and acetic acid were mixed in a ratio of 180:100:20 v/v, whereas in solvent C, the same ingredients were mixed in a ratio of 80:900:20 v/v. At 0, 5, 20, and 25 minutes, respectively, Solvent B was at 100%, 85%, 50%, and 30% of the gradient. We used 100% solvent C for thirty to 40

minutes and allowed for 25 minutes for elution. The UVAD was set to 280 nm for the examination of phenolic compounds, with the chromatogram noted between 190 and 500 nm. Phenolic compounds were identified based on retention times, UV spectra and available standards. The % peak area was quantified using the following equation:

$$Cx = \frac{Ax \times Cs\left(\mu g/_{ml}\right) \times V(ml)}{As \times Sample\ (wt.in\ g)} \qquad (1)$$

where: Cx = Sample concentration, as = Standard peak area, Ax = Sample peak area, Cs= Standard concentration (0.09 µg/mL)

DPPH free radical scavenging potential: DPPH free radical removal potential of the wild pomegranate extracts was assessed using the Brand-Williams technique (Brand-Williams *et al*., 1995). The procedure for evaluating the free radical scavenging activity of numerous plant portions (seeds, leaves, stem bark, roots, and rind) first extract solutions from various plant parts were prepared in methanol. Then these solutions were serially diluted to the concentrations: 1000, 500, 250, 125, and 62.5 µg/ml. Next, 0.1 mL of each diluted extract solution was mixed with 2 mL of DPPH solution in methanol. The mixtures were incubated for 30 minutes at 25°C. Absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid served as the standard in this experiment. The experiment was conducted three times, and the results were reported as the mean ± SEM (standard error of the mean).

The percentage of free radical scavenging activity was calculated using the following equation:

Percent DPPH scavenging activity = (A 0 - A 1) / A 0×100 -----(2)

where A0 is the absorbance of the control. And A1 is the absorbance of the sample

ABTS free radical scavenging potential: The antioxidant capacity of various wild pomegranate parts was evaluated through the application of a standardized methodology derived from the approach specified by Re *et al*., (1999). To assess the ability of the plant extracts to scavenge free radicals against ABTS (2,2'-azinobis [3-ethylbenzothiazoline]-6-sulfonic acid), a stock solution containing 1 mg/mL of the extracts was made in methanol. Following that, this stock solution was diluted to yield concentrations of 1000, 500, 250, 125 and 62.5 µg/mL. After combining ABTS (7 mM) and potassium persulfate (2.45 mM) solutions and letting them sit in the dark for the entire night, the ABTS radical cation (ABTS•+) was produced. In the assay, two milliliters of the ABTS solution and three hundred µL of each test sample were combined. A double-beam spectrophotometer was used to measure the mixture's absorbance. The experiment's positive control was ascorbic acid. Every examination was conducted thrice, and the outcomes were presented as the average ± standard error of the mean, or SEM. Using equation (2), the percentage of free radical scavenging activity was determined.

Antibacterial activity

Culturing of bacteria: *Xanthomonas oryzae* Gram-negative yellow bacteria were obtained from the Department of Agriculture Hazara University Mansehra Pakistan and sub-subculture on Potato Dextrose Agar (PDA) and Nutrient Agar media, usually. The subcultures were incubated for 48 hours at 28°C to ensure adequate growth after this, the culture is kept at 4ºC for further activity.

Disk diffusion method: The disc diffusion method was used to evaluate the antibacterial activity of the extracts against *Xanthomonas oryzae* on Mueller-Hinton Agar (MHA) (Matuschek *et al*., 2018). The discs were infused with methanolic extracts and were placed on agar plates that had been inoculated with the tested bacteria. As the disc absorbs moisture, the extract diffuses outward through the agar, creating a concentration gradient. The highest concentration of the extract is at the edge of the disc, decreasing with distance until it is no longer inhibitory, allowing bacterial growth. If the extract inhibits bacterial growth, a clear zone, known as the inhibition zone, forms around the disc after incubation. Autoclaved distilled water served as the negative control in this process. Additionally, one disc was dipped in water and kept in a medium, which did not form a clear zone. The diameter was measured in mm using a clear crystal meter rod. The experiment was performed in triplicate to confirm precision and duplicability. The average diameter of the inhibition zones was calculated and standard errors were determined to report the results with precision.

Minimum inhibitory concentration (MIC): Mic is defined as the lowest concentration of an extract that prevents the visible growth of a microorganism. This measure is essential for assessing the effectiveness of the extract in inhibiting bacterial growth (Mousavi *et al*., 2015). For MIC determination, methanolic extracts from the root, fruit rind and leaves of wild pomegranate, which demonstrated significant antimicrobial activity in the disc diffusion method, were selected. The MIC of these crude extracts was evaluated using the broth dilution method. In this method, an extract solution at 10 mg/mL was serially diluted two-fold to obtain concentrations of 200, 100, 50 and 20 µg/mL.

Results

Total phenolic content (TPC) and total flavonoid content (TFC): TPC and TFC of the methanolic extracts from several portions of the wild pomegranate were evaluated (Table 1). The wild pomegranate's seed had the highest phenolic contents i.e. 60.85 mg GAE/g, followed by leaves 60.82 mg GAE/g and rind 59.46 mg GAE/g, respectively. The stem and roots had the lowest phenolic content, measuring 46.41 and 43.35 GAE/g. The wild pomegranate leaf extract had the highest flavonoid content, measuring 64.82 mg QE/g, compared to 62.74 and 52.41 mg QE/g for the stem and root, respectively. Other parts of the fruit, like the rind and seeds, had 55.34 and 51.35 mg GAE/g.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) in different parts of wild pomegranate.

Plant parts	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Stem	46.41 ± 1.03	62.74 ± 1.04
Root	43.35 ± 1.04	52.41 ± 1.02
Leaves	60.82 ± 1.02	64.82 ± 1.06
Rind	59.46 ± 1.05	55.34 ± 1.06
Seed	60.85 ± 1.05	51.35 ± 1.03

DPPH and free radical scavenging activity: Methanolic extracts from different parts of the pomegranate were tested for their ability to scavenge free radicals using DPPH (Table 2). At the maximum concentration (1000 µg/mL), the highest scavenging activity was noted for seeds (59.22 µg/mL), followed by rind (59.12 µg/mL), leaves (58.13 µg/mL), stem bark (57.10 µg/mL) and root (50.25 µg/mL). The DPPH scavenging activity showed a concentration-dependent response for each methanolic extract. At the maximum concentration of 1000 µg/mL, ascorbic acid was used as a standard and was associated with the percentage inhibition of DPPH radicals. Ascorbic acid exhibited a high 80.30% inhibition potential and an IC50 value of 60 µg/mL. The DPPH radical scavenging activity of the different components varied, and the scavenging potential increased in a concentration-dependent manner.

ABTS free radical scavenging activity: The ABTS free radical scavenging activity of various wild pomegranate parts were measured. At the highest concentration (1000 µg/mL), the percentage of scavenging activities was as follows: 65.16 ± 0.12% for the seed, 62.33 ± 1.19% for the rind, 60.10 ± 1.13% for the leaves, 55.23 ± 1.13% for the stem bark, and 48.32 ± 1.16% for the root. The standard, ascorbic acid, exhibited an inhibition potential of 86.52 ± 0.34%. The methanolic extracts of the stem bark, leaves, seed, rind, and root had IC50 values of 680 µg/mL, 870 µg/mL, 975 µg/mL, 1000 µg/mL and 990 µg/mL, in that order. Ascorbic acid's IC50 value was 60 µg/ml (Table 2).

HPLC characterization of phenolic compounds: The retention time (Rt), distinct peak position, and identification and quantification of each phenolic constituent are compiled in (Figs. 1-4; Table 3). Seven phenolic compounds (including malic acid, vitamin C, chlorogenic acid, quercetin, ellagic acid, and pyrogallol) were found in the leaves (Fig. 1). Six phenolic compounds (malic acid, vitamin C, chlorogenic acid, quercetin, epigallocatechin gallate, and ellagic acid) were present in the root (Fig. 2). Five phenolic compounds were found in the methanolic extract of the fruit rind: pyrogallol, vitamin C, chlorogenic acid, quercetin, and malic acid (Fig. 3). Six different phenolic compounds were identified in the methanolic extract of wild pomegranate seed: malic acid, vitamin C, chlorogenic acid, quercetin, and epigallocatechin gallate (Fig. 4). Based on their UV spectra and retention periods, phenolic compounds were identified, and their peak area percentage was used to quantify them.

Table 2. Percentage DPPH and ABTS free radical scavenging activity of different parts of wild pomegranate.

Plant parts	Concentration (ug/mL)	% DPPH Scavenging (Mean ± S.E.M)	IC50 (ug/mL)	% ABTS Scavenging Mean ± S.E.M	IC50 (ug/mL)
Leave	1000	58.13 ± 1.30 **	345	60.10 ± 1.13 **	680
	500	53.25 ± 1.64 **		45.20 ± 0.03 ***	
	250	46.16 ± 0.41 ***		43.18 ± 0.04 ***	
	125	37.25 ± 0.90 ***		39.28 ± 1.18 ***	
	62.5	30.29 ± 0.40 ***		33.18 ± 1.20 ***	
Stem	1000	57.10 ± 0.44 **	750	55.23 ± 1.13 **	870
	500	47.19 ± 0.45 ***		50.18 ± 1.03 ***	
	250	40.40 ± 0.60 ***		40.28 ± 1.20 ***	
	125	33.44 ± 0.63 ***		36.21 ± 1.17 ***	
	62.5	25.15 ± 0.54 ***		30.42 ± 0.24 ***	
Seed	1000	59.22 ± 1.34 **	795	65.16 ± 0.12 ***	975
	500	46.20 ± 1.50 ***		45.35 ± 0.20 ***	
	250	43.15 ± 1.40 ***		38.33 ± 1.15 ***	
	125	37.40 ± 0.60 ***		30.36 ± 1.22 ***	
	62.5	30.15 ± 0.38 ***		28.36 ± 1.14 ***	
Root	1000	50.25 ± 1.36 ***	890	48.32 ± 1.16 ***	990
	500	46.15 ± 1.41 ***		36.23 ± 1.13***	
	250	36.21 ± 1.34***		33.51 ± 1.40 ***	
	125	25.23 ± 1.36***		22.23 ± 1.11 ***	
	62.5	20.05 ± 1.22 ***		20.16 ± 1.05 ***	
Rind	1000	59.12 ± 1.41 **	900	62.33 ± 1.19 ***	1000
	500	46.12 ± 1.48 ***		37.24 ± 1.18 ***	
	250	39.24 ± 1.38***		34.51 ± 1.44 ***	
	125	30.23 ± 1.39 ***		27.23 ± 1.15 ***	
	62.5	24.07 ± 1.31 ***		25.16 ± 1.06 ***	
Ascorbic acid	1000	80.30 ± 0.60	60	86.52 ± 0.34	60
	500	76.30 ± 0.50		70.56 ± 0.51	
	250	63.70 ± 0.60		61.51 ± 0.40	
	125	55.22 ± 0.66		60.52 ± 0.33	
	62.5	50.96 ± 0.36		53.46 ± 0.20	

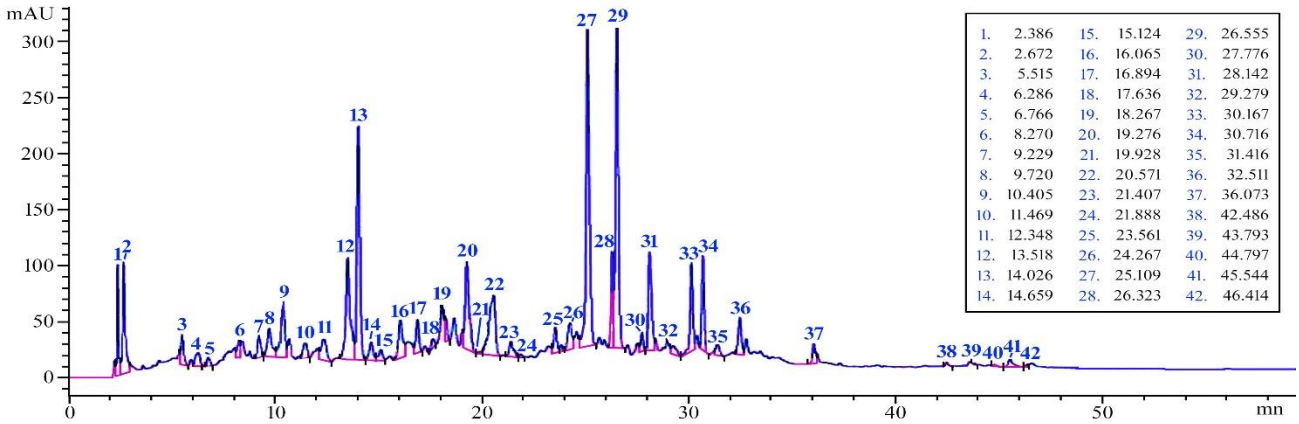

Fig. 1. HPLC chromatogram of leaves extract.

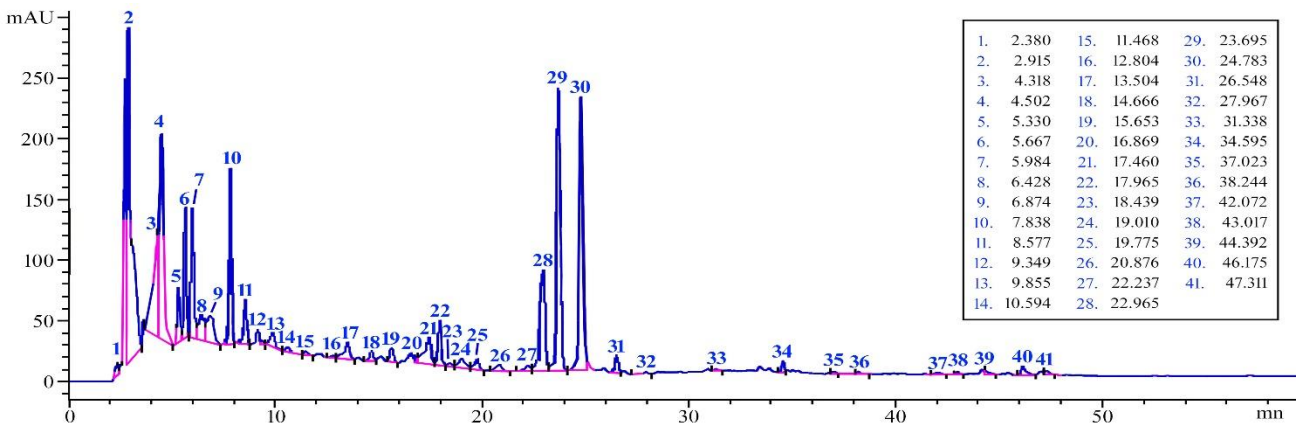

Fig. 2. HPLC chromatogram of root Methanol extract.

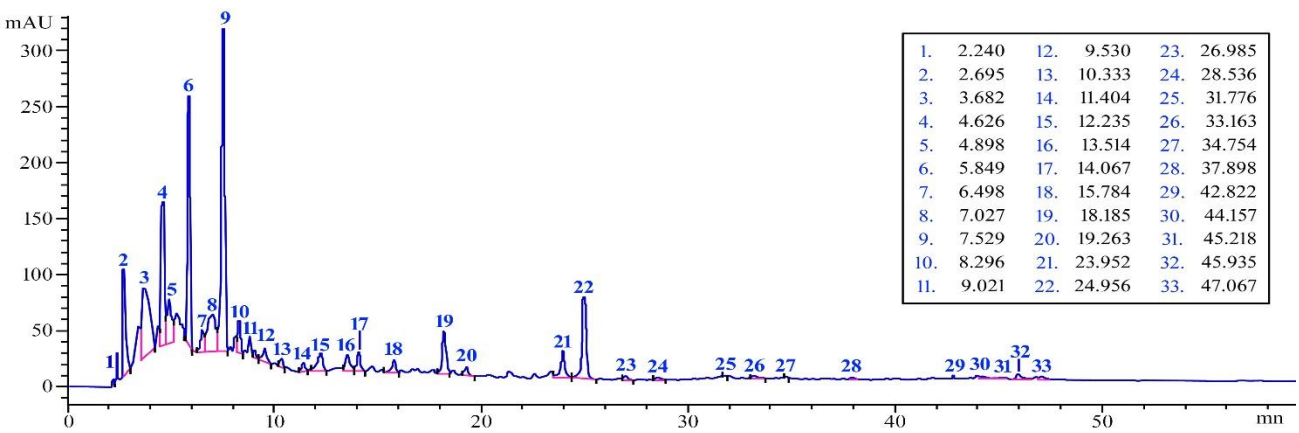

Fig. 3. HPLC chromatogram of rind extract.

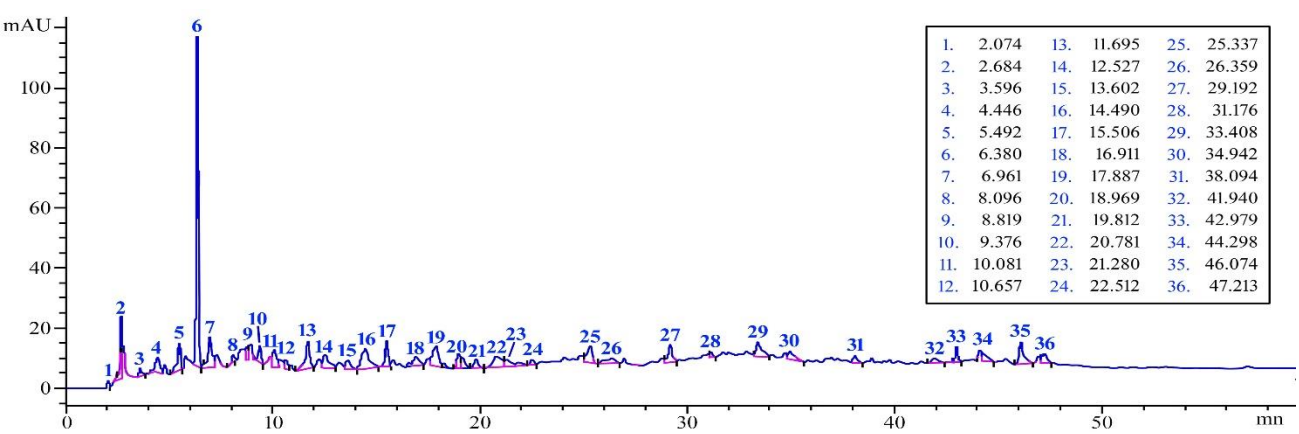

Fig. 4. HPLC chromatogram of seeds methanolic extract.

Table 3. List and measurement of phenolic substances from different wild pomegranate extracts.

Plant extract	Retention time (min)	Phenolic compound	HPLC-UV λmax (nm)	Peak area (sample)	Peak area (standard)	Concentration (µg/ml)	Identification reference
Leaves	2	Malic acid	320	453.644	40.32	10.125	Reference standard
	5	Vitamin C	320	244.718	22.376	9.842	DO
	6	Chlorogenic acid	320	81.226	12.93	5.654	DO
	8	Epigallocatechin gallate	320	163.982	7261.47	0.020	DO
	10	Quercetin	320	720.791	7089.28	0.092	DO
	16	Ellagic acid	320	319.259	319.24	0.900	DO
	28	Pyrogallol	320	1001.777	1.014	889.204	DO
Root	2	Malic acid	320	69.451	40.32	1.550	DO
	5	Vitamin C	320	378.201	22.376	15.211	DO
	6	Chlorogenic acid	320	432.074	12.93	30.075	DO
	8	Epigallocatechin gallate	320	387.822	7261.47	0.0481	DO
	10	Quercetin	320	60.406	7089.28	0.007	DO
	16	Ellagic acid	320	42.309	319.24	0.005	DO
Fruit rind	2	Malic acid	320	33.580	40.32	0.749	DO
	5	Vitamin C	320	2635.935	22.376	106.021	DO
	6	Chlorogenic acid	320	331.185	12.93	23.052	DO
	10	Quercetin	320	139.009	7089.28	0.0176	DO
	28	Pyrogallol	320	62.578	1.014	55.548	DO
Seed	2	Malic acid	320	121.344	40.32	2.708	DO
	5	Vitamin C	320	88.453	22.376	0.010	DO
	6	Chlorogenic acid	320	723.823	12.93	50.382	DO
	8	Epigallocatechin gallate	320	41.293	7261.47	0.005	DO
	10	Quercetin	320	85.815	7089.28	0.0108	DO
	16	Ellagic acid	320	56.125	319.24	0.158	DO

Table 4. Mean standard error of inhibitory activity in (mm) of different part of wild pomegranate against *Xanthomonas oryzae* gram negative bacteria.

Test pathogen	Plant parts	Concentration of extracts (mg/mL)			Negative control
		10mg/mL	20mg/mL	30mg/mL	A.D. Water
Xanthomas oryzae	Root	10 ± 0.08mm	11 ± 0.50mm	12 ± 1.04mm	0mm
	Fruit rind	12 ± 0.22mm	14 ± 0.45mm	16 ± 0.62mm	0mm
	Leaves	11 ± 0.76mm	13 ± 0.33mm	14 ± 0.57mm	0mm

Table 5. MIC of different parts of wild pomegranate at different concentrations.

Test pathogen	Plant parts	Concentration of extracts (µg/ml)				Negative control
		20 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	A.D. Water
Xanthomonas oryzae	Root	7.5 ± 3.5mm	8 ± 1.2mm	9 ± 0.52mm	9.5 ± 0.40mm	0 ± 0 mm
	Fruit rind	9.5 ± 2.1mm	10 ±0.50mm	11 ± 0.7mm	12.5 ± 0.1mm	0 ± 0
	Leaves	8.7 ± 1.52mm	9.6 ± 0.51	10 ± 0.9mm	10.8 ± 0.54mm	0 ± 0

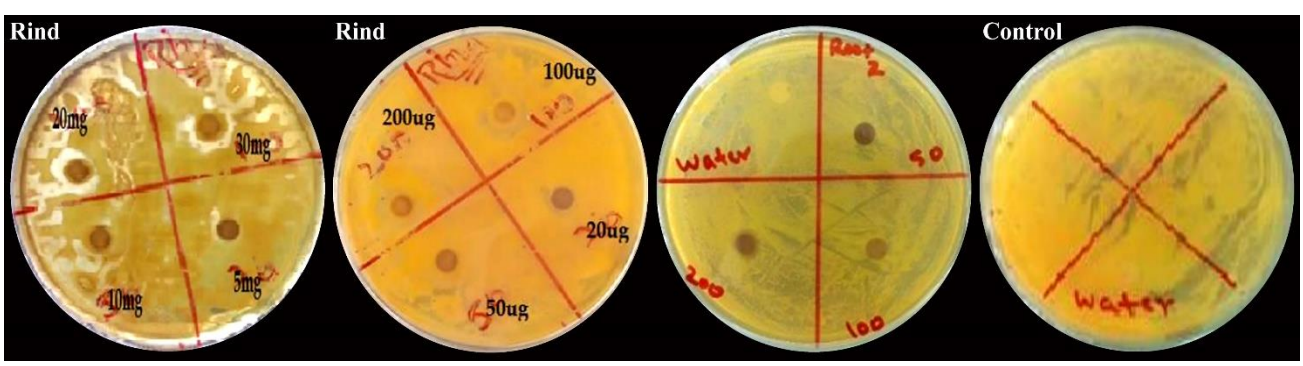

Fig. 5. Inhibition Zone of Methanolic Extracts from Wild Pomegranate Against *Xanthomonas oryzae*.

Antibacterial activity: The Zone of inhibition in Millimeter (mm) of methanolic extract of different parts (Root, fruit Rind, Leave) of wild pomegranate against *Xanthomonas oryzae* a gram-negative bacterium at different concentrations 10mg/mL, 20mg/mL, 30mg/mL (Table 3) All the parts showed inhibitory activity. The inhibitory activity of wild pomegranate root was (10±0.8, 11±0.50, 12±1.04mm) the fruit rind (12±0.22, 14±0.45, 16±0.62mm) while leaves (11±0.76, 13±0.33, 14±0.57mm). Negative control was used autoclave double distal water which showed zero inhibitory activity. The highest inhibitory activity was shown by pomegranate fruit rind (16±0.62mm) at 30mg /ml concentration while the lowest inhibitory activity was shown by wild pomegranate root (10±0.8mm) at 10mg/mL concentration (Table 5).

Minimum inhibitory concentration (MIC): The term "minimum inhibitory concentration" (MIC) describes the lowest extract concentration at which a microorganism appears to be non-growing. For each part of the wild pomegranate-root, fruit rind, and leaves-the minimum inhibitory concentration (MIC) in millimeters (mm) against *Xanthomonas oryzae* is provided. (Fig. 5; Table 5). All parts show inhibitory activity at minimum concentration, but the fruit rind shows high inhibitory activity at minimum concentration as compared to other parts such as root and leaves at that concentration.

Discussion

Many of the medicines used today were derived from natural sources, especially plants, as nature was a ridiculous repository for medicinal plants thousands of years ago (Afsar *et al*., 2016). Nowadays, it's believed that plants are a major source of antioxidant chemicals (flavonoids, anthocyanins, and phenols), which are primarily used as essences in food manufacturing to keep food from going bad. The antioxidant capacity and antibacterial activity of wild pomegranate parts (stem bark, leaves, rind, and roots) are investigated in this study, along with the HPLC characterization of various phytochemicals. Plant secondary metabolites are an important class of phytochemicals because they possess antioxidant properties. It has been demonstrated that certain phenolic compounds possess antiviral, antibacterial, antitumor, and antioxidant qualities. Since flavonoids are particularly effective scavengers for the majority of oxidized molecules, they are important antioxidants that are frequently found in flower tissues, leaves, and pollen. (Moncayo *et al*., 2021). The results showed that the leaves sample had the highest total phenols and total flavonoid content, whereas seed extracts had the lowest values. Analysis revealed that the wild pomegranate is a high source of naturally occurring phytochemicals, which is consistent with previously published investigations (Saeed *et al*., 2020).

Antioxidants can neutralize free radicals such as superoxide and hydroxyl radicals due to their structural features, including benzene rings (Ullah *et al*., 2021; Gul *et al*., 2022). To evaluate the total antioxidant activities of wild pomegranate parts (stem bark, leaves, seeds, rind, and roots), DPPH and ABTS assays were employed as free radical scavengers. The removal of DPPH and ABTS free radicals is a crucial step in assessing the antioxidant capacity of plant extracts. The study found that wild pomegranate seed extract is highly effective at scavenging free radicals. These findings are consistent with previous research by (Batool *et al*., 2010) who reported that ethanolic extracts of *Zanthoxylum alatum* fruit exhibit strong free radical scavenging activity. Other studies have also demonstrated that extracts from the leaves, seeds, and bark of *A. incana* and *A. virdis* possess significant abilities to eliminate DPPH free radicals (Sutanto *et al*., 2019) According to the published research study, methanolic extracts of wild pomegranate have maximum polyphenols content and can have high free radicals scavenging activity. Our results are similar to the findings of (Middha *et al*., 2013), that he originate a momentous quantity of phenols and flavonoids in the seed, leave and rind extracts of wild pomegranate.

HPLC characterization of phytochemicals investigates the antioxidant potential of probable phytochemicals. Among the recognized phytochemicals, the leaves of wild pomegranate contain seven phytochemicals: vitamin C, chlorogenic acid, malic acid, epigallocatechin gallate, quercetin, ellagic acid, and pyrogallol. The root has six phytochemicals: malic acid, vitamin C, chlorogenic acid, epigallocatechin gallate, quercetin and ellagic acid. The rind includes five phytochemicals: malic acid, vitamin C, quercetin, chlorogenic acid, and pyrogallol. The seed extract also contains six phytochemicals: malic acid, vitamin C, epigallocatechin gallate, quercetin, ellagic acid, and pyrogallol. Our results align with previous research that identified similar phytochemicals. For instance, methanolic extracts of *Silybum marianum* (L.) seeds have been shown to contain quercetin and morin among other phytochemicals, while *Elaeagnus umbellata* Thumb's fruit has twelve phenolic components including malic acid, gallic acid, vitamin C, chlorogenic acid, epigallocatechin gallate, quercetin, morin, ellagic acid, catechin hydrate, rutin, pyrogallol and mandelic acid (Nazir *et al*., 2018), Additionally, malic acid, chlorogenic acid, quercetin, rutin, pyrogallol, mandelic acid, hydroxybenzoic acid, and morin were identified in the methanolic extracts of *Rosa moschata* leaves and fruits.(Nazir *et al*., 2020). Phenolic compounds have also been detected in *Pisum sativum* L., which exhibited enhanced antioxidant capacity. Based on these findings, it can be concluded that malic acid, chlorogenic acid, epigallocatechin gallate, quercetin, ellagic acid, and pyrogallol are likely the primary phenolic components responsible for the antioxidant activity observed in wild pomegranate. In another result, it described that these compounds, chlorogenic acid (Gökbulut *et al*., 2017), epigallocatechin gallate (Moreno-Vásquez *et al*., 2021), quercetin (Halevas *et al*., 2021), ellagic acid and pyrogallol (Sutanto *et al*., 2019) have influential antioxidant possessions.

Wild pomegranate is a rich source of naturally occurring phytochemicals due to these phytochemicals it is a powerful antibacterial and antifungal activity. The MIC values exhibited the antibacterial activity of wild pomegranate methanolic extract of different parts over *Xanthomonas oryzae* a gram-negative bacterium, the highest activity was shown by pomegranate fruit rind. This result was reliable the former study (Dahham *et al*., 2010) The antibacterial effects

of alcoholic extracts from pomegranate fruit peel and seed juice on various bacteria, including *S. aureus*, have been investigated. Pomegranate peel extract was found to exhibit the highest antimicrobial activity (Naziri *et al*., 2012). Methanolic extracts of pomegranate peel be more effective against Gram-positive bacteria compared to Gram-negative bacteria, with *S. aureus* being particularly sensitive to these extracts (Kanatt *et al*., 2010). Furthermore, the application of pomegranate peel extract to common chicken meat products was reported to extend their shelf life due to its antibacterial properties against *S. aureus*. The varying sensitivity of bacteria to extracts from different parts of the pomegranate plant is attributed to the distinct composition of these extracts (Negi *et al*., 2003). Extracts from pomegranate peels using various polar solvents at room temperature have demonstrated antibacterial activity against *P. aeruginosa* and *S. aureus*. The minimum inhibitory concentration (MIC) values for these pomegranate extracts against *S. aureus* and *P. aeruginosa* have been reported to range from 40 to over 90 µg/mL (Duman *et al*., 2009) found that pomegranate extracts could inhibit or delay the growth of *S. aureus* at concentrations ranging from 0.01% to 1% v/v.

Conclusion

The fruit, rind, and root of the pomegranate plant are all recognized for their medicinal properties. In this study, methanolic extracts from various wild pomegranate parts-leaves, stem bark, seeds, fruit rind, and roots-were used to determine the phytochemical composition as well as the total phenol and flavonoid content. It was determined how effective these ingredients were as antioxidants against the free radicals DPPH and ABTS. DPPH and ABTS radicals were found to be effectively inhibited by the seed extract, with IC50 values of 795 µg/mL and 975 µg/mL, respectively. Phenolic compounds vary in concentration, and some samples have higher levels of phenolics and flavonoids than others, which may explain the variation in antioxidant activity among the various parts of the wild pomegranate. The findings of this study indicate that wild pomegranates are a rich source of phytoconstituents with potential medical applications, including quercetin, pyrogallol, malic acid, and chlorogenic acid. These components may be the cause of the observed antibacterial activity and antioxidant potential against the Xanthomonas oryzae bacteria. While this study did not focus on any particular disease, the measurement of antioxidant characteristics can direct the application of these plants in the treatment of diseases like diabetes that are linked to reactive oxygen species (ROS). To fully realize the antioxidants' potential in managing pertinent diseases, more investigation is required to isolate, identify, and comprehend the underlying mechanisms of these compounds.

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