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PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF POMEGRANATE PEEL

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ABSTRACT: Pomegranate (*Punica granatum L.*) has been used for the prevention and treatment of a various health issues such as Cancer, diabetes, inflammation, dental plaque, dysentery, and to fight malarial parasites and intestinal infections. It is an important source of many bioactive compounds. In the present study, the potential phytochemicals present in the different extracts (Aqueous, Acetone, Ethanol and Hexane) of pomegranate peel were carried out. Phytochemical investigations included phenols, tannins, flavonoids, anthocyanins, coumarins, quinone, saponins, steroids, glycosides, cycloglycosides, terpenoids, alkaloids, fatty acids, carbohydrates, proteins and amino acids. Among these four different extracts, aqueous extracts of pomegranate peel contains more number of phytochemicals like phenols, glycosides, flavonoids, terpenoids, carbohydrates, proteins and amino acids. Antioxidant study was evaluated by performing DPPH, flavonoids, total phenolic test and FRAP assay to identify the percentage of scavenging by the chemical constituents. DPPH activity is maximum in acetone extract whereas flavonoid activity, total phenol activity and FRAP activity of pomegranate peel is maximum in ethanol extract. Antibacterial activity of pomegranate peel extract was carried out against *Escherichia coli* and *Bacillus subtilis* using Well Diffusion method on the Nutrient agar medium. Acetone and ethanolic extracts of pomegranate peel were most effective in inhibiting the growth of a number of bacteria. It is concluded that pomegranate peel is a very essential plant medicinally. A long term research project is a must to evaluate the pharmacological uses of extracts with different solvents that can be used to isolate the pure and high yield of chemical constituents from the plants.

KEYWORDS: Pomegranate peel, Phytochemical Screening, antioxidants and antibacterial activity.

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1. INTRODUCTION

A medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs [21]. In the traditional medicine system, parts of many plants are used for the treatment of various human ailments [10]. Pomegranate (*Punica granatum* L.) is native to the Mediterranean region and has been used extensively in the folk medicine of Indian subcontinent and many other countries. The name Pomegranate is derived from the Latin words 'ponus' and 'granatus'. The world pomegranate production amounts to approximately 1,500,000 tons [7]. Among various plant sources, Pomegranate is one of the interesting colorful and an important medicinal plant, and mainly its fruit contain many therapeutic uses [21]. The pomegranate peels constituents approximately 60 % of the total weight of the pomegranate fruit. The pomegranate peel is considered as an agro-waste but it can be a potential source of antioxidants, numerous phytochemicals and also possesses antibacterial and antifungal activity [27]. The amount of Polyphenols is rich in this fruit. Phytochemical compounds such as gallotannins, ellagic acid, gallic acid, punicalins, and punicalagins present in pomegranate peel. *Punica granatum* is a rich source of bioactive compounds and contain a variety of secondary metabolites. The fruit is rich in tannins and other biochemical's [10]. The nutritional parameters are not limited to the edible part of the fruit, the vital role are played by the non-edible fractions of fruit and tree i.e. leaves, barks, buds, flower and peel. Although, these parts are considered to be waste, they contain enormous amount of nutritional value and biological active compounds compared to the edible portion of the fruit [21]. Pomegranate peel is an inedible portion of the fruit, is found to have higher medicinal value. Pomegranate peel attracts attention due to its apparent wound healing properties, immune modulatory activity and antibacterial activity, anti-atherosclerotic and anti-oxidative capacities. The entire plant has medicinal property such as the seeds and juice cure throat problems, eye diseases, gum bleeds, toning skin, cancer, cardiovascular disease, diabetes, infant brain ischemia and male infertility [29]. Considering all these facts, the present study was designed to investigate the presence of various Phytochemicals, analyze antioxidants and antibacterial activity. This study is also suggested for use of pigment present in the Pomegranate peel in textile industry for dyeing a cotton fabric in the presence of mordents.

2. MATERIALS AND METHODS

2.1. Collection and processing of sample: The Pomegranate fruit was collected from local market of erode area. From collected fruits, remove the peel and is used as a sample. Then, the peel were rinsed under running water to remove solid particles from the surface and allowed to shade dry for 5 days, grained to powder and stored in air tight container.

2.2. Preparation of plant extract: 10 g of fresh sample material (Pomegranate peel) is mixed with 100 ml of different solvents (distilled water, ethanol, hexane and acetone). This was kept in rotatory shaker for 24 hours of incubation and finally filtered using Whatmann No. 1 filter paper. The above filtrate is stored in refrigerator and used for further study.

2.3. Preliminary Phytochemical Screening: Aqueous extract, acetone extract, ethanol extract and hexane ether extract of Pomegranate peel were subjected to qualitative analysis for the identification of various active constituents like alkaloids, flavonoids, steroids, tannins, glycosides and phenols.

2.3.1. Test for Carbohydrates

(a) Molish's test: To a few drops of extract, 2 ml of molish's reagent is added. The mixture is shaken well and 2 ml of Conc. H_2SO_4 is added slowly along the sides of the test tube and allowed to stand. A reddish ring formed at the junction of two solutions indicates the presence of carbohydrates.

(b) Fehling's test: To a few drops of extract, 2 ml of Fehling's reagent is added. The mixture is shaken well and boils for 5 minutes. Brick red precipitate indicates the presence of sugar.

2.3.2. Test for Alkaloids

(a) Mayer's test: To a few drops of extract, 2 drops of Mayer's reagent is added by the side of the test tube. A green colored precipitate confirms the test as positive.

(b) Wagner's test: To a few drops of extract, 2 drops of Wagner's reagent is added by the side of the test tube. A reddish brown precipitate confirms the test as positive.

2.3.3. Test for Saponins

(a) Foam test: To a few ml of extract, 20 ml of distilled water is added in the test tube and the test tube is continuously shaken for 10 minutes. The foam formed confirms the presence of saponins.

(b) Froth test: 3 ml of extract were mixed with 10 ml of distilled water in a test tube. The test tube was shaken vigorously for about 5 minutes and it was allowed to stand for 30 minutes and observed for froth. The presence of froth indicates the presence of saponins.

2.3.4. Test for Tannins

(a) Lead Acetate test: To a few ml of extract, few drops of 1% Lead acetate is added. The mixture is shaken well. A yellowish precipitate indicates the presence of tannins.

2.3.5. Test for Flavonoids

(a) Acid test: To a few ml of extract, few drop of Dilute H_2SO_4 is added. Orange colour develops which indicates the presence of flavonoids.

2.3.6. Test for Terpenoids

(a) Acetic anhydride test: To 2 ml of extract, 2 ml of acetic anhydride and Conc. H_2SO_4 is added. Formation of blue, green rings indicate the presence of terpenoids.

2.3.7. Test for Amino Acids

(a) Ninhydrin test: To a few drops of extract, few drop of Ninhydrin solution is added in a test tube. A characteristic blue colour indicates the presence of amino acids.

2.3.8. Test for Proteins

(a) Biuret Test: Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour.

(b) Millon's test: To a few ml of extract, few drop of Millon's reagent is added. White precipitate

indicates the presence of protein.

2.3.9. Test for Glycosides

(a) Liebermann's test: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added. Formation of violet to blue to green reddish brown ring indicates the presence of glycosides.

2.3.10. Test for Cardiac glycosides: In a test tube added 5 ml of extract and 2 ml of glacial acetic acid and 1 drop of ferric chloride and 1.0 ml of Conc. H₂SO₄ is added slowly along the sides of the test tube and allowed to stand. Formation of brown, violet, greenish rings indicate the presence of cardiac glycosides.

2.3.11. Test for Phlobotannins: Few drops of extract were boiled along with 1% HCl. Formation of red precipitate indicates the presence of Phlobotannins.

2.3.12. Test for Coumarins: To 2 ml of extract, 10% of 3 ml NaOH is added. Formation of yellow indicates the presence of coumarins.

2.3.13. Test for Cycloglycosides: In a test tube added 5 ml of extract and 2 ml of acetic acid and 1 drop of ferric chloride and 1.0 ml of Conc. H₂SO₄ is added slowly along the sides of the test tube and allowed to stand. Formation of brown, violet, greenish rings indicate the presence of cycloglycosides.

2.3.14. Test for Total Phenols: To 2 ml of extract, 3% of FeCl₂ is added. Formation of deep blue colour indicates the presence of total phenol.

2.3.15. Test for Quinone: Few drops of extract added 5 ml of HCl. Formation of yellow precipitate indicates the presence of quinone.

2.3.16 Test for Anthraquinones: To 2 ml of extract, 2 ml of 10% Ammonium hydroxide is added. Formation of bright pink colour indicates the presence of anthraquinones.

2.3.17. Test for Steroids: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added reddish brown colour is formed. To this added 1 ml of Conc. H₂SO₄. Formation of violet to blue green colour indicates the presence of Steroids.

2.3.18. Test for Carotenoids: 10 ml of extract is evaporated to dryness. To this 2 to 3 drops of Conc. H₂SO₄ and chloroform was added. Formation of blue colour indicates the presence of carotenoids.

2.3.19. Test for Fatty Acids: To a few ml of extract are pressed in filter paper and dried. The transparency appeared in the filter paper indicates the presence of fatty acid.

2.3.20. Test for Cholesterol: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added. To this added 1 ml of Conc. H₂SO₄. Formation of violet to blue green colour indicates the presence of cholesterol.

2.3.21. Test for Anthocyanins: To 2 ml of extract, 2 ml of 2N NH₄Cl and ammonium is added. Appearance of pink red to blue violet colour indicates the presence of anthocyanin.

2.3.22. Test for Leuco anthocyanins: To 5 ml of extract, 5 ml of Iso amyl alcohol is added. Formation of Upper Layer Red indicates the presence of Leuco anthocyanins.

2.3.23. Test for Phenols: To 2 ml of extract, 3 ml of ethanol and a pinch of ferric chloride are added. A greenish yellow colour appears which indicates the presence of Phenols.

2.3.24. Test for Emodins: 2 ml of NH_4OH and 3 ml of benzene is added to few ml of extract. Formation of red colour indicates the presence of emodins.

2.4. Antioxidant Activity

2.4.1. DPPH Assay: To 100 μl of plant extracts added 0.4 ml of DPPH (0.1mM) solution and 0.4 ml of 50mM Tris HCl. The solutions are mixed well and incubated in dark condition at room temperature for 30 min. After 30 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The ascorbic acid is taken as standard. The % radical scavenging activity of the plant extracts was calculated using the following formula, (Where, RSA is the Radical Scavenging Activity).

$$\text{DPPH (\%)} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

2.4.2. Determination of Flavonoids: 0.5ml of each extract solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank of all extracts were prepared and their absorbance was measured at 415 nm.

Prepare a standard curve using different concentration of Catechol (20 μg , 40 μg , 60 μg , 80 μg and 100 μg). From the curve find out the concentration of flavonoids in the test sample and expressed as mg phenols/100 gm material.

2.4.3. Determination of Total Phenolic Content: 1 ml of different extracts was taken in different test tubes added 0.5 ml of Folin-Ciocalteu reagent. After 3 minutes, added 2.0 ml of 20% Sodium carbonate solution. Mixed thoroughly and placed the tubes in boiling water for exactly one minute, cooled and measured the absorbance at 765 nm against the reagent blank.

Prepare a standard curve using different concentration of Quercetin (5 μg , 10 μg , 15 μg , 20 μg and 25 μg). From the curve find out the concentration of total phenol in the test sample and expressed as mg phenols/100 gm material.

2.4.4. Determination of Ferric Reducing Antioxidant Power (FRAP): One ml of each extract at various concentrations (0 - 500 mg/l) was added to a test tube. One ml potassium phosphate buffer (0.2 M, pH 6.6) and freshly prepared potassium ferricyanide (1 ml, 1%) were added to extracts. The mixture was incubated in a water bath (50°C for 20 min). One ml of trichloroacetic acid (10% TCA) was added to the mixture followed by centrifugation at 5000 g for 5 min. From the upper layer of mixture, 1 ml was taken and mixed with 1 ml distilled water followed by 100 μl of freshly prepared FeCl_3 (0.1 %). The absorbance of samples was measured at 765 nm against blank.

$$\text{FRAP (\%)} = \frac{\text{FRAP BLANK} - \text{FRAP TEST}}{\text{FRAP BLANK}} \times 100$$

2.5. Antibacterial Assay by Disc Diffusion Method

Agar well diffusion method was used to evaluate the antibacterial activity of peel extracts against test microorganism. Nutrient agar medium (pH 7.0) was prepared and autoclaved. It was allowed to cool up to 45°C. Then it was seeded aseptically with 500µl of freshly prepared inoculums (10⁶ colony forming unit, CFU) and immediately mixed. For inoculum preparation, the colonies of bacteria such as *E. coli* and *Bacillus subtilis* were suspended in nutrient broth and turbidimetrically adjusted. Twenty five milliliters of seeded nutrient agar media was transferred into each Petri plate and solidify. The organisms were spreaded in different petri plates. Four wells were made in each plate. Test solution of 50 µl was poured into each respective well. These plates were incubated at 37°C. After 24 hours of incubation, the diameter of the clear zones that showed inhibition of bacterial growth was measured in millimeter (mm). Experiment was done in triplicate and mean value of zone inhibition was calculated with standard error.

3. RESULTS AND DISCUSSION

In the present study, evaluated the phytochemical analysis, Antioxidant activity and Antibacterial activity of different extracts of pomegranate (*Punica granatum*) peel and also deals with the investigation of biochemical constituents, Mineral composition and Secondary metabolites present in the pomegranate peel sample to assess the nutritional potential of the sample.

3.1. Phytochemical Screening: The results of phytochemical screening were obtained as follows

S. No	Test for Phytochemicals	Sample Extracts			
		Aqueous	Acetone	Ethanol	Hexane
1.	Carbohydrates				
	(a) Molish's test	+	+	+	+
	(b) Fehling's test	+	+	+	+
2.	Alkaloids				
	(a) Mayer's test	+	-	-	-
	(b) Wagner's test	+	-	-	-
3.	Saponins				
	(a) Foam test	-	-	-	-
	(b) Froth test	+	+	+	+
4.	Tannins				
	(a) Lead Acetate test	+	+	-	-
5.	Flavonoids				
	(a) Acid test	+	+	+	+
6.	Terpenoids				
	(a) Acetic anhydride test	+	+	+	+

7.	Amino Acids (a) Ninhydrin test	+	+	+	+
8.	Proteins (a) Biuret test (b) Millon's test	+	+	+	+
9.	Glycosides (a) Liebermann's test	+	+	-	-
10.	Cardiac Glycosides (a) Legal's Test	+	+	-	+
11.	Phlobotannins	-	-	-	-
12.	Coumarins	+	+	-	-
13.	Cycloglycosides	+	+	+	-
14.	Total phenols	+	-	+	-
15.	Quinone	-	-	-	-
16.	Anthraquinones	-	-	-	-
17.	Steroids	+	-	+	+
18.	Carotenoids	-	-	-	-
19.	Fatty Acids	-	-	-	-
20.	Cholesterol	-	-	-	-
21.	Anthocyanins	-	-	-	-
22.	Leuco Anthocyanins	-	-	-	-
23.	Phenols	+	+	+	+
24.	Emodins	-	-	-	-

'+'=Presence, '-'=Absence

The Phytochemical screening of various extracts shows the presence of certain important components such as phenols, glycosides, flavonoids, terpenoids, carbohydrates, proteins and amino acids. Phytochemical constituents afford imperative pharmaceutical properties for human health. These compounds can be used as drugs or as dietary supplements to heal or to prevent various diseases.

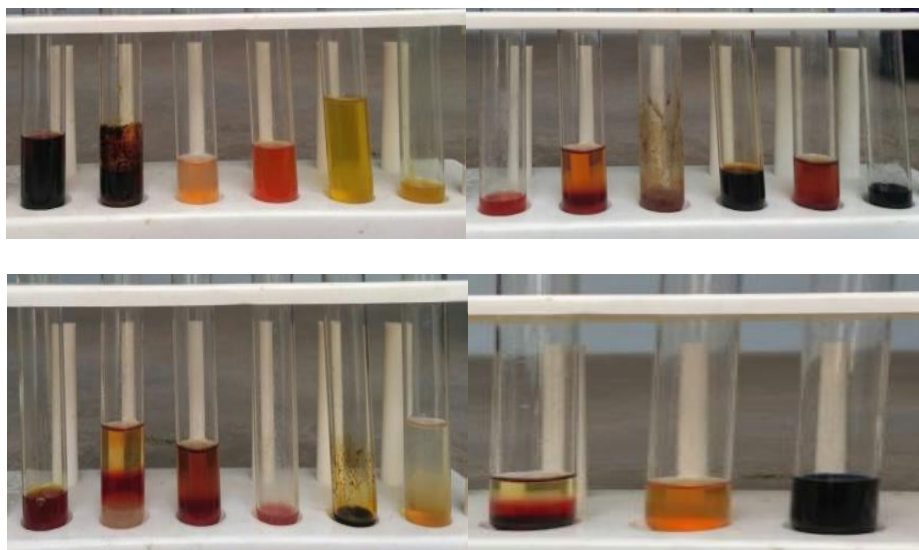


Figure 1: Results showing that phytochemical screening of Aqueous extract of pomegranate peel

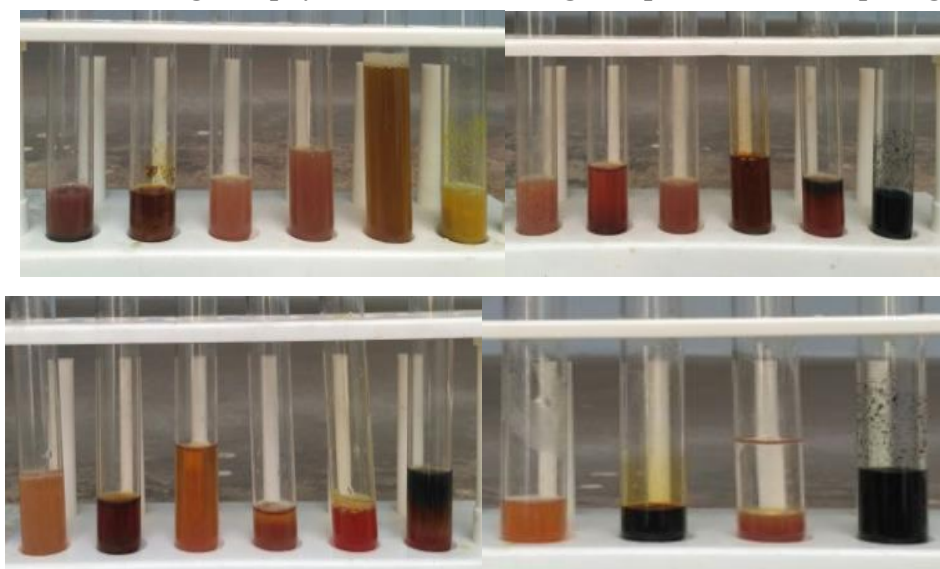
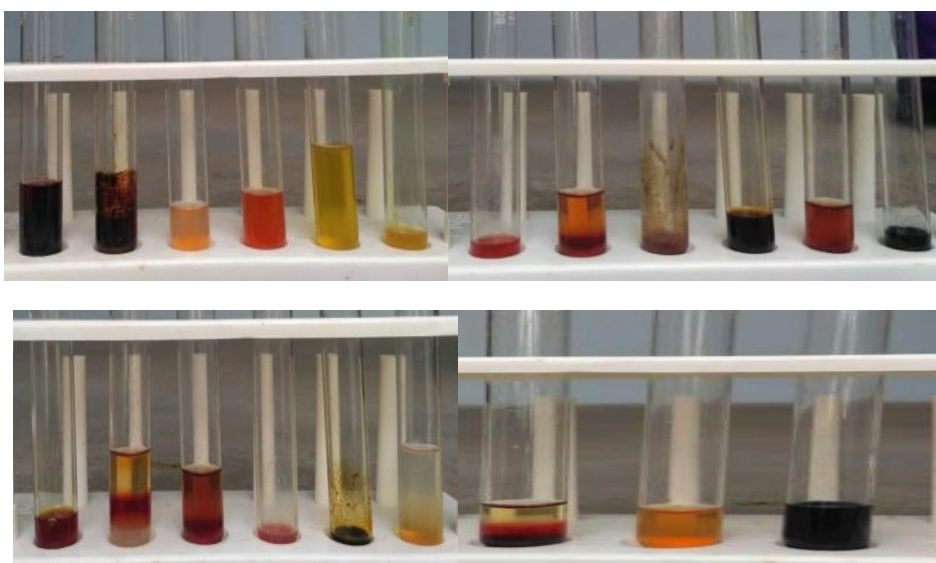
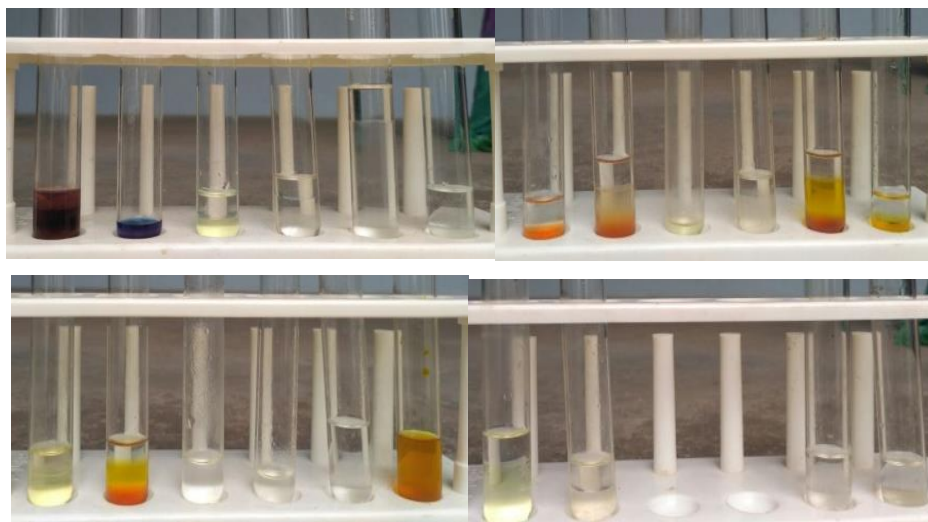


Figure 2: Results showing that phytochemical screening of Acetone extract of pomegranate peel



Figures 3: Results showing that phytochemical screening of Ethanol extract of pomegranate peel

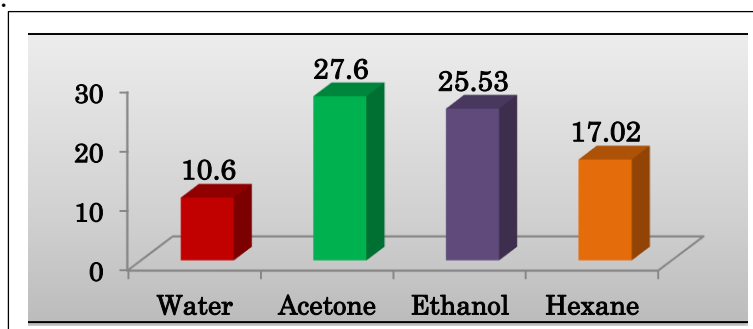


Figures 4: Results showing that phytochemical screening of Hexane extract of pomegranate peel

3.2. Antioxidant Assay: The antioxidants of Pomegranate peel was analyzed using DPPH test, Total phenol, Flavonoids and FRAP test in different extracts such as water, acetone, ethanol and hexane.

3.2.1. DPPH ASSAY: An assay on DPPH activity of different extracts of pomegranate peel was done. The results are shown below.

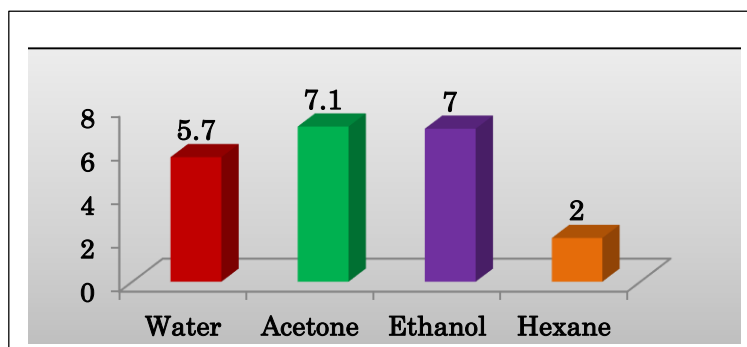
Extracts	DPPH (%)
Water	10.6
Acetone	27.60
Ethanol	25.53
Hexane	17.02



From the result it is clear that acetone extract of *Punica granatum* peel shows maximum DPPH activity when compared to other extracts.

3.2.2. Flavonoid Assay: An assay on flavonoid activity of different extracts of pomegranate peel was done. The results are shown below.

Extracts	Flavonoid (mg/100g)
Water	5.7
Acetone	7.1
Ethanol	7.0
Hexane	2.0

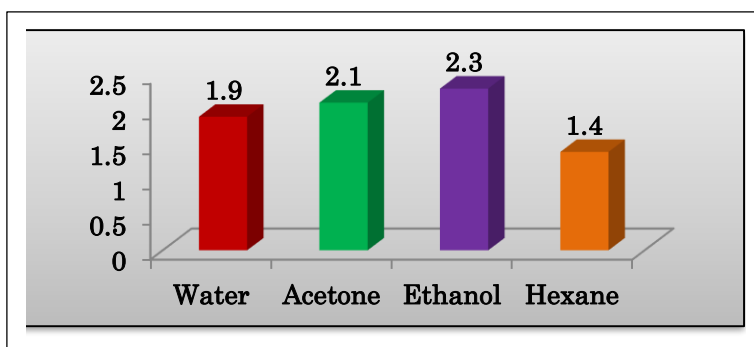


From the result it is clear that acetone and ethanol extracts of *Punica granatum* peel shows maximum Flavonoid activity when compared to other extracts.

3.2.3. Total Phenol Activity: An assay on total phenol activity of different extracts of pomegranate

peel was done. The results are shown below.

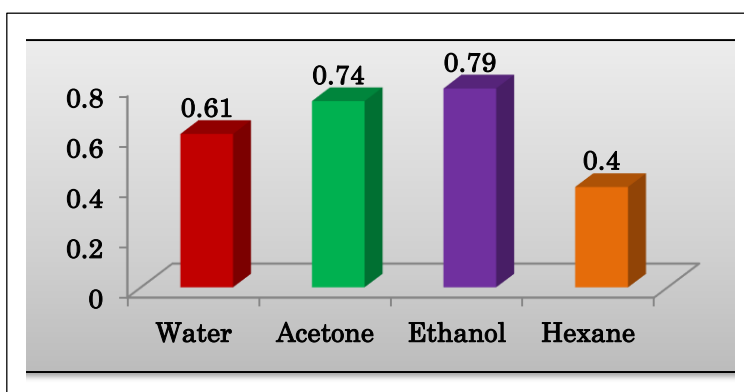
Extracts	Total Phenol (mg/100g)
Water	1.9
Acetone	2.1
Ethanol	2.3
Hexane	1.4



From the result it is clear that ethanol extract of pomegranate peel shows maximum Total phenol activity when compared to other extracts.

3.2.4 FRAP Activity: An assay on FRAP activity of different extracts of pomegranate peel was done. The results are shown below.

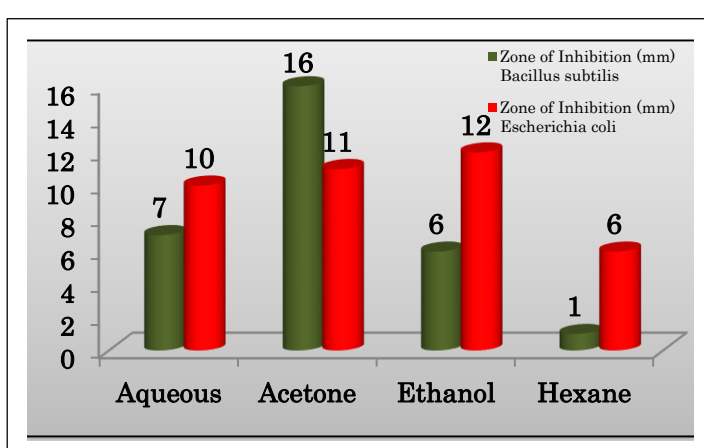
Extracts	FRAP (%)
Water	0.61
Acetone	0.74
Ethanol	0.79
Hexane	0.40



From the result it is clear that ethanol extract of pomegranate peel shows maximum FRAP activity when compared to other extracts.

3.3. Antibacterial Assay: The peel extracts of the pomegranate peel had been tested for their antibacterial activities and an interesting antibacterial profile has been observed against Gram Positive (*Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli*). The peel extracts showed enormous activity against the two bacteria tested. The activities of extracts are mentioned in the terms of zones of inhibitions (mm).

S. No	Extracts	Zone of Inhibition (mm)	
		<i>Bacillus subtilis</i>	<i>Escherichia coli</i>
1	Aqueous	7	10
2	Acetone	16	11
3	Ethanol	6	12
4	Hexane	1	6



The diameter of inhibition zones (DIZ) against *Bacillus subtilis* was 7 mm, 16 mm, 6 mm and 1 mm

for Aqueous, Acetone, Ethanol and Hexane extracts of pomegranate peel respectively. The diameter of inhibition zones (DIZ) against *Escherichia coli* were 10 mm, 11 mm, 12 mm, 6 mm for *Punica granatum* peel extracts (Distilled Water, Acetone, Ethanol, Petroleum ether) respectively.

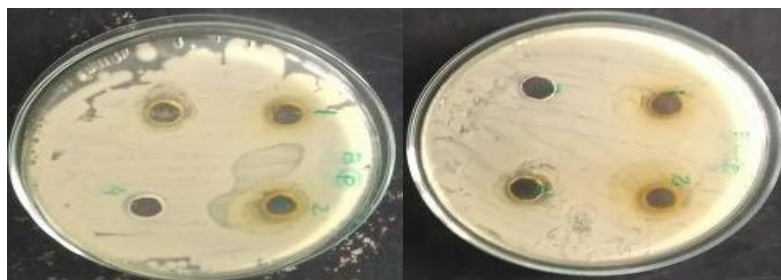


Figure 5: Results showing that antibacterial activity of the different extracts of Pomegranate peel against *Escherichia coli* and *Bacillus subtilis* respectively

From the result, we observed that the zone of inhibition of *Bacillus subtilis* (Gram Positive Bacteria) is higher in acetone extract whereas the zone of inhibition of *Escherichia coli* (Gram Negative Bacteria) is higher in ethanol extract. Pomegranate peel extracts were active and effective against the growth of tested microorganisms.

4. CONCLUSION

Pomegranate is a medicinal plant mainly its fruit contains many therapeutic uses. Phytochemical screening and antioxidant efficacy and antibacterial activity of pomegranate (*Punica granatum*) peel were performed. From this study it can be concluded that various Phytochemicals including phenols, flavonoids are present in different extracts (Aqueous, Acetone, Ethanol and Hexane) of pomegranate peel. The presence of phytoconstituents is useful for treating different ailments and it also have a potential of providing useful drugs for human use. It also concluded that inedible portion of pomegranate contains higher phytochemical constituents which can also be used as the functional food. Antioxidant activity of all four extracts of pomegranate peel was investigated and found. DPPH activity is maximum in acetone extract whereas flavonoids activity, total phenol activity and FRAP activity of pomegranate peel is maximum in ethanol extract. Antibacterial activity test showed that pomegranate peel has a measurable effect against certain Gram positive and Gram negative bacteria. From this study, concludes that pomegranate peel contain several Phytochemicals. Also it has both antioxidant and antibacterial property. A large segment of population in the world relies upon tradition system of medicine. Therefore there is a need for improvement of investigation of such plants. With more resources and time, further investigation of chemical constituents of pomegranate peel can be revealed.

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CONFLICT OF INTEREST

Authors have no any conflict of interest.

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