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Investigation of Nutrient Content, Phytochemical Content, Antioxidant Activity and Antibacterial Activity of Inedible Portion of Pomegranate (*Punica granatum L.*)

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Authors' contributions

Authors KLSRDS and DYJ carried out this research under the supervision of authors RMUSKR and AKS. All authors contributed in data analysis, interpretation and manuscript writing.

Original Research Article

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ABSTRACT

Pomegranate is a plant with high potential to have antimicrobial and antioxidant compounds. In the present study inedible parts (peel and seed) of pomegranate, variety "Bhagawa" were examined for the presence of phytochemicals, phenolic compounds, flavonoids, anthocyanin, antioxidant and anti-microbial compounds. Further, nutrition value of the samples was also examined. Results of GC-MS analysis carried out using four different solvents showed 12 and 25 fractions for peel and seed extracts respectively. Both peel and seed were with high amount of Anthocyanin, Phenolic compounds and Flavonoids. Both extracts gave 100% free radicals reduction in DPPH assay showing high antioxidant activity. Further, both extracts showed antibacterial activity against eight tested food borne pathogens. Antibacterial activity shown by peel extracts was higher than that of seed extract. Both peel and seed extracts showed highest and lowest antibacterial activity against *Staphylococcus aureus* and *Listeria monocyatogenes* respectively. Both extract showed same antibacterial activity which was shown by Kanamycin for *Shigella fexneri.* According to the present study, pomegranate peel and

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seed can be used to extract antibacterial, antioxidant compounds and flavonoids for the use in food industry.

Keywords: Pomegranate; phytochemicals; antioxidants; GC-MS; antibacterial compounds.

1. INTRODUCTION

Plants are always a rich source of valuable compounds that do not hint for primary metabolic path but incorporates thousands of secondary metabolic activities [1]. Pomegranate (*Punica granatum L.*) is one of such plants with high potential to contain valuable chemical compounds.

India is major pomegranate exporter in the world [2]. Pomegranate is a popular fruit in Sri Lankan home gardens, especially in dry and intermediate zones [3]. The ripe pomegranate fruit can be up to five inches wide with deep red, leathery skin. The arils are contained in the fruit and they are separated by white/yellow colour membranous pericarp [4].

The arils contain white, pink to purplish or crimson pulp which is juicy, sweet and variable in acidity, but some are quit tart [5]. The juice is edible but seed (without juice) and peel are considered as inedible portion. This inedible portion is found to be with higher medicinal value [6]. Pomegranates are used in ancient historical uses in several systems of medicine for a variety of ailments [7]. In Ayurvedic Medicine, Pomegranate is considered as “Blood Tonic” and to heal aphthae, diarrhea, ulcers, diabetes, dental condition, erectile dysfunction, and protection from Ultra-Violet (UV) radiation [4]. According to the historical background and the modern investigation, pomegranate has a lots of health benefits [8]. Pomegranate contain higher amount of nutrients compared to many familiar fruits. They contain no fats, low sugar and only 80 calories per 100g serving, 5g of fiber and 15% of the recommended daily allowances of vitamin C [9].

Pomegranate supplies Vitamin C as other fruits and Vitamin B5, B9, and K. It contains vital minerals potassium and Zinc. Seed oil contains phytoestrogens, similar to the estrogen naturally produce in human body [10]. Pomegranate contains anti-inflammatory activity to repair the body tissue damage and reduces the inflammatory state of gum [11].

Generally the seed and peel particles are considered as waste portion of pomegranate. Inedible portion of pomegranate also contain antioxidant, phenolic compounds and flavonoids in high quantities. Further it can be a good nutrient source. However, this waste portion has not been utilized in food industry [12]. In this study, nutrient content, phytochemical content, antioxidant activity and antibacterial activity of pomegranate (variety Bhagawa) were investigated to check its possible applications in food industry.

2. MATERIALS AND METHODS

2.1 Plant Materials

Sound fruits of pomegranate (Variety- Bhagava) collected from local farmers in Kolhapur, India were used in this study. Peels and seeds of collected fruits were separated. Those were dried in a tray drier for 24 hours at 55ºC - 60ºC. Dried peels and seeds were grinded and graded with 60 grade mesh and kept in air tight container until use.

2.2 Extraction

A documented extraction procedure was used with some modifications [13]. 10g of dried peel and seed powder was taken separately and Acetone, Diethyl ether, Methanol and acetoacetate were used as solvents. The samples were kept in vertical shaker at 40ºC for 4 hours. Extracts were filtered and concentrated using force convectional environment. Concentrated samples were kept in a freezer (at -4ºC to -7ºC) for further use.

2.3 Proximate Analysis

2.3.1 Moisture content

AOAC 927.05 method was used to measure moisture content with slight modifications [14]. A sample portion (1.5 g) was taken into pre dried crucibles. Then that was kept in an oven at 102ºC±2ºC for 2 hours and final weight was taken. The moisture content was calculated using the formula given below.

$$Moisture\ Content = \left(\frac{W1 - W2}{W1} \times 100\ \%\right)$$

Where:

W1 : Initial Sample Weight
W2 : Constant Sample Weight

2.3.2 Crude fat content

The AOAC 927.39C method was used to measure crude fat content [15]. Two grams of dried sample was fixed with the Soxhlet apparatus. Anhydrous pet ether was used as the solvent, flow rate was maintained at 3-5 drops per second and the extraction was carried out for 2 hrs. After completion of the process the round bottom flask was kept in an oven at 100ºC for 30 min. A control test with no added sample was also carried out. Crude fat content was expressed as percentage (% Crude Fat).

$$(\text{Crude Fat \% in Dry Basis}) = \left(\frac{\text{g of fat}}{\text{g of sample}}\right) \times 100$$

2.3.3 Crude fiber content

Crude fiber content was measured using a reported method with slight modifications [16]. Two grams of each sample was digested with 1.25% H_2SO_4 for 30 min at 100ºC. Then the mixture was filtered through a muslin cloth and residue was washed with hot distilled water. Residue was digested again with 1.25% NaOH as mentioned previously. The washed residue was transferred to crucible and kept in an oven at 101ºC until gaining constant weight. After weighing, crucible was kept in muffle furnace for 12 hours at 550ºC. The crude fiber content was expressed as percentage. The control samples were carried out without extraction.

$$\text{Fiber \%} = \left(\frac{(\text{W2} - \text{W1}) - (\text{W3} - \text{W1})}{\text{Wt. of the Sample}}\right) \times 100\ \%$$

Where:

W1 = Weight of the empty crucible
W2 = Weight of the crucible + residue (after drying)
W3 = Weight of the crucible + residue (after ashing)

2.3.4 Crude protein

The AOAC 991.20 method was used to measure crude protein content of the sample [17]. Each sample (0.5 g) was digested with 98% H_2SO_4 in Kjeldhal apparatus. After completion of digestion, distillation was continued. The distilled gas was trapped with 4 % Boric acid for 6 minutes. The collected mixture of boric acid was titrated with 0.1 M HCl solution. The control test was also carried out without sample. The crude protein content was expressed as percentage.

$$\binom{Crude\ Pro.}{(\%)} = \left(\frac{1.4007 \times (Vs - Vb) \times M}{W}\right) \times 6.38$$

Where:

Vs = Volume of HCl (ml) used for test portion
Vb = Volume of HCl (ml) titrant used for the blank
M = Molarity of HCl solution
W = Test portion weight (g)

2.3.5 Ash content

The AOAC 900.02 method was used to measure the ash content of the samples [18]. Sample (1.5 g) was weighed into crucible and the sample was pre heated well. Then the sample was kept in the muffle furnace at 550ºC for 12 hours. The crucible's weight was taken after the completion of ashing and the control was carried out without samples. The ash content was expressed as percentage.

$$\text{Ash Content} = \left(\frac{W1 - W2}{W1}\right) \times 100\ \%$$

Where:

W1 = Dried Sample Weight
W2 = Weight of Ash

2.4 Phytochemical Analysis by GC-MS Technique

A documented procedure was used with slight modifications [19]. A High Pressure Mass Spectrometer model MS QP-2010 (German) coupled with a High Pressure Gas Chromatograph model GC-2010 (German) fitted with Rivers, 60 m length, 0.25 µm thickness columns was used in the study. The ionization voltage was 0.7 V and iron source temperature was 250ºC. The sample size was 1 µl and Helium was used as the carrier gas. The identification of compounds in pomegranate peel and seed were carried out using Mass Spectroscopic data.

2.5 Antioxidant Activity

Antioxidant Activity of pomegranate peel and seed extracts were evaluated by 1, 1-diphenyl-2- picrylhydrazyl (DPPH) assay. The procedure followed was a method documented before with slight modifications [8]. The stock DPPH solution was prepared by dissolving 24 mg of DPPH in 100 ml methanol and stored at (-20)ºC until use. The working solution was obtained by mixing 10 ml of stock solution with 30 ml of methanol to obtain an absorbance value of 1.1±0.02 at 517 nm, using UV-Visible spectrophotometer. 500 µl extract of different concentrations of peel and seed were allowed to react with 3 ml of DPPH solution. The mixture was shaken vigorously and left to stand at room temperature for 30 min in dark. The mixture was measured at 517 nm. Control with no sample added was also analyzed and the results were expressed as percentage of Radical Scavenging Activity (RSA %).

$$\%\ RSA = \left\{\left(\frac{A\ control - A\ sample}{A\ control}\right) \times 100\right\}$$

A = Absorbance at 517 nm

A standards curve was prepared by plotting the percentage of free radical scavenging activity of ascorbic acid versus its concentration. The final results were expressed as mg ascorbic acid equivalent antioxidant capacity in 1g of sample (mg AEAC g^{-1}).

2.6 Analysis of Total Phenolic Content

The Total Phenolic content was analyzed according to a method documented before with slight modifications [20]. The peel and seed (10 g equivalent of fresh peel and seed) were extracted with MeOH for 30 min on the ultrasonic bath and filtered. Two hundred microliters of extracts were added to 1 ml of 1:10 diluted Folin - Ciocalteu reagent. After 4 min., 800 µl of sodium carbonate (7.5g/l00 ml) were added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured. Positive controls were done without samples and Gallic acid (0–100mg/l) was used for calibration of a standard curve. The results were expressed as Gallic acid equivalents per 100 g of fresh weight (mg GAE/100g FW). Triplicate measurements were taken and mean values were calculated.

2.7 Total Flavonoid Content Analysis

Total Flavonoid Content was estimated following a method documented before with slight modifications [21]. 50mg of lyophilized extract of pomegranate peel and seed were dissolved in water and diluted with ethanol in 250ml volumetric flask. Two mililiter of stock solution was added to 1ml of 2.5% aluminum chloride solution in 25ml volumetric flask. The final volume was adjusted with ethanol. After 30 minutes, readings at 425nm were taken using UV-visible spectrophotometer for each solution. Control tests were done with no added samples. The flavonoid content was calculated using following equation.

$$TFC = A \times 1.25\ /\ m$$

Where:

A = Absorbance measured
m = Mass of the sample

2.8 Analysis of Total Anthocyanin Content

Total anthocyanin content was investigated according to the procedure described in European Pharmacopoeia 6.0 with Slight modifications [22]. About 5g of pomegranate peel and seed (5g equivalent of fresh weight) was accurately weighted and 95 ml of methanol was added. The mixture was mechanically stirred for 30 min, and then filtered into 100 ml volumetric flask. Filter was rinsed and diluted to 100 ml with methanol. A 50 - fold dilution of this solution in a 0.1% v/v solution of hydrochloric acid in methanol was prepared. The absorbance of the solution was measured at 528nm, using a 0.1% v/v solution of hydrochloric acid in methanol as the blank. The percentage content of anthocyanin, expressed as cyaniding-3-glucoside chloride, was calculated using the following equation. Triplicate measurements were taken and mean values were calculated.

$$Total\ Anthocyanins\ Content = \left(\frac{A \times 5000}{718 \times m}\right)$$

Where:

A = Absorbance at 528 nm
718 = Specific absorbance of cyaniding-3-glucoside chloride at 528 nm
m = Mass of the substance

2.9 Assessing the Antibacterial Activity

A previously documented procedure was used with slight modifications to test the antibacterial activity [23]. Prepared nutrient broth tubes were inoculated with bacterial cultures of *Listeria monocytogenes, Escherichia coli, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium* and *Shigella fexneri*. After 24 h incubation at 37ºC, samples were taken from those tubes for enumeration of microbes. Immediately after sampling those tubes were stored at refrigerated conditions. According to the data obtained in enumeration test, microbial cultures containing 10^8 CFU/ml were prepared using cultures kept under refrigerated conditions. Then 0.1 ml of each microbial culture was inoculated to nutrient agar plates in three replicates. Then, three wells of 6 mm diameter were prepared in each plate with cork borer and 50 µg / ml each of pomegranate peel and seed extract were poured into one of the wells in each plate separately. 50 µg / ml of Kanamycin solution was used as the standards and the plates were incubated at 37ºC for 24 h. The zone of inhibition was measured by measuring the inhibition zone around the well (mm) including the well diameter. The readings were taken in three different fixed directions for all 3 replicates and the average values were tabulated.

2.10 Statistical Analysis

In this study, each experiment was carried out in triplicates except GC - MS analysis. The mean value and standard deviation were calculated from the data obtained. Experimental data was analyzed using Analysis of Variance (ANOVA) and regression analysis using Minitab -14 software.

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

Considerable nutrient content was found in inedible portion of pomegranate in proximate analysis indicating the possibility of using those in new food product developments. Moisture content, ash content and crude fiber content of pomegranate peel was found to be higher than that of seed in proximate analysis Table 1. According to the statistical analysis, there was a significant difference in moisture content, crude fat content, crude protein content and crude fiber content of pomegranate peel and seed (P=0.05) but ash content was not significantly different.

Table 1. Proximate composition of pomegranate peel and seeds

Parameter	Quantity (%)	
	Peel	Seed
Moisture	69.73±0.35	20.85±0.45
Protein	7.81±0.16	9.10±0.35
Crude fat	1.17±0.29	14.17±0.29
Ash	5.56±0.05	5.31±0.19
Crude fiber	19.00±0.97	12.55±0.26

3.2 Phytochemical Analysis by GC-MS Technique

The chemical constituents identified by the GC-MS analysis of various extracts of the pomegranate peel and seed are shown in Tables 2 and 3 with molecular formula, retention time, molecular weight and peak area. Pomegranate peel contains higher amount of Hexacosane (28.44%), Heneicosane (17.39%), Hexacontane (44.67%), and Tetratetracontane (26.26%) in Methanol, Dietheyl ether, Ethyl acetate and Acetone extracts respectively. Nevertheless, the seed contains in higher amount of 3-Butoxy-1, 1, 17, 7, 7-hexamethyl-3, 5, 5-tris (trimethylsiloxy) tetrasiloxane (52.90%), 1-Tricosane (15.28%), octacosane (13.90%), and Z-7-Pentadecenol (64.51%) in same extracts respectively. BHT (Butylated hydroxyltoluene) was identified in both pomegranate peel and seeds. BHT is lipophilic, organic compound chemically derived from phenol, which is useful for its antioxidant property. Those can be used as food additives in very minute quantities. However, use of this chemicals in foods has been restricted due to synthetic BHT which is most commonly used [24] is suspected to be carcinogenic [25]. These natural BHT as such would be a possible replacement for synthetic BHT in food industry. According to the GC - MS results, there were 25 and 12 phyto chemicals presented in the seed and peel respectively. Specially, Palmitic acid, linoleic acid, 3-Butoxy-1, 1, 17, 7, 7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane and Z-7-Pentadecenol were identified. Palmitic acid is saturated fatty acid and Linoleic acid is unsaturated fatty acid [26,27].

3.3 Antioxidant Activity

The DPPH assay has been widely used for the free Radical Scavenging Activity (RSA) of various plant and pure compounds to evaluate antioxidant activity [28]. The RSA values of peels and seeds of pomegranate tested in this study are given in Table 4. When concentration was increased from 0.001 to 0.01 µg / ml, the RSA values of both peels and seeds were increased from 10% to 100% and from 12%, 94% respectively. According to the statistical analysis, no significance difference was observed in RSA value of peels and seeds.

Table 2. Phytochemical compounds present in pomegranate peel extract by using different solvents

Sr. No.	RT*	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
		I – Methanol Extract			
1.	22.722	Tetracosane	C24H50	338	13.22
2.	23.747	Hexacosane	C26H54	366	28.44
3.	24.703	Tetratetracontane	C44H90	618	24.52
4.	25.717	n-Nonacosane	C29H60	408	21.18
		II – Di-ethyl ether Extract			
5.	12.320	ButylatedHydroxytoluene	C15H24O	220	4.79
6.	19.571	Docosane	C22H46	310	6.88
7.	20.518	n-Tricosane	C23H48	324	13.09
8.	21.617	Tetracosane	C24H50	338	16.79
9.	22.715	Heneicosane	C21H44	296	17.39
10.	23.750	Hexacosane	C26H54	366	16.03
11.	24.703	n-Nonacosane	C29H60	408	14.23
12.	25.721	n-Hentriacontane	C31H64	436	10.80
		III – Ethyl acetate Extract			
13.	21.559	Hexacontane	C60H122	842	44.67
14.	24.390	Cyclooctasane	C28H56	392	36.01
		IV – Acetone Extract			
15.	22.734	Hexacosane	C26H54	366	12.17
16.	23.760	Tetracosane	C24H50	338	25.35
17.	24.716	Tetracontane	C40H82	562	22.99
18.	25.733	Tetratetracontane	C44H90	618	26.26

**RT = Retention Time*

Table 3. Phytochemical compounds present in pomegranate seed with different solvents

Sr. No.	RT*	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
		I- Methanol Extract			
1.	12.331	3-Butoxy-1,1,17,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	C19H54O7Si7	590	52.90
2.	14.469	Hexadecamethylcyclooctasiloxane	C16H48O8Si8	592	25.18
3.	17.229	n-Hexadecanoic acid/Palmitic acid	C16H32O2	256	13.96
		II- Diethyl ether Extract			
4.	11.810	ButylatedHydroxytoluene	C15H24O	220	2.92
5.	12.552	Diethylphthalate	C12H14O4	222	0.64
6.	18.098	n-Octacosane	C28H58	394	0.71
7.	18.174	n-Heneicosane	C21H44	296	0.99
8.	19.021	Docosane	C22H46	310	1.62
9.	19.922	Tetracosane	C24H50	338	12.12
10.	20.739	5-Methyl octadecane	C19H40	268	15.13
11.	21.527	1-Tricosane	C23H46	322	15.28

Table 3. Continued……..

12.	22.293	Hexacontane	C60H122	842	13.05
13.	23.123	Tetrapentacontane	C54H110	758	11.32
14.	24.066	n-Nonacosane	C29H60	408	9.50
15.	25.167	n-tritriacontane	C33H68	464	7.35
16.	26.471	n-Tetracontane	C40H82	562	4.81
17.	28.050	n-Pentacosane	C25H52	352	2.88
		III- Ethyl acetate Extract			
18.	20.508	Heneicosane	C21H44	296	1.71
19.	21.606	Tetracosane	C24H50	338	4.79
20.	22.720	n-Nonacosane	C29H60	408	9.69
21.	24.316	Hexacosane	C26H54	366	6.26
22.	24.701	Octacosane	C28H58	394	13.90
23.	26.493	n-Tetracontane	C40H82	562	8.44
24.	26.875	Tetratetracontane	C44H90	618	9.08
		IV- Acetone Extract			
25.	16.775	n-Hexadecanoic Acid	C16H32O2	256	3.91
26.	17.810	13-Hexyloxacyclotridec-10-en-2-one	C18H32O2	280	6.90
27.	18.377	Linoleic Acid	C18H32O2	280	4.82
28.	18.447	9-Octadecenoic Acid (E)-	C18H34O2	282	6.28
29.	20.122	Z-7-Pentadecenol	C15H30O	226	64.51
30.	22.227	Hexahydro-2,7(1H,3H)-Naphthalenedione	C10H14O2	166	2.62
31.	22.663	Z-7-Hexadecenal	C16H30O	238	10.96

**RT = Retention Time*

Table 4. Percentage radical scavenging activity of pomegranate by DPPH

Sample	Inhibition per concentration			
	1 µg/ml	**10 µg/ml**	**100 µg/ml**	**1000 µg/ml**
Ascorbic acid	1.75	41.53	93.84	94.50
Pomegranate peel	10.80	61.38	100.00	100.69
Pomegranate seed	12.83	42.17	92.17	94.35

3.4 Analysis of Total Phenolic Content

According to the statistical analysis, no significant difference of TPC was observed between peel and seed extracts (P = 0.05). The relationship between TPC and RSA values of both peels and seeds found to be positive and R^2 value for this was 46.2% and 53.0% respectively. These results Table 5 were not in agreement with the findings of some other authors [1,29] where they have found a variation of TPC values. Some other authors reported that TPC in peel and seeds are 311, 89 mg GAE/g respectively [30]. In some studies it has been reported the TPC values of peels and seeds are 85.60 and 11.84 mg GAE/g [1]. In another study it has been reported that the content of TPC in the peel of Chinese pomegranate was 249.4 mg /g TAE/g FW [31].

Table 5. Total phenolic content of pomegranate peel and seed

Sample	Total Phenolic content at different concentrations (µg/ml)			
	1µg/ml	10µg/ml	100µg/ml	1000µg/ml
Pomegranate peel	0.054	0.066	0.167	0.495
Pomegranate seed	0.044	0.054	0.118	0.368

3.5 Analysis of Total Flavonoid Content and Total Anthocyanin Content

Flavonoids is one of the important chemical found in plants which possess antimicrobial, anti-allergic, and anti-inflammatory activity. Anthocyanin which is responsible for the highest red colour of pomegranate juice also possess pharmacological properties hence used for therapeutic properties [4]. Both total flavonoids and total anthocyanin content of pomegranate seeds and peels were tested in this study. Results of these tests are shown in Table 6. According to the results, mean values for TFC of peels and seeds were 1.03±0.83 µg/ml and 0.45±0.32 µg/ml respectively. According to the statistical analysis no significant difference in TFC of peels and seeds were observed (P = 0.05). The mean TAC of peels and seeds were 0.28±0.0 and 0.17±0.07 µg/ml respectively. According to the statistical analysis of the data, no significant difference was observed between the extracts of pomegranate peels and seeds TAC as well.

Table 6. Total flavonoid and total anthocyanin content of pomegranate peels and seeds

Sample	TFC	TAC
Pomegranate peel	1.03±0.83	0.28±0.00
Pomegranate seed	0.45±0.32	0.17±0.07

3.6 Estimation of Antibacterial Activity

The results of the antibacterial activity test of pomegranate peels and seeds extracts are given in Table 7. In this test Kanamycin was used as the standard and positive control. Both extract showed antibacterial activity against all tested bacteria. Peel extracts, showed higher antibacterial activity against all tested microbes except *Listeria monocytogenes.* The antibacterial activity of pomegranate peel extracts was higher than the seed extract. Both peel and seed extracts showed highest antibacterial activity against *Staphylococcus aureus.* This was followed by *Bacillus megaterium* and *Bacillus subtilis* which were affected in same level by both extracts. Those two bacteria were followed by *Bacillus cereus* and *Shigella fexneri* which were also affected by both extracts in same level. Lowest antibacterial activity was shown against *Listeria monocyatogenes* by both extracts. As per the results of this study, tested pathogenic bacteria can be suppressed by higher concentration of pomegranate peel and seed extracts. As such those can be used as antibacterial compounds against tested bacteria, especially against *Staphylococcus aureus, Bacillus megaterium* and *Bacillus subtilis.* As both extracts showed same antibacterial activity which was shown by Kanamycin for *Shigella fexneri,* the extracts can be recommended to be used against *Shigella fexneri* as well.

Table 7. Antibacterial activity of methanol extracts of pomegranate peels and seeds

Microorganism	Inhibition zone in mm			
	Peel (100 µg/ml)	Seed (100 µg/ml)	Methanol	Kanamycin (50 µg/ml)
Listeria monocyatogenes	10	08	--	14
Escherichia coli	12	10	--	16
Bacillus cereus	12	12	--	15
Bacillus megaterium	13	12	--	16
Bacillus subtilis	13	12	--	16
Staphylococcus aureus	14	13	--	14
Salmonella typhimurium	13	12	--	13
Shigella fexneri	12	12	--	12

Well size = 6 mm (Diameter), Sample quantity = 50 µl for each well

4. CONCLUSION

Inedible portion of pomegranate (variety Bhagava) contains higher amount of nutrients. Pomegranate peel and seed contain higher phytochemical constituents. As such those can be used in new food product developments. Peels and seeds extracts of pomegranate (Variety Bhagawa) contain high amount of important phytochemicals, antioxidants, phenolic compounds, flavonoids, and anthocyanin. Peel and seed extract of pomegranate (variety Bhagava) can be used against some pathogenic bacteria such as *Staphylococcus aureus, Bacillus megaterium* and *Bacillus subtilis.*

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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