Evaluation of Antimicrobial Properties of Peels and Juice Extract of *Punica Granatum* (POMEGRANATE)

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***Abstract: - Punica granatum* is commonly known as Pomegranate which belongs to the Punicaceae family. Pomegranate has extensively been used as a source of traditional remedies and in the ancient Ayurveda system of medicine. The main objective of this study was to assess the phytochemical analysis and antimicrobial activity of the peel and juice extract of the pomegranate on selected bacterial and fungal strains. The peel and juice extracts of methanol and mixture of methanol and chloroform extracts shown highest antimicrobial activity compared to the other extracts. Various bacterial and fungal species like *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Escherichia coli* and *Aspergillus niger* showed the zone of inhibition of peel and juice extract. Phytochemical analysis of the extracts used showed the presence of Alkaloids, Flavonoids, Terpenoid, Proteins, Free amino acids, Carbohydrates and Vitamin C. The extract of peel and juice were further characterised by FTIR analysis.**

***Key words*: *Punica granatum*, Phytochemicals, Antimicrobial activity, MIC, Antioxidant activity, FTIR spectroscopy.**

I. INTRODUCTION

To cure number of human diseases medicinal plants are most widely used in traditional medicine system which is the nature's gift [1]. Plants are capable of synthesising a wide variety of chemical compounds which are commonly known as phytochemicals or bioactive molecules that are used to defend against attack from the various organisms [2].

Punica granatum is commonly known as Pomegranate belongs to the Punicaceae family. *Punica protopunica* is considered as the precursor to the Pomegranate and is the only other species in the Punica genus. Pomegranate has extensively been used as a source of traditional medicine for thousands of years in the ancient Ayurveda system of medicine [3].

Pomegranate is a shrub or small tree that grows up to 6 and 8 metres in height. Depending on cultivars types the tree bears numerous bulbous, reddish, purple or orange-yellow coloured fruits [4]. The trunk is covered by a red-brown bark which later becomes gray. The branches of the tree are stiff, angular and often spiny. Pomegranate may begin to bear in 1 year after planting out and under suitable conditions the fruit should mature in 5 to 7 months after bloom. Pomegranates are also long-lived. There are specimens in Europe that are known to be over 200 years of age [5].

Each fruit measures about 6-10 cm in diameter and weighs about 200gm crowned at the base by the prominent calyx [4]. The rind or skin of the fruit is yellowish, leathery and tough overlaid with light or deep red. The internal part is separated by membranous walls and white, soft, bitter tissue into compartments packed with sacs filled with sweetly acid, juicy, red, pink or whitish pulp. In each sac there is one angular, soft or hard seed with an inner woody part. Aril juice contain about 85% water, 10-12% total sugar, 0.2-2% organic acid, fatty acid, amino acid and antioxidant phenolics. Peels corresponds to about 50% of the fruit weight. Peel and mesocarp are important sources of bioactive molecules [6].

The fruit's astringent properties have been used to treat various diseases in folk medicine such as cuts, sore throats, tapeworms, dysentery, and gum disease. Pomegranate juice is a major source of antioxidant nutrients which protect against heart disease and other disorders [2]. Pomegranate peels are exploited in the traditional medicine for the treatment of cardiovascular disease, diabetes, and various forms of cancer. Pomegranate helps in promoting neurologic health, maintains joint integrity and function, exhibits estrogenic properties, blocks herpes simplex virus replication and adsorption, enhances immune function and treats periodontal disease. Pomegranate is a unique fruit plant which produces a broad spectrum of biologically active substances which prevents the harmful effects of radioactive substances [7]. Thus in the view of usefulness of pomegranate, present study focused on extraction of peel and juice components on various solvents. These extracts were further characterised by phytochemical analysis and FTIR studies. The extracts were further evaluated for their antimicrobial and antioxidant properties.

II. MATERIALS AND METHOD

Collection of Plant Material

Fresh fruits of pomegranate were collected from the fruit market of Vapi, Gujarat, India. The peels and seeds of pomegranate were manually separated. The peels were then cut in to small pieces and then first washed with tap water

followed by washing with distilled water. The peels were then allowed to air dry for 5-7 days in shade. The dried peels were then grinded in mixer grinder and powder was made. The powder was stored in an air tight container and kept at 4 °C. for further use. The pomegranate seeds were crushed and squeezed to yield the juice. Juice was filtered, pasteurised, concentrated and stored at 4 °C. until use.

Preparation of Plant Extracts

Extraction was carried out using Maceration technique. Four types of solvents were used for the extraction of various components.

Methanol Extract

To extract the peel and juice of pomegranate 20g powder material was macerated in conical flask with 100ml methanol and 30 ml of juice was macerated in another flask containing 70ml methanol. Both flasks were allowed to stand for 3-7 days with occasional shaking. The liquid was then strained off, the solid material was pressed and then liquid was clarified by using muslin cloth. The filtrates were air dried at room temperature and residual moisture was removed in a vacuum oven at 50 to52 °C. Then dried extract was dissolved in DMSO (Dimethylsulphoxide) and kept at 4 ºC until use.

Chloroform Extract

To extract the peel and juice extract of pomegranate 20g powder material was macerated in conical flask with 100ml chloroform and 30ml of juice was macerated in another flask containing 70ml chloroform. The further extraction procedure was carried out same as described earlier.

Methanol : Chloroform (1:1) Extract

To extract the peel and juice extract of pomegranate 20gm powder material was macerated in conical flask with 50ml methanol and 50ml chloroform (1:1 ratio); 30ml of juice was macerated in 35ml methanol and 35ml chloroform. The further extraction procedure was carried out same as described earlier.

Aqueous Extract

30g powder material was macerated in conical flask containing 100ml distilled water and 30ml of juice was macerated in 70ml of sterile distilled water. The further extraction procedure was carried out same as described earlier.

Microorganisms and Culture Conditions

Eleven strains of bacteria and three fungal strains were procured from MTCC (Microbial Type Culture Collection Centre and Gene Bank) Chandigarh, India. The bacterial strains used were *Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Peudomonas aerugenosa, Salmonella typhi, salmonella typhi A, Salmonella typhi B.* The fungal strains used were *Aspergillus niger* and *Rhizopus oryzae*.

All the bacterial strains were maintained by subculturing on nutrient agar slant and stored at 4 °C. All the fungal strains were maintained by subculturing on PDA medium and stored at 4 °C.

Phytochemical Analysis of different Crude extracts

Phytochemical analysis of each extract was performed to detect the presence of several phytochemicals like Tannins, Flavonoids, Alkaloids, Triterpenes and Steroids, Glycosides, Saponins, Proteins, Free amino acid, carbohydrates and Vitamin C.

Ferric chloride test for Tannins

About 0.5g of crude extract was dissolved in 10ml of boiling water. The solution was filtered and to the filtrate few drops of 6% $FeCl_3$ was added. Development of deep green colour shows presence of Tannins [8].

Ferric chloride test for Flavonoids

Crude extract was mixed with few drops of Ferric chloride solution which results in the formation of blackish red colour indicating the presence of flavonoids [9].

Hager's test for Alkaloids

Crude extract was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate would show a positive result for the presence of alkaloids [9].

Salkowki's test for Steroids and Triterpenoids

About 0.5g of Crude extract was mixed with 2ml of Chloroform. Then concentrated H_2SO_4 added to form two layers. Formation of green colour at the upper layer and reddish brown colour at the lower layer indicates the presence of Steroids and Triterpenoids respectively [10].

Keller Killani test for Glycosides

Crude extract was treated with few drops of glacial acetic acid. Ferric chloride solution was added and mixed. Then concentrated sulphuric acid was added and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides [9].

Foam test for Saponins

Crude extract was mixed with water and shaken and then observed for the formation of froth, which is stable for 15 minutes for a positive result [9].

Biuret test for Proteins

Crude extract 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 [9].

Ninhydrin test for Free Amino Acids

Crude extract was boiled with 0.2% solution of Ninhydrin for few minutes which would result in the formation of purple colour suggesting the presence of free amino acids [9].

Benedict's test for Carbohydrate

Crude extract was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath and then observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate [9].

DNPH test for Vitamin C

Crude extract was treated with 2,4Dinitrophenyl hydrazine (DNPH) dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C [9].

FTIR analysis

The extracts of peel and juice of *Punica granatum* was further characterized by FTIR spectroscopy (Fourier transform infrared spectroscopy, Perkin Elmer Spectra GX). The FTIR analysis was carried out in the mid IR region of 400–4,000 cm-1. The dried extract samples were mixed with spectroscopically pure KBr in the ratio of 5:95 to form a uniform pellets, which was then fixed in sample holder of FTIR spectroscope, and the analysis was carried out [11].

Determination of Anti-bacterial Activity

Agar-well diffusion method was employed for the determination of antibacterial activities of peel and juice extracts of pomegranate [12].The test organisms were grown on nutrient broth prior to start the experiment. The organisms were kept in incubator at 37°C for 24 hours. The inoculums for each test culture were prepared which have approximately 10^6 CFU/ml (0.5 Mac-Farland Standard). 100µL of standardized inoculums of each bacterium was spread uniformly on sterile Muller-Hinton Agar plate. Wells (8mm in diameter) were cut from the agar with a sterile borer. 150µL volume of the extract was introduced in to the well. Standard antibiotic Tetracycline (100mg/ml) was used as positive control and DMSO was used as negative control. The agar plates were then incubated at 37°C for 24 hours. The zone of inhibition was recorded to the nearest size in mm.

Determination of Minimum Inhibitory Concentration (MIC)

Broth dilution method was employed for the determination of MIC (Minimum Inhibitory Concentration) of pomegranate extracts. Muller Hinton broth was used for the determination of MIC. A total of 5ml of broth was dispensed in to separate tube and sterilised at 121° C for 15 minutes and then allowed to cool. One fold serial dilution of the extracts in the broth was made from the stock concentration of the extracts to obtain 100mg/ml for the peel and juice extract. 100µl (0.1ml) of the standardized inoculums of the each test organisms were inoculated in to different concentration of the extracts in the broth. The test tubes in the broth were incubated at 37°C for 24 hours and after incubation the tubes were observed for the turbidity. The lowest concentration (highest dilution) that showed no turbidity (no growth) in the test tube was recorded as MIC.

Determination of Anti-fungal Activity

Agar-well diffusion method was employed for the determination of antifungal activities of peel and juice extracts of pomegranate [13]. Tested fungi was subcultured on PDA plates and incubated at 28 °C for 3-5 days. Wells (8mm in diameter) were cut from the agar with a sterile borer. 150µL volume of the extract was introduced in to the well. Standard antifungal Fluconazole (10mg/ml) was used as positive control and DMSO was used as negative control. The plates were then incubated at 28 °C. The appearance of zones of inhibition was regarded as presence of the antifungal action in the test substance.

H_2O_2 scavenging Activity

H_2O_2 scavenging ability of methanol extracts of peels and juice of pomegranate was determined according to the method of Ali *et al*., (2009)[14]. A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). The extracts at 10mg/ml concentration were added to H_2O_2 solution (0.6ml) and the total volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer. A blank solution containing phosphate buffer, without H_2O_2 was prepared. The percentage of H_2O_2 scavenging of peels and juice extracts of pomegranate and standard compounds were calculated using the formula:

$$\% \text{ scavenging of } H_2O_2 = [(A_{control}-A_{sample})/A_{control}] \times 100$$

Where;

$A_{control}$ = Absorbance of control (H_2O_2 solution)

A_{sample} = Absorbance in the presence of plant extracts.

III. RESULTS AND DISCUSSION

Phytochemical analysis

Many researchers investigated that *Punica granatum* and their parts are medically valuable. The identification of phytochemicals of peel and juice extracts of pomegranate were done for the screening of phytochemicals such as Carbohydrates, Sugars, Glycosides, Proteins, Amino acids, Tannins, Alkaloids, Flavonoids, Saponins, Sterols and oils. In the present study methanol, chloroform, methanol : chloroform (1:1) and aqueous extracts of peels and juice of the pomegranate were subjected to preliminary phytochemical screening.

Phytochemical analysis of Methanol extract

The methanolic extract of *Punica granatum* peels showed presence of steroids, triterpenoides, alkaloids, flavonoids, saponins, tannins and carbohydrates whereas methanolic extract of *Punica granatum* juice showed the presence of alkaloids, saponins and vitamin C (Table 1).

Table 1 Phytochemical Analysis of Methanol extract of *Punica granatum*

S.NO	Phytochemical Parameters	Peel extract	Juice extract
1	Steroids and Triterpenoides	+	-
2	Alkaloides	+	+
3	Glycosides	-	-
4	Flavonoids	+	-
5	Saponins	+	+
6	Tannins	+	-
7	Proteins	-	-
8	Free amino acids	-	+
9	Carbohydrates	+	-
10	Vitamin C	-	+

Note: + indicates = Positive results, - indicates = Negative results

Phytochemical analysis of Chloroform extract

The chloroform extract of *Punica granatum* peels showed presence of alkaloids, tannins and carbohydrates whereas chloroform extract of *Punica granatum* juice showed the presence of flavonoids (Table 2).

Table 2 Phytochemical Analysis of Chloroform extract of *Punica granatum*

S.NO	Phytochemical Parameters	Peel extract	Juice extract
1	Steroids and Triterpenoides	-	-
2	Alkaloides	+	-
3	Glycosides	-	-
4	Flavonoids	-	+
5	Saponins	-	-
6	Tannins	+	-
7	Proteins	-	-
8	Free amino acids	-	-
9	Carbohydrates	-	-
10	Vitamin C	+	-

Note: + indicates = Positive results, - indicates = Negative results

Phytochemical analysis of Methanol : Chloroform (1:1) extract

The methanol : chloroform (1:1) extract of *Punica granatum* peels showed presence of glycosides, flavonoids, tannins, proteins and carbohydrates whereas juice extract of *Punica granatum* showed the presence of proteins and free amino acids (Table 3).

Table 3 Phytochemical Analysis of Methanol : Chloroform (1:1) extract of *Punica granatum*

S.NO	Phytochemical Parameters	Peel extract	Juice extract
1	Steroids and Triterpenoides	-	-
2	Alkaloides	-	-
3	Glycosides	+	-
4	Flavonoids	+	-
5	Saponins	-	-
6	Tannins	+	-
7	Proteins	+	+
8	Free amino acids	-	+
9	Carbohydrates	+	-
10	Vitamin C	-	-

Note: + indicates = Positive results, - indicates = Negative result

Phytochemical analysis of Aqueous extract

The aqueous extract of *Punica granatum* peels showed presence of steroids, triterpenoides, flavonoids, tannins, and carbohydrates whereas juice extract of *Punica granatum* showed the presence of alkaloids, flavonoids and tannins (Table 4).

Table 4 Phytochemical Analysis of Aqueous extract of *Punica granatum*

S.NO	Phytochemical Parameters	Peel extract	Juice extract
1	Steroids and Triterpenoides	+	-
2	Alkaloides	-	+
3	Glycosides	-	-
4	Flavonoids	+	+
5	Saponins	-	-
6	Tannins	+	+
7	Proteins	-	-
8	Free amino acids	-	-
9	Carbohydrates	+	-
10	Vitamin C	-	-

Note: + indicates = Positive results, - indicates = Negative results

Negi and Jayprakash (2003) [15], reported that the primary phytochemicals in pomegranate are polyphenols, including anthocyanins pigments, flavonols glycosides, procyanidins, phenolics and ellagic acid derivatives. The most important quality criterion is that an attractive red colour of juice which contains anthocyanins as a major constituent [16].

FTIR analysis

The FTIR analysis of peel extract showed major peeks at 3419 cm^{-1} which corresponds to the OH group. Peaks in region of 1047 cm^{-1} showed the presence of CH stretch of an aliphatic compound. The peaks present in the region 1624 cm^{-1} corresponds to the nitro compound present in the extract (Fig.1).

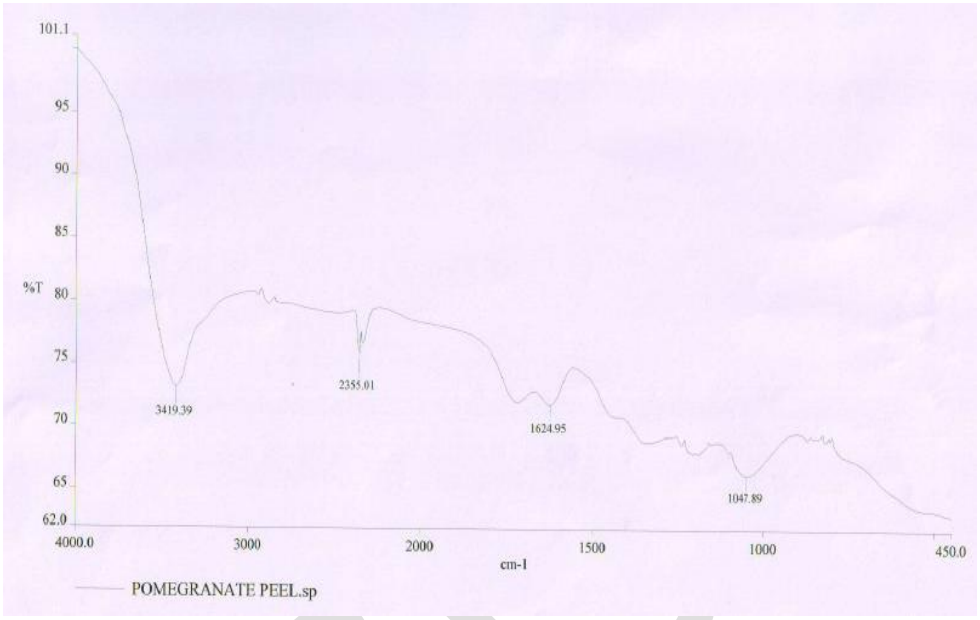

Fig. 1 FTIR analysis of methanolic extract of peel of *Punica granatum*

The FTIR analysis of juice extract showed major peaks in the region of 3235 cm^{-1} and 2924 cm^{-1} which corresponds to the OH and CH groups in the phenol and alkenes like structure. The peaks observed in the region of 1010 cm^{-1}, 935 cm^{-1} and 779 cm^{-1} corresponds to the CH stretch in the alkenes like structure (Fig.2).

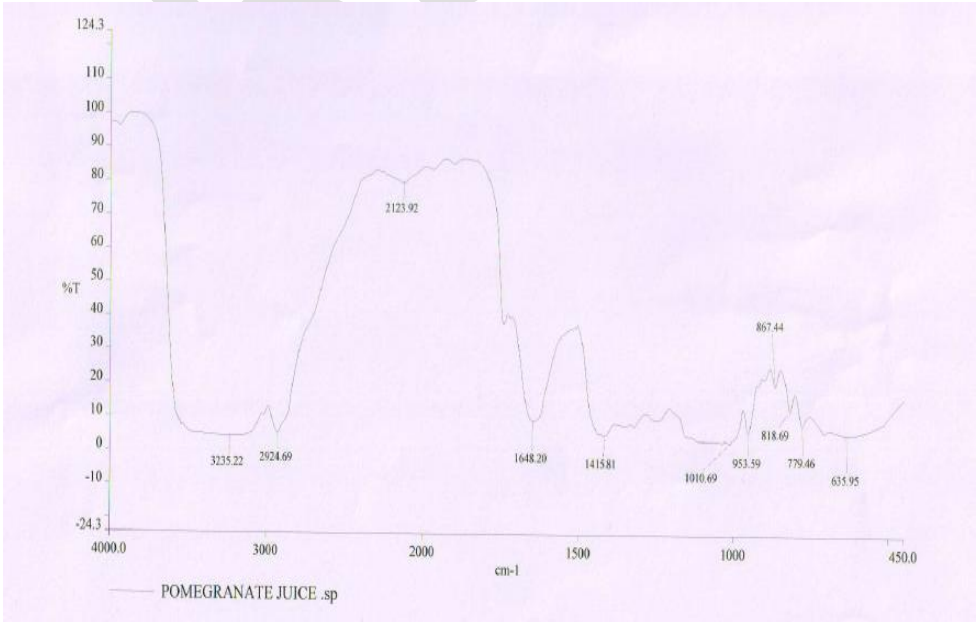

Fig. 2 FTIR analysis of methanolic extract of juice of *Punica granatum*

Anti-bacterial activity

In the present study, the antibacterial activity of *Punica granatum* peels and juice extracts towards clinically significant microbes is reported. The extracts obtained from peels and juice of *Punica granatum* was found to be effective against the bacteria *Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Peudomonas aeruginosa, Salmonella typhi, Salmonella typhi A, Salmonella typhi B.*

Antibacterial activity of Methanol extract

Methanolic extracts obtained from the peels of *Punica granatum* were found to be effective against the bacteria *B.cereus, B.subtilis, B.megaterium, P.vulgaris, P.aeruginosa, S. typhi, S. typhi A, S.typhi B* showing zone of inhibition ranging from 6mm to 18mm. Similarly the extracts obtained from juice of *Punica granatum* were found to be effective against bacteria *B.cereus, B.subtilis, B.megaterium, S.aureus, P.aeruginosa, S. typhi, S. typhi A, S.typhi B* showing zone of inhibition ranging from 2mm to 13mm (Table 5).

Table 5 Anti-bacterial activity of Methanol extract of *Punica granatum*

Test Organisms	Zone of Inhibition in mm		
	Peel extract	Juice extract	Tetracycline (P.C)
B.cereus	12	13	16
B.subtilis	17	14	14
B.megaterium	14	10	16
S.aureus	-	-	20
E.coli	-	14	10
E.aerogenes	-	-	10
P.vulgaris	6	-	18
P.aeruginosa	16	10	14
S.typhi	12	-	16
S.typhi A	16	2	12
S.typhi B	14	-	14

Note: - indicates = No Zone of inhibition, P.C indicates = Positive Control

Antibacterial activity of Chloroform extract

Chloroform extracts obtained from peels and juice of *Punica granatum* was found to be less effective against the microorganisms and lesser zone of inhibition was found in *S.aureus and P.vulgaris* by peels and juice extracts of pomegranate (Table 6).

Table 6 Anti-bacterial activity of Chloroform extract of *Punica granatum*

Test Organisms	Zone of Inhibition in mm		
	Peel extract	Juice extract	Tetracycline (P.C)
B.cereus	-	-	12
B.subtilis	-	-	10
B.megaterium	-	-	18
S.aureus	5	6	18
E.coli	-	-	12
E.aerogenes	-	-	10
P.vulgaris	6	4	14
P.aeruginosa	-	-	18
S.typhi	-	-	14
S.typhi A	-	-	16
S.typhi B	-	-	10

Note: - indicates = No Zone of inhibition, P.C indicates = Positive Control

Antibacterial activity of Methanol : Chloroform (1:1) extract

Methanol : Chloroform extract obtained from peels of *Punica granatum* was effective against bacteria *B.cereus* and *B.megaterium* showing zone of inhibition 12mm and 14 mm respectively. The juice extract was found to be effective against *B.cereus* showing 8mm zone of inhibition (Table 7).

Table 7 Anti-bacterial activity of Methanol : Chloroform (1:1) extract of *Punica granatum*

Test Organisms	Zone of Inhibition in mm		
	Peel extract	Juice extract	Tetracycline (P.C)
B.cereus	12	8	14
B.subtilis	8	5	16
B.megaterium	14	12	14
S.aureus	14	6	10
E.coli	4	4	18
E.aerogenes	-	4	12
P.vulgaris	-	-	12
P.aeruginosa	-	-	10
S.typhi	5	4	18
S.typhi A	6	4	16
S.typhi B	7	5	14

Note: - indicates = No Zone of inhibition, P.C indicates = Positive Control

Antibacterial activity of Aqueous extract of Punica granatum

Aqueous extracts of peels of *Punica granatum* showed zone of inhibition ranging from 5mm to 12mm. Juice extract was found to be less effective than peels extract (Table 8).

Table 8 Anti-bacterial activity of Aqueous extract of *Punica granatum*

Test Organisms	Zone of Inhibition in mm		
	Peel extract	Juice extract	Tetracycline (P.C)
B.cereus	10	8	10
B.subtilis	9	6	12
B.megaterium	12	-	14
S.aureus	-	-	16
E.coli	-	-	12
E.aerogenes	-	-	14
P.vulgaris	6	-	10
P.aeruginosa	5	5	12
S.typhi	6	-	14
S.typhi A	5	5	16
S.typhi B	8	6	12

Note: - indicates = No Zone of inhibition, P.C indicates = Positive Control

There was significant difference between the inhibitions effects on microorganisms by different extracts used in this study. The highest zone of inhibition was obtained by methanolic extracts of peels and juice extracts and also by the methanol : chloroform extracts of juice of *Punica granatum*. The highest zone of inhibition of methanolic extract of juice of pomegranate against *B.megatarium, B.cereus* and *B.subtilis* were 15mm, 18mm and 17mm respectively. The highest zone of inhibition of methanol : chloroform peel extract by *B.megatarium* and *S.aureus* were 14mm and 12 mm respectively. However the lowest zone of inhibition was obtained by chloroform extracts of peels and juice. The study shows the pharmacological importance of peel of *Punica granatum*, and therefore exploring bioactive phytochemicals of the waste material (peel) in antimicrobial activity of traditional medicinal use.

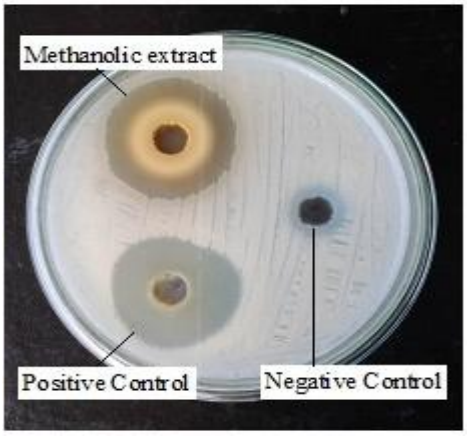

Fig. 3 Zone of inhibition of Methanol extracts of the juice of *Punica granatum* on *Escherichia coli.*

The antimicrobial effects of pomegranate extracts were previously studied. Mathabe *et al*., (2005) [17] reported that the bark, leaves, flowers and fruits of pomegranate are widely used as phytotherapeutic agents. Prashanth *et al*., (2009) [18] reported that methanolic extracts of *Punica granatum* fruit rind to be active against all microorganisms tested in their study. Their results provide evidence for the presence of antimicrobial compounds in the crude methanolic extracts of these plants. Ahmet *et al*., [19] reported the *In vitro* antibacterial activity of extracts obtained from six pomegranate cultivators against the bacteria *B.megaterium, P. aeruginosa, S. aureus, C. xerosis, E. coli, E. faecalis* and *M. Luteus*, showing inhibition zones ranging from 13-26 mm. There are many reports of antimicrobial activity of pomegranate showing that pomegranate juice is inhibitory to *Staphylococcus aureus* and *Bacillus megaterium* [12].Similar results were recorded in our study for the pomegranate juice extract.

Dahham *et al.,* (2010) [12] reported that a number of antibiotics have lost their effectiveness due to the development of resistant strains of bacteria, which has primarily occurred through the expression of resistance. Therefore there is a need to develop antimicrobial drugs for the treatment of infectious diseases.

Minimum Inhibitory Concentration (MIC) Determination

The MIC determination was carried out using broth dilution method. The minimum inhibitory concentration of the methanolic extracts of peels of pomegranate on bacterium *B.cereus, B.megaterium, P.vulgaris,* and *P.aeruginosa* was observed in the range of 50,0000 to 12,500 (µg/ml) whereas the MIC of peel extracts on bacterium *B.subtilis, S. typhi, S. typhi A,* and *S.typhi B* was found to be in the range of 50,000 to 25,000 (µg/ml) (Table 9).

Table 9 MIC of Methanolic extracts peels of *Punica granatum*

Test organisms	Concentration of extract (µg/ml)									
	50,000	25,000	12,500	6,250	3,125	1,562.5	7,81.2	3,90.6	1,95.3	97.65
B.cereus	-	-	-	+	+	+	+	+	+	+
B.subtilis	-	-	-	+	+	+	+	+	+	+
B.megaterium	-	-	-	+	+	+	+	+	+	+
P.vulgaris	-	-	-	+	+	+	+	+	+	+
P.aeruginosa	-	-	-	+	+	+	+	+	+	+
S.typhi	-	-	+	+	+	+	+	+	+	+
S.typhi A	-	-	+	+	+	+	+	+	+	+
S.typhi B	-	-	+	+	+	+	+	+	+	+

Note: - indicates = no turbidity, + indicates = turbidity

MIC value of methanolic extract of peels of pomegranate was reported by Prashanth *et al*, (2001)[18] at the concentration of 50mg/ml. In contrast the present study showed the MIC value at the concentration of 12,500 and 25,000 (µg/ml).

Anti-fungal activity

Anti-fungal activity was determined using agar - well diffusion technique. Two tested fungi *Aspergillus niger* and *Rhizopus oryzae* were used to study the effects of methanol, chloroform, methanol : chloroform and water extracts of peels and juice of *Punica granatum*.

Anti-fungal activity of Methanol extracts of Punica granatum

Methanolic extracts of peels of *Punica granatum* found to be effective against both the fungal strains used. *Aspergillus niger* and *Rhizopus oryzae* showed zone of inhibition 8mm and 6mm respectively. Juice extracts obtained from this fruit are not effective against *Aspergillus niger*. However *Rhizopus oryzae* showed zone of inhibition of 8mm (Table 10).

Table 10 Anti-fungal activity of Methanol extracts of *Punica granatum*

Test Organisms	Zone of Inhibition in mm		
	Peel extract	Juice extract	Fluconazole (P.C)
A.niger	8	-	10
R.oryzae	6	8	8

Note: - indicates = No Zone of inhibition, P.C = Positive Control

Anti-fungal activity of Chloroform extract of Punica granatum

Chloroform extracts obtained from peels and juice of *Punica granatum* were showed 5mm zone of inhibition on *A.niger* and 6mm zone of inhibition was observed on *R.oryzae* by juice extract (Table 11).

Table 11 Anti-fungal activity of Chloroform extract of *Punica granatum*

Test Organisms	Zone of Inhibition in mm		
	Peel extract	Juice extract	Fluconazole (P.C)
A.niger	5	5	6
R.oryzae	-	6	8

Note: - indicates = No Zone of inhibition, P.C = Positive Control

Anti-fungal activity of combination of Methanol : Chloroform (1:1) extract of Punica granatum

Methanol : Chloroform extracts of peels of *Punica granatum* was effective agaist both the fungal strains used showing zone of inhibition 8mm and 6mm for *A.niger* and *R.oryzae* respectively. Juice extract found be not effective on both the fungal strains tested (Table 12).

Table 12 Anti-fungal activity of combination of Methanol : Chloroform (1:1) extract of *Punica granatum*

Test Organisms	Zone of Inhibition in mm		
	Peel extract	Juice extract	Fluconazole (P.C)
A.niger	8	-	6
R.oryzae	6	-	8

Note: - indicates = No zone of inhibition, P.C = Positive Control

Anti-fungal activity of Aqueous extract of Punica granatum

Aqueous extracts of peels of *Punica granatum* found to be effective on both the fungal strains used. Zone of inhibition of both fungal strains was 5mm. Aqueous extracts of juice of *Punica granatum* was found to effective on *A.niger* giving 4mm zone of inhibition (Table 13).

Table 13 Anti-fungal activity of Aqueous extract of Punica granatum

Test Organisms	Zone of Inhibition in mm		
	Peel extract	Juice extract	Fluconazole (P.C)
A.niger	5	4	6
R.oryzae	5	-	6

Note: - indicates = No Zone of inhibition, P.C = Positive Control

Aspergillus niger showed more sensitivity as compared to the *Rhizopus oryzae*. The highest zone of inhibition of *A.niger* was observed in methanolic extract of peels of pomegranate compared to the chloroform, aqueous and combination of methanol and chloroform extracts. The zone of inhibition of *A.niger* was ranging from 4mm to 8mm at the concentration of 150µg/µl.

The inhibitory effect of *Punica granatum* against mycelial fungi was reported previously. Tayel *et al*., (2009) [20] investigated that applying pomegranate peel extracts as an alternative, reduced-risk antifungal agent for controlling citrus green mould invasion. Navindra *et al* (2006) [21] reported that the main active constituents in pomegranate extract are tannins and alkaloids. The main phytochemical constituent in the peel of *P. granatum* are gallotannins, ellagic acid derivatives, catechins & procyanidins and flavonols.

H_2O_2 scavenging activity

In the present study the methanolic extract of peels and juice of pomegranate were studied for the antioxidant activity. Antioxidant compounds present in extracts can donate electrons to H_2O_2. Both the extracts of peels and juice of pomegranate showed antioxidant activity 45% and 25%

respectively. Reference standard (20 mM Ascorbic acid) yielded 63% H_2O_2 scavenging activity (Fig. 4).

The Antioxidative system protects the animal against reactive oxygen species (H_2O_2, superoxide, OH, singlet oxygen and nitrogen species) induced oxygen damage. Various synthetic antioxidants (such as BHT) are identified but they could not be widely used because they are suspected to be carcinogenic. Therefore natural antioxidants have gained importance [21].

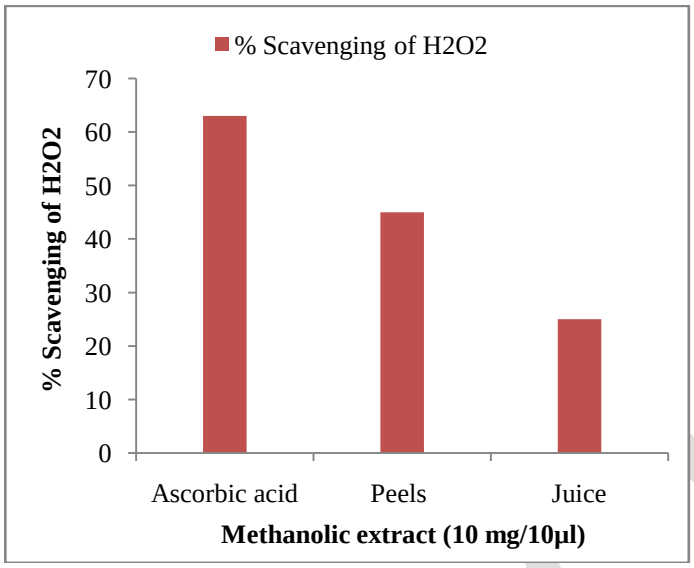

Fig. 4 Free radical scavenging activity of Methanolic extracts of peel and juice extract of *Punica granatum*

IV. SUMMARY AND CONCLUSION

The main objective of the present study were to estimate phytochemical analysis and the antibacterial and antifungal activity of pomegranate peels (rind, husk or pericarp) and juice on selected bacterial and fungal cultures to find out the most significant part of the fruit with highest antimicrobial activity. Phytochemical analysis of peels of *Punica granatum* showed the presence of Alkaloids, Saponins and Free amino acid whereas juice extract showed the presence of Alkaloids, Saponins and Free amino acids. Chloroform extract of peels and juice showed the presence of Glycosides, Protiens, and free amino acids and Vitamin C . Methanol : Chloroform extracts showed the presence of Glycosides, Flavonoids, Tannins Protiens Free amino acids and Carbohydrates. The presence of phytochemicals make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. The highest zone of inhibition was obtained by methanolic extracts of peels and juice extracts and also by the methanol : chloroform extracts of juice of *Punica granatum*. Minimum Inhibitory Concentration of the methanolic extracts of the peels of *Punica granatum* was found to be effective on bacterial strains *B.cereus, B.megatarium, P.vulgaris* and *P.aeruginosa* at the concentration of 12,500 µg/ml. Whereas the bacterial strain *B.subtilis, S.typhi, S.typhi A,,S.typhi B* was found to be at the concentration of 25,000(µg /ml) However no MIC results were obtained by methanolic extracts of juice. *Aspergillus niger* showed more sensitivity as compared to the *Rhizopus oryzae*. Methanolic extracts of peels and juice of pomegranate showed antioxidant activity 45% and 25% respectively. Ascorbic acid used as reference standard yielded 63% H_2O_2 scavenging activity.

In conclusion the results obtained from the present study showed that the peels and juice of *Punica granatum* have extensive antimicrobial potential and thus it is of medicinal use.

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