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Evaluation of In-vitro Antibacterial Activity of Pomegranate (*Cv. Ganesh*) Peel Extract

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ABSTRACT

The study aimed to assess the effect of drying (freeze, tray and sun) and solvent (methanol, ethanol, water, acetone and hexane) on the antibacterial activity of extract from pomegranate by products. Antibacterial activity against six strains of pathogen (S. thermophilus P. aeruginosa, E. coli, Bifidobacteria, E. faecalis and L. acidophilus) were determined. All pomegranate peel extracts demonstrated selective antimicrobial activity against all pathogenic bacteria. The extraction of freeze drying powder in methanol showed maximum inhibitory concentration at 20, 25, 30 and 35 µl volumes followed by ethanol, water and acetone. Extraction in hexane solvent did not showed inhibitory concentration against selected pathogenic strains.

1.0 INTRODUCTION

Today's the food borne malady is the primary concern for consumer health. The microbial activity is responsible for the deterioration in food products to reduce the food safety and quality. Recently, the use of natural resources from plants, herbs and spices are the widely used in food processing and pharmaceutical sector due to biological functions as well as antioxidant and antimicrobial activity (Chidamabaramurthy et al., 2002; Negiand Jayaprakash, 2003). These sources have ability to inhibit the growth of pathogens due to presence of polyphenols, tannins and flavonoids contents (Ahmad and Beg, 2001; Machado et al., 2003; Naz et al., 2007; Shan et al., 2007). The pomegranate peel is excellent source of polyphenols, tannins (hydrolysable & condensed) and flavonoid compounds, which showed miraculous antimicrobial activity (Cowan, 1999; Machado et al., 2003; Voravuthikunchai et al., 2004). The pomegranate peel is a potential agent to resistance against various pathogens such as L. monocytogenes, *S. auereus, E.coli, S. thermophilus, S. typhimurium, P. aeruginosa, Bifidobacteria, E. faecalis, L. acidophilus , S. mutans Clarke , P. fluorescens, B. subtilis. P. vulgaris, K. pneumoniae , S. flexneri, T. rubrum , B. cereus, S. anatumand Y. enterocolitica* (Choi et al., 2009; Gould et al., 2009; Lansky et al., 2004; Satish et al., 2007; Jurenka, 2008; McCarrell et al., 2008; Sharma et al., 2010; Negi et al., 2003; Dahham et al., 2010; Khan et al., 2011; Yehia et al., 2011; Nozohour et al., 2018; Kaur et al., 2018; Ferrazzano et al., 2017; Chaudhary and Rahul, 2017; Hayouniet al., 2011; Kanattet al., 2010; Al-Zoreky, 2009). The various types of polar and non-polar solvents such as methanol, ethanol, water acetone, hexane, ether, chloroform, (either alone and combinations) are used for the assessment of antimicrobial activity of pomegranate peel against various pathogens (Al-Zahrani, 2011; Malviya et al., 2014). The main aim of this study was to investigate antimicrobial effect of different dried pomegranate peel powder extraction with various solvents.

2.0 MATERIAL AND METHODS

2.1 Drying and preparation of PGP powder

The fresh ripened pomegranate (*Cv. Ganesh*) was procured form AzadpurMandi New Delhi. The pomegranate washed with water and peeled manually. The obtained peel was drying by three various conditions to obtained powder. The fruit peel was freeze dried for 94 hours at -45ºC by using freeze dryer (Benchtop, Vir Tis, USA), sun drying for 72 hours and tray oven drying at 60ºC for 29 hours. The obtained peel powder was stored for extraction.

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2.2 Preparation of PGP extract

For the assessment of anti-bacterial activity of different dried pomegranate peel (0.2gm) were sonicated in 10 ml of different solvents such as methanol, ethanol, water, acetone and hexane by ultrasonic bath (CUB-5, Citizen, 40KHz, 220-240 volt) for 30 minutes at - 45ºC (Kumar and Neeraj, 2018; Kumar and Neeraj, 2019). After the ultra-sonication pomegranate peel extract was centrifuged (Sigma, 3-18, KS, Germany) at 8654 rpm speed and 5ºC for 10 minutes (Al-Zoreky, 2009). The obtained extract was filtered by using whatmaan paper for clear extract and stored in refrigeration conditions for further estimation of antimicrobial activity.

2.3 Bacterial strains

The pure lyophilized NCDC bacterial culture *S. thermophilus (NCDC158), P. aeruginosa (NCDC 105), E. coli (NCDC 134), Bifidobacteria (NCDC 229), E. faecalis (NCDC 223), and L. acidophilus (NCDC 334)*were obtained from Microbiology lab, NIFTEM, Sonipat.The obtained bacterial strains were cultured at 37°C on broth agar media, till reach the growth at logarithmic stage. The turbidity of growth media was adjusted by sterile broth media, and compare with 0.5 McFarland standard (625 nm Abs is 0.1), which delivered final concentration of bacteria is per ml 2x108 CFU.

2.4 Preparation of mediaand plates

The Muller Hinton agar media was prepared according to the commercially available dehydrate base and instructions of manufacturers. 38g powder of Muller Hinton agar powder was suspended in 1000 ml. distilled water in conical flask and autoclaved for sterilization. The sterilized agar was poured in sterilized petriplate (20-30 ml). The process was done in laminar air flow. The petriplate were stored in incubator at 36°C after solidified of media.

2.5 Determinations antibacterial *(In-Vitro*of) activity

The antibacterial activity of different dried pomegranate peel extract in various solvents were determined using agar well diffusion assay method followed by Al-Zoreky (2009) according to the National Committee for Clinical Laboratory Standards against *P. aeruginosa, E. coli, E. faecalis, S. thermophilus, Bifidobacteria and L. acidophilus* pathogenic bacterium. The 100 micro liter bacterial culture suspension was spread on Muller-Hinton agar medium plate's surface. The 6 mm diameter well made in the solidified media by using sterilize stainless steel borer. The different volumes of pomegranate peel extract (20µl, 25µl, 30µl and 35µl) were applied in the prepared well. After that, petriplate were incubated at 30 ±2°C for 12-48 hours until visible growth of pathogens and antibacterial resistance of applied pomegranate peel extract. The solvents were used as a negative and Ampicillin (10µg) was used as a control to measure the resistance, sensitivity and intermediate of pomegranate peel extract against used pathogenic strains. The antibacterial activity of pomegranate peel was expressed as diameter of zone inhibition in mm. The experiments were performed in triplicate.

3.0 STATISTICAL ANALYSIS

The one-way analysis of variance (ANOVA) and Duncan triplicate test was used to perform statically analysis of data by using IBMSPSS statistical software version (IBMSPSS 24.0). The mean and standard deviation was reported with ($P<0.05$) levels of significance.

4.0 RESULT AND DISCUSSION

4.1 Antibacterial Assay

The Ampicillin (10µg) was used as positive control to compared antibacterial activity of pomegranate peel extract against *S. thermophilus, P. aeruginosa, E. coli, Bifidobacteria, E. faecalis, and L. acidophilus* bacterial strains at 20µ, 25µl, 30µl and 35µl concentrations. Table 1 represented the sensitivity, intermediate and resistance of *Ampicillin* antibiotic against different bacterial strains in terms of zone inhibition in mm.

Table-1: As per CLSI, Inhibition activity of Ampicillin (10µg) antibiotic against different bacterial strains.

S. No	Strains	Ampicillin (10µg) (mm)		
		Sensitive	Intermediate	Resistant
1	*P.aeruginosa*	22	16-21	15

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2	*E. coli*	17	14-16	13
3	*Bifidobacteria*	30	25	20
4	*S.thermophilus*	24	-	-
5	*E.faecalis*	17	-	16
6	*L. acidophilus*	15		

4.2 *Pseudomonas aeruginosa*

P. aeruginosa is a gram negative, rod shape, *monoflagellated*and*asporogenous*bacterium. Its widely founds in nature (soil and water).*P. aeruginosa* normally grow well at 25°C to 37°C and it also has ability to grow at 42°C (Wu et al., 2015; Planet et al., 2018). It is ubiquitous environment bacterium, which produces many toxins in environment and cause human infection, damage tissue and enhances persistence (Hoiby and Koch, 1990; Tsakris et al., 2000; Ehsan and Clancy, 2015).The antibacterial activity of different dried pomegranate peel extract with different solvents against *P. aeruginosa* bacterial strains is present in Table 2. The methanolic extraction of pomegranate peel powder in freeze dried and tray oven dried pomegranate peel powder showed highest antimicrobial activity against *P. aeruginosa*bacterial strain at allconcentrations (20µl, 25µl, 30µl, 35µl)of pomegranate peel extract. The FDM and TDM showed 22.83mm, 22.66mm zone of inhibition at 20µl, 23mm zone of inhibition at 25µl, 24 mm, and 23.83mm zone of inhibition at 30µl and 24mm zone of inhibition at 35µl concentrations respectively.The ethanolic extract of different dried pomegranate peel such as FDE, TDE and SDE were showed 19.83mm, 18.83 and 18.83mm zone of inhibition respectively at 20µl concentrations. The aqueous and acetone extraction FDW (20.83mm), FDA (21mm), TDW (20.83mm), TDA (20mm),SDW (21.33mm) and SDA (19.83mm) zone of inhibition showed against *P. aeruginosa*bacterial strain at 20µl. The hexane extraction of pomegranate peel not showed antimicrobial activity against *P. aeruginosa* bacterial at concentration of 20µl. At the concentration of 25µl, and 30µl FDA, TDW, TDA, SDM and SDA were showed equal antimicrobial activity against *P. aeruginosa*bacterial with 21mm zone of inhibition. The highest activity were observed at 30µl and 35µl concentration in methanolic extraction of freeze dried and tray oven dried pomegranate peel in methanolic extraction with 24mm zone of inhibition . The above results indicated that methanolic extraction of freeze and tray dried pomegranate peel at all concentrations were found sensitive against *P. aeruginosa* bacteria followed by FDA and SDM at 40µl. The aqueous extraction of sun dried pomegranate peel also showed sensitivity against bacteria at 25µl, 30µl and 35µl extract concentration, whereas hexane fractions of pomegranate were not able to inhibit the bacterial growth at all the used extract concentrations (Negi et al., 2003). The obtained results indicated the freeze drying method and methanol solvents are the best for extraction of pomegranate peel for detection of antibacterial activity. The various studies has been investigated on this perspective to assessment of inhibition activity of pomegranate peel against *P. aeruginosa* bacteria and obtained similar results (Negi et al., 2003; Dahham et al., 2010; Khan et al., 2011; Leiteet al., 2014; Nozohour et al., 2018; Kaur et al., 2018; Alexandre et al., 2019).

Table-2: Antibacterial activity of pomegranate peel extract against *P. aeruginosa* bacterial strain

S. No.	**Sample ID**	**Concentrations (µl)**			
		20	**25**	**30**	**35**
		(Size of zone inhibition in mm)			
1	GFDM	22.83±0.29a	23.00±0.00a	24.00±0.00a	24.00±0.00a
2	GFDE	19.83±0.28e	20.16±0.28ef	20.33±0.57fg	21.16±0.28d
3	GFDW	20.83±0.28d	21.16±0.28c	21.33±0.28d	21.33±0.28d
4	GFDA	21.00±0.00cd	21.00±0.00c	21.83±0.29c	22.00±0.00c
5	GFDH	00.00±0.00g	0.00±0.00h	0.00±0.00l	0.00±0.00h
6	GTDM	22.66±0.57a	23.00±0.00a	23.83±0.28a	24.00±0.00a
7	GTDE	18.83±0.28f	19.00±0.00g	19.00±0.00j	19.00±0.00f
8	GTDW	20.83±0.28d	21.00±0.00c	20.16±0.29gh	20.33±0.57e
9	GTDA	20.00±0.00e	20.00±0.00 f	21.00±0.00de	21.16±0.28d
10	GTDH	00.00±0.00g	0.00±0.00h	0.00±0.00l	0.00±0.00h

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11	GSDM	20.83±0.28d	21.00±0.00c	21.00±0.00de	22.00±0.00c
12	GSDE	18.83±0.28f	19.83±0.28f	20.00±0.00ghi	20.00±0.00e
13	GSDW	21.33±0.57cd	22.00±0.00b	22.83±0.28b	23.00±0.00b
14	GSDA	19.83±0.28e	20.83±0.28cd	21.00±0.00de	21.83±0.28c
15	GSDH	00.00±0.00g	0.00±0.00h	0.00±0.00l	0.00±0.00h

(Here: F= Freeze dried, T= Tray oven dried, S= Sun dried, M= Methanol, E= Ethanol, W= Water, A= Acetone, H=Hexane). (N=3, Mean ± SD)

4.3*E. coli*

Methanol extraction of pomegranate peel in freeze dried and tray dried showed highest zone of inhibition against *E. coli*at concentration 20µl,25µl,30µl,35µl. The FDM and TDM showed 22.83mm zone of inhibition at 20µl and 25µl concentration. FDM showed 24mm and TDM showed 23.83mm zone of inhibition at 25µl concentration In FDM and TDM, Size of zone of inhibition was 24mm at concentration of 30µl and 35µl. Both concentrations showed stagnant result in FDM and TDM. SDM showed no inhibition. Ethanolic extract of differently dried pomegranate peel FDE,TDE and SDE showed variable result at concentration 20µl,25µl, 30µl,35µl. Zone of inhibition was approx. same (19.60mm, 19.83mm) in FDE at concentration 20µl, 25µl and 21mm, 21.16mm at 30µl,35µl. In TDE zone of inhibitionwas 20.93mm at 20µl and with little difference at other concentrations 25µl,30µl, and 35µl. It means in SDE, higher zone of inhibition was at concentration (23mm) 35µl followed by 22.16mm,22mm and 21mm at concentration 30µl,25µl,20µl. Water extract of pomegranate peel in case of sun drying, showed highest zone of inhibition at different concentrations. It was maximum (24mm) at concentration of 30µl and 35µl as compared to water extract of freeze dried and tray dried samples. Acetone extract of pomegranate peel in freeze dried, tray dried and sun dried samples showed antimicrobial against *E. coli*. The hexane extraction of pomegranate peel not showed antimicrobial activity against *E. coli* at all selected concentration and was not able to inhibit the bacterial growth at all the used extract concentrations. The zone of inhibition in FDA was 20mm,21mm,22mm,and 23mm at concentration 20µl,25µl, 30µl,35µl. The highest antimicrobial activities were observed at concentration of 30µl and 35µl with inhibition zone of 24 mm in methanol and water extract of peel dried differently (Table 3). The result indicated that freeze drying and tray drying method with methanol extraction and sun drying method with water extraction of peel showed maximum antimicrobial activity. It was almost 18mm at different concentration 20µl,25µl,30µl,35µl of FDW. Although all aqueous extract of pomegranate peel showed antimicrobial activity against E. coli but it was higher in SDW.Many studies confirmed that the pomegranate peel had antimicrobial activity against gram positive and gram negative bacteria (McCarrell et al., 2008; Khan and Hanee, 2011; Yehia et al., 2011; Pagliarulo et al., 2016).

Table-3: Antibacterial activity of pomegranate peel extract against *E. coli*bacterial strain

S. No.	**Sample ID**	**Concentrations (µl)**			
		20	**25**	**30**	**35**
		(Size of zone inhibition in mm)			
1	GFDM	23.83±0.28a	24.00±0.00a	24.00±0.00a	24.00±0.00a
2	GFDE	19.60±0.69fg	19.83±0.28h	21.00±0.00f	21.16±0.28e
3	GFDW	18.00±0.00h	18.00±0.00j	18.16±0.00i	18.33±0.57h
4	GFDA	20.00±0.00f	21.00±0.00fg	22.00±0.00d	23.00±0.00c
5	GFDH	0.00±0.00i	0.00±0.00k	0.00±0.00j	0.00±0.00i
6	GTDM	22.83±0.28b	23.83±0.28a	24.00±0.00a	24.00±0.00a
7	GTDE	20.93±0.11e	21.20±0.34fg	21.46±0.50e	21.33±0.58e
8	GTDW	20.00±0.00f	21.00±0.00fg	21.00±0.00f	22.00±0.00d
9	GTDA	19.00±0.00g	19.00±0.00i	19.86±0.23g	20.00±0.00f
10	GTDH	0.00±0.00i	0.00±0.00k	0.00±0.00j	0.00±0.00i
11	GSDM	21.00±0.00de	19.00±0.00i	19.00±0.00h	18.00±0.00h
12	GSDE	21.00±0.00de	22.00±0.00d	22.16±0.28d	23.00±0.00c
13	GSDW	22.83±0.28b	23.16±0.28b	24.00±0.00a	24.00±0.00a

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14	GSDA	21.33±0.57cde	21.50±0.50ef	22.00±0.00d	22.00±0.00d
15	GSDH	0.00±0.00i	0.00±0.00k	0.00±0.00j	0.00±0.00i

(Here: F= Freeze dried, T= Tray oven dried, S= Sun dried, M= Methanol, E= Ethanol, W= Water, A= Acetone, H=Hexane). (N=3, Mean ± SD)

4.4 *Bifidobacterium*

Methanol extraction of pomegranate peel in freeze dried and tray dried showed highest zone of inhibition against *Bifidobacterium*at concentration 20µl,25µl,30µl,35µl. The FDM,TDM and SDM showed 22.90mm, 21.90mm and 16.90mm sized zone of inhibition at 20µl. FDM showed 24mm sized zone of inhibitionat 30µl and 35µl concentration and TDM showed 23.80mm zone of inhibition at 35µl concentration (Table 4).SDM showed minimum sized zone of inhibition (16.96mm,17.80mm,19.96mm, and 20mm)at concentration of 20µl,25µl, 30µl, and 35µl. Ethanolic extract of differently dried pomegranate peel FDE,TDE and SDE showed variable result at concentration 20µl,25µl,30µl,35µl. Zone of inhibition was approx same (20mm, 20.06mm,and 21mm) in FDE at concentration 25µl,30µland35µl and 21mmat35µl.In TDE zone of inhibitionwas approx 20.85mm and 20.95mm at concentration of 25µl and 30µl with little difference at concentrations35µl. FDE and TDE showed equal level of antimicrobial resistance due same size of zone size at concentration of 35µl.In SDE, higher zone of inhibition was at concentration (21.93mm) 35µl followed by 21.33mm,21mm and 20mm at concentration 30µl, 25µl,20µl. Water extract of pomegranate peel in case of sun drying, showed highest zone of inhibition at different concentrations. It was maximum(24.73mm) at concentration of 35µl as compared to water extract of freeze dried and tray dried samples. Acetone extract of pomegranate peel in freeze dried, tray dried and sun dried samples also showed antimicrobial activity against*Bifidobacterium*. SDA showed highest antimicrobial activity against *Bifidobacterium*as compared to FDA and TDA reflected on the basis of size of zone of inhibition. Highest zone of inhibition was at concentration of 35µl in SDE. The hexane extraction of pomegranate peel not showed antimicrobial activity against *Bifidobacterium* at all selected concentration and was not able to inhibit the bacterial growth at all the used extract concentrations. The highest antimicrobial activities were observed at concentration of 35µl and with inhibition zone of 24.73 mm in water extract of peel dried differently. The result indicated that freeze drying method with methanol extraction and sun drying method with water extraction of peel showed maximum antimicrobial activity (Reddy et al., 2007; Bialonska et al., 2010). FDM and SDW were showing maximum antimicrobial activity against *Bifidobacterium*.

Table-4: Antibacterial activity of pomegranate peel extract against *Bifidobacterium* bacterial strain

S. No.	Sample ID	Concentrations (µl)			
		20	25	30	35
		(Size of zone inhibition in mm)			
1	GFDM	22.90±0.17a	23.00±0.00a	24.00±0.00 a	24.00±0.00b
2	GFDE	19.93±0.11d	20.00±0.00d	20.06±0.11 f	21.00±0.00f
3	GFDW	20.00±0.00d	20.80±0.34c	21.00±0.00 e	21.93±0.11e
4	GFDA	20.00±0.00d	20.03±0.05d	21.00±0.00 e	21.00±0.00f
5	GFDH	0.00±0.00h	0.00±0.00h	0.00±0.00h	0.00±0.00i
6	GTDM	21.90±0.17b	22.83±0.28a	23.73±0.46 a	23.80±0.34b
7	GTDE	19.00±0.00e	20.85±0.25c	20.95±0.08 e	21.00±0.00f
8	GTDW	21.00±0.00c	21.00±0.00c	21.00±0.00 e	21.00±0.00f
9	GTDA	18.75±0.43f	19.10±0.17e	19.20±0.34g	20.00±0.00g
10	GTDH	0.00±0.00h	0.00±0.00h	0.00±0.00h	0.00±0.00i
11	GSDM	16.96±0.05g	17.80±0.34f	19.96±0.05 f	20.00±0.00g
12	GSDE	20.00±0.00d	21.00±0.00c	21.33±0.05d	21.93±0.11e
13	GSDW	22.85±0.25a	23.00±0.00a	23.80±0.34 a	24.73±0.46a
14	GSDA	20.00±0.00d	20.95±0.08c	21.00±0.00 e	21.98±0.02e
15	GSDH	0.00±0.00h	0.00±0.00h	0.00±0.00h	0.00±0.00i

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(Here: F= Freeze dried, T= Tray oven dried, S= Sun dried, M= Methanol, E= Ethanol, W= Water, A= Acetone, H=Hexane). (N=3, Mean ± SD)

4.5 *Streptococcus thermophilus*

The results of the antibacterial activity of pomegranate peel extract against *S. thermophilus* are shown in Table 5. Methanolic extraction of pomegranate peel in freeze dried and tray dried showed highest zone of inhibition against *S. thermophilus* at concentration 20µl,25µl,30µl,35µl. FDM,TDM and SDM showed 22.80mm, 22.75mm and 18.96mmsized zone of inhibition at 20µl. FDM and TDM showed 24mm sized zone of inhibition at 35µl and 23.83mm,23mm at 35µl concentration butSDM showed 22.73mm zone of inhibition at concentration of25µl. The results reflected thatTDM showed minimum antimicrobial activity against *S. thermophilus* as compared to FDM and TDM. Ethanolic extract of differently dried pomegranate peel FDE, TDE and SDE showed variable result at concentration 20µl,25µl,30µl,35µl. Zone of inhibition was approx same (22mm, 22.23mm) in TDE and FDE at concentration of 35µl. In TDE zone of inhibition was recorded 22.03mm and 22.23mm at concentration of 25µl and 35µl.TDE showed highest antimicrobial activity against *S.thermophilus* as compared to FDE and SDE. Methanolic and ethanolic extract of pomegranate peel in case of tray drying method, showed highest zone of inhibition at concentrations of 35µl. It was maximum(24mm) in TDM and 22.23mm in TDE at concentration of 35µl. Water extract of pomegranate peel in case of sun drying, showed highest zone of inhibition at different concentrations. It was maximum (24 mm) at concentration of 35µl followed by 23.93mm, 23.10mm, 22.70 at concentrations 30µl,25µl,and 20µl respectively. Water extract of freeze dried and tray dried samples showed minimum inhibition as compared to sun dried samples SDW showed maximum antimicrobial activity against *S.thermophilus*. Acetone extract of pomegranate peel in freeze dried, tray dried and sun dried samples also showed antimicrobial activity against*S.thermophilus*. FDA showed highest antimicrobial activity *S.thermophilus*as compared to SDA and TDA reflected on the basis of their size of zone of inhibition. Highest zone of inhibition 23.13mm was at concentration of 35µl in FDA. The hexane extraction of pomegranate peel not showed antimicrobial activity against *S.thermophilus*at all selected concentration and was not able to inhibit the bacterial growth at all the used extract concentrations. The highest antimicrobial activities were observed at concentration of 35µl with inhibitionzone sizeof 24 mm in FDM,TDM and SDW. The result indicated that freeze drying and tray drying method with methanol extraction and sun drying method with water extraction of peel showed maximum antimicrobial activity against *S. thermophilus*.

Table-5: Antibacterial activity of pomegranate peel extract against *S. thermophilus*bacterial strain

S. No.	Sample ID	Concentrations (µl) 20	25	3035	
		(Size of zone inhibition in mm)			
1	GFDM	22.80±0.20a	23.46±0.50a	23.83±0.28a	24.00±0.00b
2	GFDE	20.00±0.00d	21.00±0.00e	21.00±0.00d	22.00±0.00
3	GFDW	19.93±0.11d	20.00±0.00f	20.00±0.00e	21.00±0.00
4	GFDA	21.00±0.00c	22.00±0.00d	22.10±0.17c	23.13±0.23c
5	GFDH	0.00±0.00g	0.00±0.00h	0.00±0.00h	0.00±0.00
6	GTDM	22.75±0.43a	23.00±0.00bc	23.00±0.00b	24.00±0.00b
7	GTDE	20.86±0.23c	22.03±0.05d	22.16±0.28c	22.23±0.40
8	GTDW	19.90±0.17d	20.00±0.00f	20.00±0.00e	20.96±0.05
9	GTDA	20.00±0.00d	20.00±0.00f	21.00±0.00d	21.00±0.00
10	GTDH	0.00±0.00g	0.00±0.00h	0.00±0.00h	0.00±0.00
11	GSDM	18.96±0.57e	22.73±0.46c	19.40±0.69f	19.86±0.80
12	GSDE	21.00±0.00c	21.00±0.00e	21.93±0.11c	22.10±0.17
13	GSDW	22.70±0.51a	23.10±0.17b	23.93±0.11a	24.00±0.00b
14	GSDA	20.83±0.28c	20.93±0.11e	21.00±0.00d	21.90±0.17
15	GSDH	0.00±0.00g	0.00±0.00h	0.00±0.00h	0.00±0.00

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(Here: F= Freeze dried, T= Tray oven dried, S= Sun dried, M= Methanol, E= Ethanol, W= Water, A= Acetone, H=Hexane). (N=3, Mean ± SD)

4.6 *Enterococcus faecalis*

Methanol extraction of pomegranate peel in freeze dried and tray dried showed highest zone of inhibition against *E. faecalis* at concentration 30µl and 35µl. FDM, TDM and SDM showed 24.03mm, 24, and 23 mm sized zone of inhibition at 35µl (Table 6). TDM showed 24mm sized zone of inhibition at concentration of 30µl and 35µl similarlySDM showed 23mm sized zone of inhibition at all four concentration used. FDM also showed 23mm sized zone of inhibition at concentration 30µl and 35µl having equal antimicrobial activity against *E.faecalis* as in SDM at all concentrations.The results reflected that FDM showed maximum antimicrobial activity against*E. faecalis*as compared to SDM and TDM. Ethanolic extract of differently dried pomegranate peel FDE, TDE and SDE showed variable result at concentration of 20µl,25µl,30µl, and 35µl. Zone of inhibition was same (20mm) in TDE and (21mm)FDE at concentration of 25µl,30µl, and 35µl. In SDE zone of inhibition was approx 23mm at concentration of 35µl and 22mm at concentration of 20µl,25µl,30µl. SDE showed highest antimicrobial activity against *E. faecalis*as compared to FDE and TDE. Aqueous extract of pomegranate peel in case of sun drying, showed highest zone of inhibition at different concentrations. It was maximum (23mm) at concentration of 35µl followed by 22.96mm, 22.10mm and 22mm at concentrations of 30µl,25µl, and 20µl respectively. Water extract of freeze dried and tray dried samples showed minimum inhibition as compared to sun dried samples. SDW showed maximum antimicrobial activity against *E. faecalis*. FDW and TDW shoed equal antimicrobial activity against *E. faecalis*at all concentrations because size of zone of inhibition was same. Acetone extract of pomegranate peel in freeze dried, tray dried and sun dried samples also showed antimicrobial activity against*E. faecalis*. FDA showed highest antimicrobial activity as compared to SDA and TDA reflected on the basis of their size of zone of inhibition. Highest zone of inhibition 22.06mm was at concentration of 35µl in FDA. TDA and SDA also showed less antimicrobial activity against as compared to FDA.The hexane extraction of pomegranate peel not showed antimicrobial activity against *E.faecalis*at all selected concentration and was not able to inhibit the bacterial growth at all the used extract concentrations. The highest antimicrobial activities were observed at concentration of 35µl with inhibition zone size of 24.03 mm in FDM,24mm in TDM, 23mm in SDE and SDW. The result indicated that freeze drying, tray drying and sun drying method with methanol, ethanol and water extraction of peel showed maximum antimicrobial activity against *E.faecalis*. FDM and TDM showed maximum antimicrobial activity againststrainas compared to others. The obtained results were similar with previous investigation conducted by Duman et al. (2009). Barathikannan et al. (2016) reported in-vivo and in-vitro antibacterial activity of pomegranate peel extract with different solvents.

Table-6: Antibacterial activity of pomegranate peel extract against *E. faecalis* bacterial strain

S. No.	Sample ID	Concentrations (µl)			
		20	25	30	35
		(Size of zone inhibition in mm)			
1	GFDM	23.06±0.41a	23.00±0.20a	23.00±0.34a	24.03±0.30a
2	GFDE	20.00±0.65d	21.00±0.36c	21.00±0.36c	21.00±0.52d
3	GFDW	20.00±0.43d	20.00±0.65d	20.00±0.65d	20.00±0.65e
4	GFDA	20.00±0.60d	21.00±0.66c	21.13±0.55c	22.06±0.41c
5	GFDH	0.00±0.00g	0.00±0.00g	0.00±0.00g	0.00±0.00g
6	GTDM	22.00±0.65b	23.00±0.65a	23.00±0.62a	24.00±0.36a
7	GTDE	19.00±0.65e	20.00±0.70d	20.00±0.52d	20.00±0.00e
8	GTDW	20.00±0.52d	20.00±0.26d	20.00±0.81d	20.00±0.70e
9	GTDA	20.00±0.36d	20.00±0.72d	21.00±0.55c	21.00±0.62d
10	GTDH	0.00±0.00g	0.00±0.00g	0.00±0.00g	0.00±0.00g
11	GSDM	23.00±0.45a	23.00±0.91a	23.00±0.88a	23.00±0.00b
12	GSDE	22.00±0.72b	22.00±0.65b	22.00±0.91b	23.00±0.55b

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13	GSDW	22.00±0.00b	22.00±0.55b	22.96±0.35a	23.00±0.72b
14	GSDA	19.00±0.43e	20.06±0.49d	20.10±0.52d	21.03±0.55d
15	GSDH	0.00±0.00g	0.00±0.00g	0.00±0.00g	0.00±0.00g

(Here: F= Freeze dried, T= Tray oven dried, S= Sun dried, M= Methanol, E= Ethanol, W= Water, A= Acetone, H=Hexane). (N=3, Mean ± SD)

4.7*Lactobacillus acidophilus*

The results of the antibacterial activity of different dried pomegranate peel extract with 5 different solvents are shown in Table 7. Methanol extraction of pomegranate peel in freeze dried and tray dried showed highest zone of inhibition against *L. acidophilus*at concentration 30µl and 35µl. FDM, TDM showed24mmsized zone of inhibition at 30µl and35µl concentration and 23mm sized zone of inhibition at concentration of 20µland 25µl. SDM showed 22mm sized zone of inhibition at concentration of 35µl.SDM also showed 20mm sized zone of inhibition at concentration 25µl and 30µl having equal antimicrobial activity against *L. acidophilus*.The results reflected that FDM and TDM showed maximum antimicrobial activity against *L. acidophilus*as compared to SDM. Ethanolic extract of differently dried pomegranate peel FDE, TDE and SDE showed almost same result at all concentration. Zone of inhibition was found same (21mm) in FDE and TDE at concentration of 30µl,35µl. It was equal to SDE at concentration of 20µl,25µl, and 30µl. In SDE maximum size of zone of inhibition was approx 22mm at 35µl concentration, which was highest among three ethanol extract samples. SDE showed highest antimicrobial activity against *L. acidophilus* as compared to FDE and TDE. Aqueous extract of pomegranate peel in case of sun drying, showed highest zone of inhibition(23mm) at 30µl and 35µl concentrations. SDW showed highest antimicrobial activity against *Lactobacillus acidophilus* as compared to FDW and TDW. Water extract of freeze dried and tray dried samples showed minimum inhibition as compared to sun dried samples. FDW and TDW showed equal antimicrobial activity against *L. acidophilus* at concentrations of 30µl,35µl because size of their zone of inhibition was same(21mm at 30µl and 22mm at35µl). Acetone extract of pomegranate peel in freeze dried, tray dried and sun dried samples also showed antimicrobial activity against*L. acidophilus* but it was less as compared to other samples. FDA showed highest antimicrobial activity *L. acidophilus* as compared to SDA and TDA reflected on the basis of their size of zone of inhibition. Maximum sized zone of inhibition was 22mm was at concentration of 35µl in FDA. TDA and SDA showed less antimicrobial activity against *Lactobacillus acidophilus*as compared to FDA.The hexane extraction of pomegranate peel not showed antimicrobial activity against *L. acidophilus*at all selected concentration and was not able to inhibit the bacterial growth at all the used extract concentrations. The highest antimicrobial activities were observed at concentration of 35µl with inhibition zone size of 24 mm in FDM and TDM, 23mm in aqueous extract of sun dried peel powder. The result indicated that pomegranate peel extraction with methanol, ethanol, acetone and water showedexcellent antimicrobial activity against *L. acidophilus* (Barathikannanet al., 2016).

Table-7: Antibacterial activity of pomegranate peel extract against L. *acidophilus* bacterial strain

S. No.	Sample ID	Concentrations (µl) 20	25	30	35
		(Size of zone inhibition in mm)			
1.	GFDM	23.00±0.30a	23.00±0.20a	24.00±0.30a	24.00±0.46a
2.	GFDE	20.00±0.00d	21.00±0.30c	21.00±0.40d	21.00±0.40d
3.	GFDW	21.00±0.30c	21.00±0.00c	21.00±0.20d	22.00±0.10c
4.	GFDA	20.00±0.30d	21.00±0.00c	21.00±0.20d	22.00±0.53c
5.	GFDH	0.00±0.00h	0.00±0.00h	0.00±0.00g	0.00±0.00g
6.	GTDM	23.00±0.00a	23.00±0.00a	24.00±0.30a	24.00±0.61a
7.	GTDE	20.00±0.00d	20.00±.0.00d	21.00±0.00d	21.00±0.00d
8.	GTDW	21.00±0.00c	21.00±0.00c	21.00±0.00d	22.00±0.00c
9.	GTDA	19.00±0.87e	19.00±0.30e	20.00±0.53e	20.00±0.50e
10.	GTDH	0.00±0.00h	0.00±0.00h	0.00±0.00g	0.00±0.00g

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11.	GSDM	21.00±0.10c	20.00±0.40d	20.00±0.50e	22.00±0.40c
12.	GSDE	21.00±0.60c	21.00±0.50c	21.00±0.70d	22.00±0.41c
13.	GSDW	22.00±0.30b	22.00±0.40b	23.00±0.80b	23.00±0.45b
14.	GSDA	20.00±0.00d	20.00±0.40d	21.00±0.80d	21.03±0.60d
15.	GSDH	0.00±0.00h	0.00±0.00h	0.00±0.00g	0.00±0.00g

(Here: F= Freeze dried, T= Tray oven dried, S= Sun dried, M= Methanol, E= Ethanol, W= Water, A= Acetone, H=Hexane). (N=3, Mean ± SD)

5.0 CONCLUSION

Pomegranate peel is very beneficial by product of pomegranate fruits, which is excellent source of phenolic compounds and attractive source of value added compounds. The study revealed that the extraction of phenolic compounds from pomegranate peel directly influenced by drying condition and solvent. The results indicated,extraction in methanol solvent of freeze dried pomegranate peel powder significantly showed higher antibacterial activity against six strains as compared to tray and sundried powder. Extraction with hexane solvent did not affect the growth of pathogens when compared with other solvents. The resistance of pomegranate peel can be potential for reducing the risk of pathogenic contamination in food matrix.

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