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Evaluation of Antioxidant Activity of Solvent Extracted Pomegranate Peel and its Effect against Plant Pathogenic Bacteria and Fungi

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Pomegranate peel the byproduct of pomegranate industry when extracted with different solvents revealed that methanol: water (80:20) and acetone: water (80:20) to be equally effective solvents for extraction of various antioxidants. The peel extract of Ganesh variety of pomegranate had more amount of antioxidant *viz.*, phenol, proathocyanidin and flavonoid with more antioxidant activity in terms DPPH and FRAP value when compared with the synthetic butylated hydroxyl toluene (BHT). The methanolic extract when tested against different plant pathogens *viz.*, *Xanthomonas* spp.(*Xanthomonas axonopodis pv. punicae, Xanthomonas axonopodis pv. malvacearum* and *Xanthomonas axonopodis pv. citri*), *Aspergillus flavous* and *Fusarium oxysporum* f.sp. *ciceri* was found to inhibit the growth of bacteria and fungi.

Key words: Pomegranate peel, antioxidant, photochemical, DPPH, FRAP.

Pomegranate (*Punica granatum* L.) is an economically important fruit crop and largely cultivated in many tropical and subtropical regions of world. The total area under cultivation of pomegranate in India is 113.2 thousand ha with production is about 745 metric tons and productivity is 6.6 metric tons [1]. Pomegranate has a great antioxidant potential and good potency for cancer prevention due to high levels of phenolic compounds, flavonoids, anthocyanins, tannins, ascorbic acids, and gallic acid present in the fruit [2].

P. granatum has been used extensively as a traditional medicine in many countries for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory pathologies. In addition, *P. granatum* is reported to have antioxidant anti-atherosclerotic , antibacterial and antiviral properties. The constituents of *P. granatum* include gallocatechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol, which are very well known for their therapeutic properties[3].

During the industrial processing of pomegranate, large volumes of industrial wastes are produced, which have a wide range of nutritional values. Therefore in the recent past attention has been focused on the industrial byproducts of pomegranate that have a high potential of antioxidant and antimicrobial properties, great interest has recently been focused on the addition of polyphenols to foods and biological systems, due to their well-known abilities to scavenge free radicals [4]. Pomegranate (*Punica granatum*) has been known to have considerable pharmacological properties with antimicrobial, antiviral, anticancer, potent antioxidant and

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antimutagenic effects[5]. A number of extracts of pomegranates tested against a range of human pathogenic bacteria *S. aureus, E.coli, Klebsiella pneumoniae, Proteus vulgaris, Bacillus subtilis and Salmonella typhi* [6]. Pomegranate extracts were able to inhibit not only the growth of *S. aures* but also the production of enterotoxin[7] . The present work has been proposed to analyze the effective solvent for extracting the antioxidants and testing the *in vitro* effects of these extracts against pathogenic microorganisms.

MATERIALS AND METHODS

The fresh mature pomegranate fruits of three cultivars *viz*., Ganesh, Bhagawa and Mridula and one wild germplasm *viz*., Jodhpuri Red used in this study were obtained from the Horticultural farm, MPKV, Rahuri. The peel of fruits were manually removed, shade dried and powdered in grinder to 40-mesh. Three different solvents *viz.* Methanol:Water (80:20), Acetone:Water (80:20) and Ethyl acetate were used for extraction. Dried powder of peels (15 g) were extracted with100 mL of each solvent at room temperature for 1 hour. The extract was filtered through Whatman No.42 filter paper to remove fine particles. The residue was re-extracted with the same solvent and the extracts were added to each other. After extraction, the solvent was evaporated on a rotary evaporator under vacuum and at 30^0C up to 20 ml and the concentrated extracts were stored at -20^0 C until use. The extract were used for estimation of phytochemicals ug., total phenols, proanthocyanidins, flavonoids and antioxidant activity both by DPPH and FRAP assay and tested for its efficiency against plant pathogens bacteria and fungi (*Xanthomonas axonopodis pv. punicae, Xanthomonas axonopodis pv. malvacearum* , *Xanthomonas axonopodis pv. citri, Aspergillus flavous* and *Fusarium oxysporum* f.sp. *ciceri*) using standard analytical methods and tests.

Phytochemical analysis

Estimation of total phenols was carried out with Folin Ciocalteu reagent [8]. The concentrated peel extract from different solvents was diluted 50 fold in distilled water and 0.1ml of the diluted extract was taken for estimation of phenols. The 0.1ml of the diluted aliquots of samples were mixed with 2.5 ml of 10-fold diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 min at room temperature and the absorbance was measured at 760 nm. The final results were expressed as tannic acid (100µg/ml) equivalents from standard curve.

Determination of proanthocyanidins was based on the procedure reported by [9] Sun *et al.* (1998). A volume of 250µl of the concentrated extract was mixed with 750 µl water to make a total volume of 1ml. The above solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml 3.6N hydrochloric acid. The mixture was allowed to stand for 15 min. and the absorbance was measured at 500 nm. The final results were expressed as catechin equivalents based on the standard curve prepared by using 0,30,60,90 and 120 µg of catechin. The standard curve with 3.6 N HCl was taken into consideration for calculating proanthocyanidins in the sample to express as catechin equivalent.

The flavonoids content was measured using a modified colorimetric method[10]. The sample extract was diluted (1:10) with 9 ml respective solvent *viz*; methanol, acetone and ethyl acetate. From this 1:10 diluted extract 0.4 ml of the solution was transferred to a 25 ml flask containing 5 ml of 30% ethanol and mixed with 0.75 ml of 5% sodium nitrite for 5 min. Then 0.75ml of 10% aluminium nitrate was added. After 6 min the reaction was stopped by addning 5 ml of 1M sodium hydroxide. The mixture was further diluted with 30% ethanol up to 25ml. The absorbance of the mixture was immediately measured at 510 nm.The flavonoids content was calculated and expressed as rutine (10 µg/ml) quivalents from the standard curve.

Assessment of Antioxidant activity

Ferric Reducing Antioxidant Power (FRAP)

Antioxidant activity was estimated by FRAP (Ferric Reducing Antioxidant Power) assay [11]. Aliquot of 40 ul samples were diluted (1:25) with respective solvents and the volume was made 1ml. 40 ul of the diluted extract was mixed with 0.2 ml distilled water and 1.8 ml FRAP reagent prepared in 2.5 ml of 10 mM TPTZ solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3M acetate buffer, pH 3.6 and was prepared fresh and warmed at 37°C prior to use. The absorbance of reaction mixture was measured at 593 nm after incubation at

37 °C for 10min. The standard 1 mM $FeSO_4$ solution was used to take the absorbance readings at 593 nm in a similar way to express the results of FRAP activity. The final result were expressed as the concentration of the antioxidants having a ferric reducing ability (µM/g) equivalent to that of 1 mM $FeSO_4$.

DPPH Activity

The free radical scavenging activity of the peel extract and the standard reference compound (BHT) was analyzed by the DPPH assay[12]. In quantitative assay 40 µl of concentrated extract was diluted to 1ml with respective solvent. From this, a volume of 39 (1.17 mg), 52 (1.56 mg) and 100 µl (3.0 mg) was combined with 2.7 ml of methanol and 1ml of 0.1% of methanolic DPPH. Incubated for 30 min. in dark and the absorption maxima were measured at 517 nm. The control contained all reagents except the extract fraction while methanol was used as blank. Positive samples with observed discoloration from purple to yellow were used for quantitative analysis. 0.16% BHT was used as the standard.

$$\text{Antioxidant Activity (\%)} = \frac{\text{O.D. of control} - \text{O. D. of sample} \times 100}{\text{O.D. of control}}$$

Determination of antibacterial and antifungal activity

The antibacterial activity of the pomegranate peel extract was tested on different strains of *Xanthomonas* spp. using the disc diffusion method. Active cultures for the experiments were prepared by taking suspension of bacterial strains and mixed with sterilized Nutrient Agar (NA). This NA containing bacterial strains was poured in petri plates. The sterile discs (4-5 mm diameter) dipped in different sample extracts having 4-5µl volume of extract and placed on the surface of NA medium. Plates were incubated at 25 ± 2 °C. After 48 hr the inhibition zones around the disc were measured. One disc with streptocycline placed in middle and one disc of control also placed on surface of NA in petri plates. The same procedure was followed for *Fusarium oxysporum* using Potato Dextrose Agar (PDA) to determine antifungal activity. The antifungal activity of extract also tested on *Aspergillus flavous* by mixing different concentrations of extract (15µl/100ml PDA, 50µl/100ml PDA) in PDA. Poured this mixture in petri plates and a bit of culture placed in the centre of petri plate. Allow it to grow in incubator at 25 ± 2 °C. After 4 days growth of the zone measured in mm and same procedure was followed for control. All determinations were carried out in triplicate and data were subjected to statistical analysis by Microsoft excel data analysis.

RESULTS AND DISCUSSION

Estimation of total phenol content in the dried peel extracted with different solvents (as tannic acid equivalent).

Three different solvents were used for the extraction of phenols which recorded differential response. Methanol:Water (80:20) extract recorded significantly maximum content of phenols with a mean value of (74.97 mg/g) whereas ethyl acetate recorded the minimum content of (17.54 mg/g). As regard to the content of phenols in different varieties is concerned, significantly maximum content of phenols were recorded in Ganesh (64.06 mg/g) and the least content of phenols (45.92 mg/g) were recorded in Mridula . Both variety and solvent recorded significant differences in the total phenols and solvent into variety interaction also were significant. It was observed that methanol:water (80:20) and acetone:water (80:20) were equally effective in extracting the phenol whereas the ethyl acetate was least effective The maximum phenol content of (87.04mg/g) was recorded in Ganesh with acetone whereas the least phenol content of 13.30 mg/g was recorded in Mridula with ethyl acetate (Table1). The phenolics content of 249.4 ± 17.2 mg/g as tannic acid equivalent have been recorded earlier in the dried pomegranate peel extract when extracted with combination of methanol, ethanol and acetone[13].

Phenolic compound are secondary metabolites which are synthesized in plant. They possess biological properties such as antioxidant, anti-aging, anti-carcinogen, anti-inflammation, cardiovascular protection, improvement of the endothelial function as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilies [14]. Some typical phenolic compound which are highly correlated with antioxidant activity are phenolic acids; for example gallic acid and polyphenol; for example

flavonoids. There were several studies showing that antioxidant activity was strongly correlated with the content of the total phenolic compound.

The maximum phenolic content of 8.26% has been reported in the pomegranate peel extract with methanol whereas ethyl acetate was found to be least effective with a yield of total phenolics of 1.18 % [15]. In general methanol had a higher capacity for extracting phenolic compounds from dried peel than water and ethanol [16]. The results of the present investigation are in accordance with the reported values and methanol is a better solvent is in conformity with the earlier reports. It has been reported that highest polyphenol were extracted

Table 1. The content of phytochemicals in dry peel of pomegranate extracted with different solvents.

Phenols(mg/g)				
Solvent	Ethyl Acetate	Acetone	Methanol	Mean
Ganesh	22.23	87.54	82.39	64.06
Bhagawa	19.57	84.95	81.25	61.92
Mridula	13.30	66.69	57.76	45.92
Jodhpuri red	15.07	69.54	60.45	48.35
Mean	17.54	72.68	74.97	55.06
	Solvent	Variety	S x V	CV %
SE(±)	0.69	0.25	1.39	4.37
CD @5%	2.02	0.73	4.05	
Proanthocyanidins (mg/g)				
Ganesh	3.99	10.12	8.85	7.65
Bhagawa	3.20	7.86	4.05	5.04
Mridula	4.82	25.07	12.88	14.26
Jodhpuri red	1.33	8.40	3.05	4.26
Mean	3.34	12.87	7.21	7.80
	Solvent	Variety	S x V	CV %
SE(±)	0.22	0.08	0.44	9.72
CD @5%	0.64	0.23	1.28	
Flavonoids (mg/g)				
Ganesh	0.98	10.58	4.41	5.32
Bhagawa	0.87	10.13	4.10	5.04
Mridula	0.48	5.78	3.78	3.35
Jodhpuri red	0.23	4.71	3.27	2.74
Mean	0.64	7.80	3.89	4.11
	Solvent	Variety	S x V	CV %
SE(±)	0.04	0.01	0.08	3.38
CD @5%	0.12	0.04	0.23	

Table 2. FRAP value (µM/g) equivalent to that of 1mM FeSO4 of dry peel of pomegranate extracted with different solvents

FRAP value (µM/g)				
Solvent				
Varieties	Ethyl Acetate	Acetone	Methanol	Mean
Ganesh	48.33	58.56	57.33	54.74
Bhagawa	47.89	57.67	56.89	54.15
Mridula	44.78	57.00	56.78	52.85
Jodhpuri red	44.67	57.00	56.89	52.85
Mean	46.42	57.56	56.97	53.65
	Solvent	Variety	S x V	CV %
SE(±)	0.04	0.01	0.08	0.25
CD @5%	0.11	0.04	0.23	

with acetone : water (70:30), which were 28.47mg/ 10ml and the least with ethyl acetate i.e. 4.55 mg/ 10ml [17] . It has been also been reported that water gave the highest extract yield of polyphenols (17.78%) and ethyl acetate was least effective giving extract yield of 0.75% [18].

Estimation of proanthocyanidins in the dried peel extracted with different solvents (as catechin equivalent)

Proanthocyanidins are potent free radical scavengers and are belived to be contributors to the health benifits of fruits and vegetables [19] . Proanthocyanins antioxidant capabilities has been reported to be 20 times more potent than vit.E [20] . Three different solvents were used for the extraction of proanthocyanidins which recorded differential response.

When the dried peel of the pomegranate was extracted with the three solvents, it was observed that methanol and acetone were equally effective in extracting the proanthocyanidins whereas the ethyl acetate was significantly least effective. As regard to the different varieties, variety Mridula recorded a mean proanthocyanidin content of 12.87 mg/g whereas variety Jodhpuri Red recorded the mean proanthocyanidins content of 3.34 mg/g. The solvent, variety and their interaction effects were significant (Table 1). The proanthocyanidin content of 10.9 ± 0.5 mg/g as catechin equivalent have been recorded in the dried pomegranate peel extract when extracted with combination of methanol, ethanol and acetone[13]. The proanthocyanidin content of acetone extract were higher than the contents of the water extract and methanol extract[15]. The proanthocyanidins were extracted from pomegranate peel in acetone :water (70:30), ethanol: water (50:50) and ethyl acetate. It was observed that acetone: water (70:30) contain maximum proanthocyanidins of 5.82 ± 0.13 mg/10 ml[17].

Flavonoids content in the dried peel extracted with different solvents (as rutin equivalent)

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized

Table 3. Antioxidant activity (%) of dry peel of pomegranate extracted with different solvent analyzed by DPPH

Concentration (mg)				
Solvent Methanol				
Varieties'	Ganesh	Bhagawa	Mridula	Jodhpuri Red
1.17mg	20.98	5.61	0.95	1.06
1.56mg	64.09	53.51	1.90	4.55
3.0mg	81.54	80.36	63.56	71.69
Mean	55.54	46.49	22.14	25.77
	V	E	V x E	CV%
SE	3.95	3.42	6.84	31.62
CD5%	11.46	9.93	19.86	
Solvent Acetone				
1.17mg	76.47	75.15	75.67	75.46
1.56mg	77.47	77.00	76.66	76.47
Mean	76.97	76.07	76.17	75.97
	V	E	V x E	CV%
SE	0.32	0.22	0.45	1.01
CD5%	NS	0.66	NS	
Solvent Ethyl acetate				
1.17mg	1.16	1.11	0.42	0.32
1.56mg	6.24	6.13	4.58	5.06
3.0mg	10.95	10.61	6.06	6.55
Mean	6.12	5.95	3.69	3.98
	V	E	V x E	CV%
SE	7.40	6.40	12.82	450.02
CD5%	NS	NS	NS	
BHT (0.16%)				
74.16				

according to chemical structure into flavonols, flavones, flavonones, isoflavones, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). The capacity of flavonoids to act as antioxidant depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant diatary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity. Flavonoids extraction from dried peel of the pomegranate using with three solvent revealed that methanol (80:20) and acetone (80:20) were more effective in extracting the flavonoids whereas the ethyl acetate was least effective. As regard to the different varieties, variety Ganesh recorded a mean flavonoids content of 7.80 mg/g whereas variety Jodhpuri Red recorded least mean flavonoids content of 0.64 mg/g. The solvent, variety and their interaction effects were significant (Table 1). The flavonoids content of 59.1 ± 4.8 mg/g as a rutin equivalent have been recorded in the dried pomegranate peel extract when extracted with combination of methanol, ethanol and acetone[13] . The maximum flavonoid content have been reported in non pungent peppers when extracted with methanol [21].

Antioxidant activity - Ferric reducing antioxidant power (FRAP) of dried peel

FRAP assay uses antioxidants as reductants in a redox linked colorimetric assay. At low pH, the reduction of (Fe III TPTZ) complex to (Fe II TPTZ) which has an intense blue colour can be monitored by measuring the change in absorption at 593 nm. Three different solvents were used for the estimation of ferric reducing antioxidant power recorded differential response.

When the dried peel of the pomegranate was extracted with three solvents, it was observed that methanol and acetone were equally effective

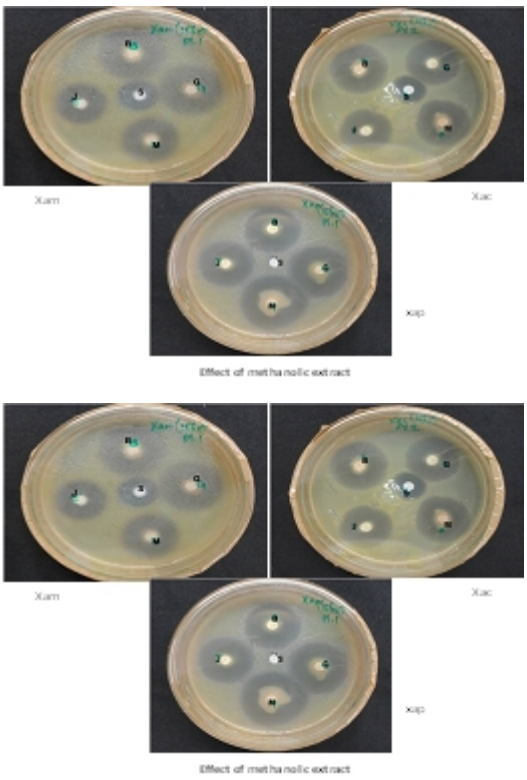
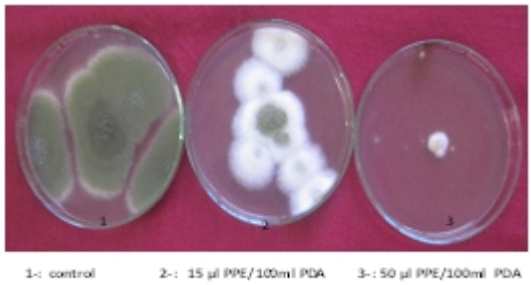
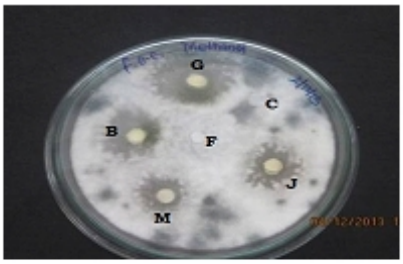


Plate 1. Effect of methanolic and acetone extract of pomegranate peel on *Xanthomonas* spp.



Effect of methanolic extract of Ganesh on *Aspergillus flavous*



Effect of methanolic extract on *fusarium oxysporum*

G – disc of extract of Ganesh
B – disc of extract of Bhagawa
M– disc of extract of Mridula
J– disc of extract of Jodhpuri Red
C - disc of control
F - disc of fungicide (captan)

Plate 2. Effect of methanolic extract of pomegranate peel on *A.flavous* and *F. Oxysporum.*

in ferric reducing antioxidant power whereas the ethyl acetate was least effective. As regard to the different varieties, variety Ganesh recorded a mean ferric reducing antioxidant power of 54.74 ¼M/g whereas variety Jodhpuri Red recorded the ferric reducing antioxidant power 52.85 μM/g. The solvent,variety and their interaction effects were significant.(Table 2).The differences in ferric reducing antioxidant power of the dried peel extract with three solvent was significantly higher with methanol and acetone. The peel extract obtained by use of mixture composed of methanol, ethanol, acetone and water was significantly higher in ferric reducing antioxidant power than those obtained using individual solvents, namely using methanol, ethanol or acetone [13].

Estimation of antioxidant activity by DPPH assay of dry peel

DPPH (2,2-diphenyl-1-picrylhydrazyl) has two major applications. One is monitoring of chemical reactions involving radicals most notably in common antioxidant assay and as a standard in electron paramagnetic resonance signal. DPPH is a well known radical and a trap (scavenger) for other radicals. The rate reduction of a chemical reaction upon addition of a DPPH is used as an indicator of the radical nature of that reaction. Three different solvents were used for the estimation of antioxidant activity of dry peel of pomegranate by DPPH assay. Acetone: water (80:20) extract of dried peel of pomegranate recorded maximum percent antioxidant activity whereas the ethyl acetate recorded the minimum percentage antioxidant activity. As regards to % antioxidant activity in different varieties is concerned maximum % antioxidant activity was observed in Ganesh for all concentrations (Table 3). Both variety and solvent recorded a significant difference in percent antioxidant activity in methanol:water (80:20) extract and solvent into variety interaction also were significant in methanol:water (80:20) extract. Maximum antioxidant activity (77.47%) was observed in Ganesh with acetone for 52 μl (1.56 mg) of diluted extract whereas the least % antioxidant activity of 0.32% was recorded in Jodhpuri Red with ethyl acetate for 39 μl (1.17mg) of diluted extract.

The percent antioxidant activity of Butylated Hydroxyltoluene (BHT) (0.16%) was recorded 74.16%. From the results it was observed that % antioxidant activity of acetone:water (80:20) extract is higher than Butylated Hydroxytoluene. Percent antioxidant activity of methanol:water (80:20) extract with a mean value is 74.29% for 100 μl of diluted extract, containing 3.0mg of peel powder. The antioxidant activity of 85.4± 3.1 % have been recorded earlier in the dried pomegranate peel extract with a methanol and 25.2 ± 2.0 % with a aqueous extract [22]. Study of antioxidant activities of the extracts was carried to investigate the correlations between the antioxidant activity and the content of phenolics, proanthocyanidins and flavonoids. The results indicate a strong correlation between DPPH and total phenolics, but no correlation existed with proanthocyanidins and flavonoids and in agreement with previous report[15].

The antioxidant activity of pomegranate peel correlated to the total phenolics. Comparing methanol with water as the solvent in pomegranate. The total extract yield (dried extract/100g PPE) were 43.18% and 46.51%, the yield of total phenolics (g total calculated weight of phenolic/100g PPE) were 5.90% and 8.26%, the content of phenolics (g phenol/100g dried extract) were 13.63% and 17.78% and the DPPH antioxidant activities were 53.74% and 65.30%, respectively [23]. By investigating the phenolic content and antioxidant capacity of pomegranate peel extracts, it was observed that the presence of phenolic compounds in this extract is an important factor in creating the antioxidant activity. In other words, more phenolic content causes more antioxidant activity[22].

The DPPH radical scavenging activity and phenolic compound content in different leaf extracts of blackberry was evaluated. Water has been reported as the best solvent with strongest antioxidative activity. The study also confirmed a strong correlation between antioxidant activity and the content of phenolics in the extracts. The leaf extract are good source of antioxidants compared to synthetic antioxidant BHA and about 7 times less active than vit. C [24]. Antioxidative components from pomegranate peel were extracted by using different solvents like methanol, ethanol, acetone, chloroform and ethyl acetate. All the extracts were tested for total phenolic content and the methanol which proved to be best in extracting the phenolic compounds was used to assess the antioxidant property previously [25].

Determination of antibacterial and antifungal activity of dry peel extracted with different solvent

Different solvents used for the determination of antibacterial and antifungal activity of dry peel of pomegranate extract by disc diffusion method. Methanolic extract of peel showed the highest inhibition zone than acetonic and ethyl acetate extract. As ragards to the antibacterial activity of different varieties is concerned maximum zone of inhibition in *Xanthomonas* spp. were observed by Ganesh with methanol *(X. citri*- 31.67 mm, *X. punicae*- 31.67mm, *X. malvacearum*-32.33 mm). Both variety and solvent recorded significant differences in zone of inhibition with all three solvent (Plate 1& 2). When the pomegranate peel extract of same three solvent tested for antifungal activity on *Fusarium oxysporum* f.sp.*ciceri* it was observed that only methanol:water (80:20) extract showed zone of inhibition and there is no zone of inhibition with acetone and ethyl acetate extract of fresh peel (Plate 3). Antifungal activity on *Aspergillus flavous* was determined by methanol:water (80:20) extract of Ganesh with different concentrations of extract. It was observed that mean value of zone of growth of *Aspergillus flavous* is 38.17 mm at a concentration of 15 µl/100 ml PDA and there is a least zone of growth of *Aspergillus flavous* (18.00 mm) at a concentration of 50 µl/100 ml PDA. When the control was tested it was observed that zone of growth of *Aspergillus flavous* was 64.0 mm. The methanolic peel extract has shown highest antimicrobial activity compared to aqueous extract[26] . The pomegranate peel extract alone did not exhibit antimicrobial activity against any of the isolates. Variation in results between studies on pomegranate extracts are not only seen in disc diffusion assay, but have also been recorded with minimum inhibition concentration determination[27]. Methanolic extract of pomegranate peels exhibit good bacteriostatic and bactericidal effects and could potentially be a good substitute for the synthetic antibiotic against pathogenic resistant bacteria [28] . Pomegranate ethanol extract group was tested with concentrations of 12.5, 25, 50 and 100 ¼l/ml. The results showed 60% motility of *Trichomonas tenax* trophozoite after treatment with 12.5 µg/100 ml of pomegranate ethanol extract group and 25 µg/ml showed higher anti-*T. Tenax*. The ethanol extract of pomegranate peel showed remarkable effect on *T. Tenax* [29] . The antifungal effect of petroleum ether, ethyl acetate and n-butanol fractions isolated from pomegranate pericarp and flower against Candida albicans. The maximum inhibition zone of Candida albicans was obtained by peel's n-butanol fraction, 35 mm. Petroleum ether fraction had no any antifungal activities [30].

CONCLUSION

Both the methanol : water and acetone : water (80:20) was found to be the best solvent mixture for extracting the phytochemicals from pomegranate peel and the peel extract of Ganesh variety of pomegranate recorded maximum content of phenols, proanthocyanidins, flavonoids and also had more antioxidant potential.

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